# THE HEADSPACE ANALYSIS OF POTATO VOLATILES

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# SUMMARY

The work in this project is concerned with the headspace analysis of potato volatiles. In the context of the thesis, the term potato volatiles deals with the headspace volatiles that are naturally evolved by raw potato tubers, and the anthropogenic volatile sprout suppressant chemicals that are applied to stored potato tubers. Both aspects of potato volatiles were investigated and consequently this thesis is divided into two sections: Section 1 deals with the headspace analysis of the volatiles that are produced by raw potato tubers; Section 2 deals with the headspace analysis of two widely used commercial potato sprout suppressants.

After harvesting, potato tubers undergo a period of dormancy which is defined as the time when the tuber buds are not growing: when dormancy is broken, the buds grow and elongate into sprouts. The mechanism of tuber dormancy is extremely complex, and the transition from the dormant to non-dormant state is associated with several physiological changes in the composition of the tuber. The first section, Section 1, of the project is an account of the investigations of the volatile compounds that are naturally evolved by raw potato tubers, in relation to the question of tuber dormancy. It has been shown that the volatiles produced by dormant potato tubers have sprout suppressant properties, thereby delaying the breaking of tuber dormancy (Burton, W.G., 1952), (Burton, W.G., Meigh, D.F., 1971). Further to this, it has been shown that the dimethylnaphthalene class of compounds is present in raw potato tubers and has been shown to possess potent sprout suppressant

properties (Meigh, D.F., et al, 1973), (Beveridge, J. 1980). However, it was thought that dormant potato tubers evolved volatiles, apart from the dimethylnaphthalenes, that had sprout suppressant properties. The aim of the project was the isolation and identification of the headspace volatiles that are naturally evolved by raw potato tubers and to relate these volatiles to tuber dormancy.

The bulk of the work was taken up with the development of the analytical techniques for the isolation and separation of raw tuber headspace volatiles. Various systems were tested for the containment and separation of the headspace volatiles, from the outset the porous polymer adsorbent Tenax G.C. was used to trap the headspace volatiles, and high resolution gas chromatography using capillary columns was used for their separation. Three systems were developed for sampling: the first using an aluminium tank proved to be unsuitable due to background volatiles arising from the tank, the second and third which were constructed from glass and PTFE were found to be successful from the point of view of isolating a sample of potato tuber volatiles free from background volatiles. These two systems were used to study the headspace volatiles produced by sprouted and dormant potato tubers and to relate the breaking of dormancy to any changes in the volatiles produced. In addition, a gas chromatographic system was developed for the transfer of volatiles adsorbed on a Tenax G.C. precolumn into a capillary column, the final system adopted involved the

thermal desorption of the volatiles from the Tenax G.C. into a small volume cold trap to concentrate the volatiles into a small volume and then a rapid reheat of the cold trap for the introduction of the volatiles into the capillary column.

Chapters 1 and 2 review the volatiles that have been isolated from potatoes and the techniques used for their isolation. Chapters 3 - 7 describe the development of the analytical techniques used in this project and Chapter 8 is an assessment of these analytical techniques compared to a more widely used method for the isolation of potato volatiles. Chapters 9 and 10 describe the analysis of the volatiles produced by both dormant and sprouted potato tubers.

In Section 2, the headspace and residue analyses of two widely used commercial sprout suppressants are described. Currently a substantial portion of the potato crop is used for the production of processed potato products (i.e. potato crisps, granules and chips) after harvesting the potatoes are stored in large scale commercial potato stores, the storage periods ranging from 2 - 8 months. Consequently, the stored tubers are treated with sprout suppressants to prevent a deterioration in the tuber quality due to increased water loss through the permeable surface of the sprout. The two most widely used sprout suppressant chemicals are tecnazene (1,2,4,5-tetrachloro-3-nitro-benzene) and chlorpropham ((3-chlorophenyl) carbamic acid 1-methyl ethyl ester); both these chemicals are volatile and act in the vapour phase. A study of the headspace and residue levels

of tecnazene and chlorpropham in large scale commercial potato stores was undertaken. It was found that under commercial storage conditions the levels of tecnazene were $\sim$ 10 ug $dm^{-3}$ and chlorpropham were $\sim$ 0.1 ug $dm^{-3}$ in a potato store headspace, in addition a considerable build up of residue chlorpropham in the fabric of potato stores was found to occur over several storage seasons, and that this residue chlorpropham made a significant contribution to the headspace chlorpropham in the store.

# LIST OF PLATES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| A | amps |
| bp | boiling point |
| °C | degree centigrade |
| cc | cubic centimetre |
| cm | centimetre |
| c.v. | cultivar |
| C.V. | coefficient of variation |
| C.W.20M | carbowax 20M |
| $dm^3$ | cubic decimetre |
| F.I.D. | flame ionisation detector |
| g | gram |
| G.C. | gas chromatography/gas chromatograph |
| G.C.M.S. | gas chromatography-mass spectroscopy |
| hr | hour |
| I.D. | internal diameter |
| IR | infra-red |
| kg | kilogram |
| lb | pound (0.453 kg) |
| m | metre |
| mg | milligram |
| min | minute |
| mm | millimetre |
| $mm^3$ | cubic millimetre |
| mp | melting point |
| M.S. | mass spectroscopy |
| Mol. Wt. | molecular weight |
| ng | nanogram |
| O.D. | outside diameter |
| PTFE | polytetrafluoroethylene |
| RI | retention index |
| RT | retention time |
| S.C.O.T. | support coated open tubular |
| W | watt |
| W.C.O.T. | wall coated open tubular |
| wk | week |
| u | carrier gas velocity |
| $\bar{x}$ | arithmetic mean |

| | | |
|---|---|---|
| 1/8 inch | 3.175 mm | These units are according to the manufacturers specifications for the equipment to which they refer |
| 1/4 inch | 6.35 mm | |
| 3/8 inch | 9.525 mm | |

# CHAPTER 1

# POTATO VOLATILES

## 1.1 INTRODUCTION

This chapter is primarily concerned with the identity and properties of the volatile chemicals produced by potato tubers. The techniques used for the collection, separation and identification of these volatiles are discussed in greater detail in Chapter 2.

The field of potato volatile research has encompassed two areas of work, which although similar in the analytical techniques that have been employed, differ in terms of the final aim of the research.

The earliest line of research was concerned with the potato volatiles that were physiologically involved with potato tubers, in particular the volatiles that were involved with the control of tuber dormancy and sprouting. The aim of this aspect of potato volatile research was the identification of the volatiles produced by potato tubers that were responsible for sprout inhibition. The bulk of the latter work was concerned with the contribution of cooked or processed potato volatiles to the odour and flavour of these potato products, i.e. boiled potatoes, baked potatoes, potato granules and potato crisps. The aim of this work was the identification of the volatiles responsible for characteristic flavours or off-flavours in these potato products.

Although there are some overlaps of interest between the work on "physiological" and "flavour" potato volatiles, they can be regarded as two separate lines of research and will be dealt with as such in this chapter.

A list of the potato volatiles that have been identified from both raw and processed potatoes is included at the end of this chapter (Table 1).

## 1.2 PHYSIOLOGICALLY SIGNIFICANT POTATO VOLATILES

The earliest work in relation to potato volatiles was concerned with the effects of ripe apple volatiles and ethylene upon the sprouting and subsequent growth of the potato plant from potato tubers.

It was found that both ripe apple volatiles and ethylene induced similar growth inhibiting effects upon sprouting tubers and potato plants. The tubers exhibited loss of apical dominance; growing potato plants exposed to ripe apple volatiles ceased normal growth and showed symptoms of epinasty. These effects were also induced by ethylene, acetylene, propylene, butylene and carbon monoxide (Huelin, F.E., 1932), (Elmer, O.H., 1932), (Elmer, O.H., 1936).

As the effects of ripe apple volatiles and ethylene were similar it was assumed that the active constituent of ripe apple volatiles was ethylene. Ethylene was confirmed to be present in ripe apple volatiles by the formation of a diphenyl ethylene amine derivative from the volatiles produced by 60 lbs of ripe apples (Gane, R., 1934).

The growth inhibiting effect of other compounds that had been isolated from apples were assessed also. These compounds were geraniol, citral, ethanol and acetaldehyde, which although they inhibited sprouting did not produce the characteristic abnormal sprout growth induced by ripe apple volatiles (Huelin, F.E., 1932). The constituents and physiological age of apples were investigated. Immature or decayed apples had no effect on the potato plant, neither did a steam distillate of apples nor cider.

Only ripe apples or the endocarp or mesocarp tissue of ripe apples produced the characteristic growth inhibiting effects (Elmer, O.H., 1936).

An investigation of the growth inhibiting effects of other fruit volatiles found that ripe pears and hawthorn fruit induced growth inhibition in potato plants but the volatiles produced by bananas, oranges, onions or sweet potatoes did not (Elmer, O.H., 1936). However, it was later established that bananas and oranges produced ethylene, and the volatiles from oranges and other citrus fruit induced epinasty in potato plants (Niedrl, J.B. et al, 1938), (Miller, E.V., Winston, J.R., 1940).

Subsequent to this, the next contribution to the work on the growth inhibiting volatiles involved with potatoes was concerned with the growth inhibiting effects of the volatiles produced by potato tubers themselves. Although it was known that high concentrations of carbon dioxide inhibited potato tubers from sprouting, it was proposed that there were two groups of volatile metabolic products produced by tubers, apart from carbon dioxide, that also had an inhibiting effect upon the sprouting of potato tubers (Burton, W.G., 1952). One group included ethylene, and in view of work on steam distillates of potatoes (Wegner, H., 1949), the other included n-amyl alcohol (1-pentanol); the latter was shown to have a sprout inhibiting effect when applied to potato tubers (Burton, W.G., 1952). Continued work on a variety of aliphatic alcohols showed that in addition to n-amyl alcohol, nonyl alcohol (3,3,5-trimethylhexan-1-ol) inhibited the sprouting of potato tubers, and that it showed

promise as a commercial sprout suppressant (Burton, W.G., 1956), (Burton, W.G., 1958).

Further investigation of the volatiles produced by tubers showed that ethylene and n-amyl alcohol, although proven to have sprout suppressant properties, were not produced in sufficient quantities by the tuber itself to suppress sprouting. However, twenty to thirty unidentified volatile compounds were found to be produced by potato tubers. The volatile or volatiles responsible for sprout suppression were thought to be olefinic, etheral or sulphur containing (Burton, W.G., Meigh, D.F., 1971). Later a clearer picture of the nature of potato tuber volatiles was obtained, despite difficulties experienced in isolating sufficient amounts of volatiles for analysis. In addition to twenty-five other volatiles, two volatiles were found with potent sprout suppressant properties. These were 1,4-dimethylnaphthalene and 1,6-dimethylnaphthalene. However, it was noted that the levels of dimethylnaphthalene that had to be applied to potato tubers for the suppressions of sprouting were far higher than the levels of dimethylnaphthalene naturally occurring in potatoes. This led the authors to conclude that dimethylnaphthalene was not solely responsible for natural sprout suppression and could act synergistically with some as yet unidentified chemical or chemicals in the volatile fraction from potato tubers (Meigh, D.F., et al, 1973).

Subsequent to this work, very little has been published to further elucidate the nature of the sprout suppressant properties of potato tuber volatiles.

The role of potato volatiles in the potato/pest, potato/pathogen relationship have recently been studied, with respect to the volatiles produced by or involved in the relationship. The volatiles produced by potato tubers infected with soft rot have been shown to include abnormally large amounts of n-butane, ethanol, acetone, 2-butanone and 3-hydroxybutanone, under both laboratory and commercial potato storage conditions. 2-butanone was proposed as a suitable volatile for predicting and monitoring the progress of a soft rot infection in a commercial potato store, thereby aiding store management (Varns, J.L., Glynn, M.T., 1979). One of the few areas of work not concerned with potato tuber volatiles was a study of the volatiles produced by potato plant foliage and the relationship between these volatiles and the orientation of the colorado beetle (Leptinotarsa decemlineata) to the plant. Five volatiles were isolated from the potato foliage, these being trans-2-hexen-1-ol, 1-hexen-1-ol, cis-3-hexen-1-ol, trans-2-hexenal and linalool. It was found that the attractive response of the colorado beetle to the potato plant was due to a precise combination of these and other potato foliage volatiles rather than one individual chemical (Visser, J.H., Ave, D.A., 1978), (Visser, J.H., et al, 1979).

## 1.3 POTATO FLAVOUR VOLATILES

The investigation of potato volatiles in relation to the odour and flavour of processed potato products has constituted by far the bulk of the work on potato volatiles and also to the majority of the volatiles that have been identified. Several classes of compounds have been isolated, the most active in terms of their contribution to potato flavour being the aldehydes and pyrazines. The origin of most of these processed potato volatiles can be attributed to the effect of the cooking process on the potatoes, e.g. boiling, frying, baking and dehydration, and under these conditions the volatiles produced could not be expected to reflect the volatiles produced by raw unadulterated tubers. However, the nature of these flavour volatiles is of interest and most of the volatiles found to occur in raw tubers have also been isolated from processed potatoes.

Preliminary isolates of potato volatiles by the steam distillation of raw tubers indicated the presence of a variety of fatty acids, esters, alcohols, phenols, unsaturated compounds and sulphur compounds (Kroner, W., Wegner, H., 1942), (Wegner, H., 1949). From this early work to the present day, the number and classes of volatile compounds that are known to be involved in potato flavour has greatly expanded. The main factors contributing to this expansion are the increasing degree of sophistication and sensitivity of the analytical techniques, and the diversifying fields of interest in the choice of processed potato flavour to be studied.

The aldehydes, pyrazines and other heterocyclics that have been identified in processed potato volatiles will be discussed as they are generally agreed to be the most flavour active constituents of processed potato volatiles. Volatiles belonging to other classes of compounds are, with a few exceptions, not major contributors to potato flavour.

### 1.3.1 The Aldehydes

The aldehydes produced by processed potatoes were the earliest class of compounds to be investigated, in particular their contribution to the off-flavour of stored potato granules and chips.

Initially it was shown that an off-flavour rapidly developed in stored potato granules under conditions of high temperature, high headspace oxygen concentration and low moisture content (Burton, W.G., 1949). Later, the off-flavour development was linked to the oxidation of the unsaturated fatty acids in the stored granules. The degree of off-flavour in the granules was expressed by the ratio of two readily oxidised unsaturated fatty acids, linoleic acid and linolenic acid and to two relatively unoxidised saturated fatty acids, palmitic acid and stearic acid. This was called the unsaturated ratio. A decrease in the unsaturated ratio was shown to follow the development of an off-flavour (Buttery, R.G., et al, 1961), (Buttery, R.G., 1961). Headspace analysis above reconstituted granules showed that there were four aldehydes involved in the potato granule off-flavour. These were 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and n-hexanal, which were produced by the oxidation of unsaturated fatty acids (Buttery, R.G., Teranishi, R., 1963). Much of the later work on potato granule storage off-flavour centred on the production of these four aldehydes under different storage regimes (Boggs, M.M., et al, 1964), (Sapers, G.M., et al, 1970), (Sullivan, J.F., et al, 1974). However, although these four aldehydes

contributed to some extent to the off-flavour, their production was taken as an indication of off-flavour in potato granules and not the sole cause. This point is best illustrated by an investigation of the off-flavour in explosion puffed dehydrated potato granules. Although 2-methylpropanal, 2-methylbutanal and 3-methylbutanal were known to be involved in the off-flavour, further investigation showed that in addition to these aldehydes, a mixture of alkyl pyrazines produced a more organoleptically accurate off-flavour (Sapers, G.M., et al, 1971).

The contribution of the aldehydes to the flavour of potato crisps has also been the subject of much investigation, particularly for the isolation of the desirable flavour components of potato crisps, thereby enabling the production of low fat crisps which possessed a desirable odour and flavour.

As with potato granules, potato crisps developed an off-flavour during storage, this off-flavour being stale rather than rancid in nature. In comparison to fresh crisps, stale crisps contained higher levels of saturated aldehydes, saturated ketones, 2-enals and lower levels of 2,4-decadienal. The latter was said to possess a desirable deep fried odour, and its source was thought to be from the degradation of linoleic acid in the frying oils (Mookherjee, B.D., et al, 1965). Further investigation of the volatile compounds with desirable crisp odours indicated that methional, 3-methylbutanal, phenylacetaldehyde and 2,4-decadienal in combination were important to a good potato crisp flavour (Buttery, R.G., Ling, L.C., 1972). However, organoleptic

trials on mixtures of compounds including 2,4-decadienal, methional, phenylacetaldehyde and two pyrazines found that although a similar odour to that of crisps could be obtained, it was inferior to the true odour of crisps (Guadagni, D.G., et al, 1972). There is evidence for further as yet unidentified compounds that may have an important role in crisp flavour. A methanol extract of crisps, as opposed to the more widely used pentane extract, yielded an extract with a desirable crisp odour. The compound or compounds responsible for the crisp odour was unstable at room temperature and could not be identified (Buttery, R.G., Ling, L.C., 1972). A frying oil was imparted with a crisp like odour by frying methionine in the oil which under these conditions breaks down to methional. However, it was found that the simple addition of methional, a known component of desirable crisp odour, to the oil did not impart the same crisp like odour.

Further investigation showed that the desirable crisp odour of the oil was due to an as yet unidentified high molecular weight compound rather than methional (Lee, S.C., et al, 1973).

Therefore, although certain individual components of the desirable odour of crisps have been identified and characterised, the complete nature of crisp odour has not (Buttery, R.G., 1973), (Deck, R.E., et al, 1973).

### 1.3.2 The Pyrazines

The pyrazines are the most interesting class of volatiles involved in potato flavour, although they were found to be present in potatoes at an early stage of the work (Shoruign, P., et al, 1933). Their full significance in potato flavour was not realised until later. Generally, the pyrazines are present at very low levels, 1 - 100 ug $kg^{-1}$ in raw or processed potatoes. However, they make a significant contribution to the overall odour and flavour of potato products by virtue of their very low odour thresholds (Seifert, R.M., et al, 1970) (Guadagni, D.G., et al, 1972).

So far forty-three pyrazines have been identified in potatoes, many having characteristic odours that contribute to the desirable odour and flavour of the potato product. Some of the substituted pyrazines have been used to enhance the flavour of a variety of potato products (Guadagni, D.G., et al, 1971). 2-methoxy-3-ethylpyrazine and 2-acetyl-3-ethylpyrazine have been patented as processed potato product flavourants (Guadagni, D.G., et al, 1973), (Mookherjee, B.D., et al, 1973).

The "raw earthy" aroma of potatoes was initially attributed to 2,5-dimethylpyrazine which was isolated from potato crisps (Deck, R.E., Chang, S.S., 1965), (Deck, R.E., et al, 1973). It has been reported that 2-methoxy-3-ethylpyrazine possesses a "raw earthy" potato odour; it has been tentatively identified in boiled potatoes (Buttery, R.G., et al, 1970), (Buttery, R.G., Ling, L.C., 1973) and raw potato peel (Meigh, D.F., et al, 1973). However, it has only been positively

identified in boiled potato sprouts (Nursten, H.E., Sheen, M.R., 1974). Other pyrazines that have been reported to have an "earthy" potato odour, either by themselves or in combination with other pyrazines are 2,3-diethylpyrazine (Coleman, E.C., Ho, C.T., 1980), 2-ethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2,5-diethylpyrazine (Deck, R.E., et al, 1973) and 2-methoxy-3-isopropylpyrazine (Buttery, R.G., Ling, L.C., 1973), (Meigh, D.F., et al, 1973), (Murray Whitfield 1975)

The pyrazines have not been shown to contribute to the desirable flavour of potato granules; however, they have been implicated in the toasted off-flavour that developed in explosion puffed dehydrated potato granules. 2-methylpyrazine, 2,5-dimethylpyrazine and three other unidentified pyrazines were thought to be involved in this off-flavour (Sapers, G.M., et al, 1971).

The pyrazines are involved in the desirable odour and flavour of potato crisps and baked potatoes. 2-ethyl-3,6-dimethylpyrazine has been shown to be the most important of the pyrazines in crisp and baked potato aroma (Buttery, R.G., et al, 1971), (Buttery, R.G., et al, 1973), (Pareles, S.R., Chang, S.S., 1974), (Coleman, E.C., et al, 1981), 2-ethyl-5-methylpyrazine and 2,3,5-trimethylpyrazine contribute a baked odour to crisps (Deck, D.E., et al, 1973). The most significant pyrazines in baked potato aroma apart from 2-ethyl-3,6-dimethylpyrazine are 2-isobutyl-3-methylpyrazine, 2,3-diethyl-5-methylpyrazine and 3,5-diethyl-2-methylpyrazine. These three pyrazines combined have an odour closer to baked potato odour than any single compound (Pareles, S.R., Chang, S.S., 1974), (Coleman, E.C., Ho, C.T., 1980).

The contribution of other classes of volatiles to the odour and flavour of baked potatoes has recently been studied, although it was found that the substituted pyrazines are dominant in baked potato odour and flavour. Other heterocyclic compounds have also been shown to be important in potato flavour. A "cracker" aroma was contributed to potato crisps by 2-acetylpyridine (Buttery, R.G., et al, 1971). Possible structural/flavour relationships have been perceived amongst these heterocyclic compounds. Oxazoles, pyrroles and thiophenes with acetyl or formyl substituents had nutty aromas had thiazoles with similar alkyl substituents to pyrazines and similar odours to these pyrazines (Coleman, E.C., Ho, C.T., 1980), (Ho, C.T., Coleman, E.C., 1980), (Coleman, E.C., et al, 1981).

## 1.4 CONCLUSIONS

On consideration of the work on "physiological" and "flavour" potato volatiles, it was concluded that both aspects of the work had advanced considerably since the initial investigations. The dimethylnaphthalenes isolated from potato tubers have been shown to possess potent sprout suppressant properties, and several potato volatiles have been shown to be of key importance to the flavour of processed potato products. Further to this it was thought that with a few exceptions the work on potato flavour volatiles was more extensive, detailed and of a higher quality than the work on physiological potato volatiles. However, in both fields of work, there remain many points that are worthy of further investigation. Although several compounds have been shown to make a major contribution to the flavour of processed potato products, the complete nature of potato flavour has not been defined in absolute chemical terms. The nature of potato dormancy has not been resolved in terms of the naturally produced dimethylnaphthalene potato sprout suppressants, it was thought that there were other compounds with sprout suppressant properties present in the volatile fraction of potato tubers.

The scope of this section of the project was the investigation of the volatiles present in the headspace surrounding potato tubers, with a view towards elucidating the sprout suppressant properties of these potato tuber headspace volatiles.

TABLE 1

## 1.5 DESCRIPTION

Table 1 consists of two parts; Part 1 and Part 2.

Part 1 is a list of the volatiles that have been isolated from raw and processed potatoes. Column 1 assigns a number to each volatile. Column 2 gives the name of each volatile, where practicable using the nomenclature under which the volatile was originally reported. However, in certain cases, especially the sulphur containing volatiles, the nomenclature used for the same compound was not consistent. Therefore the name assigned to the volatile may not be the same as that used when it was originally reported. Columns 3 - 7 refer to the form of the potato product from which the volatile was isolated:-

R - raw potatoes
Bl - boiled potatoes
Bk - baked potatoes
Cr - potato crisps
Gr - potato granules.

If the extraction procedure involved boiling the potatoes, in the case of raw tuber volatile isolates, the volatiles are listed as being isolated from boiled potatoes, irrespective of whether they were reported as being raw potato volatiles.

Part 2 is a cross reference list where the number assigned to each volatile in Part 1 is matched to the number of the paper, in the reference list, where the volatile was reported. When the number corresponding to the paper is in brackets this means that the volatile was identified tentatively in the paper.

TABLE 1

PART 1

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| | SATURATED HYDROCARBONS | | | | | |
| 1 | methane | | | | | * |
| 2 | ethane | * | | | | * |
| 3 | propane | | | | | * |
| 4 | butane | * | | | | * |
| 5 | pentane | | | | * | * |
| 6 | heptane | | * | | * | |
| 7 | octane | | * | | | |
| 8 | nonane | | | | * | |
| 9 | decane | | * | | * | * |
| 10 | undecane | | | | * | * |
| 11 | dodecane | | | | | * |
| 12 | tetradecane | | * | | * | * |
| 13 | methyl-cyclopentane | | | * | | |
| 14 | 2-methyltetradecane | | | * | | |
| 15 | 9-octylheptadecane | | | * | | |
| 16 | 3-methyleicosane | | | * | | |
| 17 | 2,4-dimethylheptane | | | * | | |
| 18 | 4,6-di-n-propyldodecane | | | * | | |
| 19 | cyclododecane | | | * | | |
| 20 | 5,7-dimethylhexadecane | | | * | | |
| 21 | 7,9-dimethylhexadecane | | | * | | |
| 22 | 2,6,9-trimethylundecane | | | * | | |
| 23 | 2,6,10-trimethylundecane | | | * | | |
| 24 | 2,6,10,14-tetramethylpentadecane | | | * | | |
| 25 | 2,6,11,15-tetramethylhexadecane | | | * | | |
| 26 | 1-cyclopentyl-4-octyldodecane | | | * | | |
| | UNSATURATED HYDROCARBONS | | | | | |
| 27 | ethylene | * | | | | |
| 28 | 2-methyl-1-butene | | | | * | |
| 29 | 1,4-dimethyl-4-vinylcyclohexene | | | * | | |
| 30 | 3,3,5-trimethyl-1-hexene | | | * | | |
| 31 | 3-ethyl-3-octene | | | * | | |
| 32 | 4-ethyl-3-octene | | | * | | |
| 33 | 1-octadecene | | | | * | |
| 34 | 1-decyne | | | * | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 35 | myrcene | | | * | | |
| 36 | farnesene | | | * | | |
| 37 | 3-carene | | | * | | |
| 38 | limonene | | * | * | * | |
| 39 | 3-p-menthene | | | | * | |
| 40 | γ-humulene | | | * | | |
| 41 | β-phellandrene | | | * | | |
| 42 | 2-pinene | | * | | | |
| 43 | 2-(10)-pinene | | * | * | | |
| 44 | α-terpinene | | | | * | |
| | AROMATIC HYDROCARBONS | | | | | |
| 45 | benzene | | * | * | | |
| 46 | ethylbenzene | | * | | * | |
| 47 | isopropylbenzene | | | * | | |
| 48 | butylbenzene | | | | * | |
| 49 | Sec-butylbenzene | | | * | | |
| 50 | Tert-butylbenzene | | | * | | |
| 51 | nonylbenzene | | | * | | |
| 52 | acetylbenzene | | | | * | |
| 53 | cyanobenzene | | | | | |
| 57 | 1-methyl-4-ethylbenzene | | | * | | |
| 58 | o cymene | | | * | | |
| 59 | 3-ethylstyrene | | | * | | |
| 60 | 1,2,4-trimethylbenzene | | | | * | * |
| 61 | 1,2,3-trimethylbenzene | | * | | | |
| 62 | 1,3,5-trimethylbenzene | | * | | | * |
| 63 | 1-ethyl-3,5-dimethylbenzene | | | | * | |
| 64 | 1,2,3,5-tetramethylbenzene | | | * | | |
| 65 | hexamethylbenzene | | | * | | |
| 66 | benzyl alcohol | | * | * | | |
| 67 | trimethylbenzyl alcohol | | | * | | |
| 68 | 3-methoxy-4-isopropylbenzyl alcohol | | | * | | |
| 69 | benzaldehyde | | * | * | * | * |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 70 | ethylbenzaldehyde | | | * | | |
| 71 | 2,5-dimethylbenzaldehyde | | | * | | |
| 72 | phenylacetaldehyde | | * | * | * | * |
| 73 | p-menthylacetophenone | | | * | | |
| 74 | salicylaldehyde | | | * | | |
| 75 | methylsalicylate | | * | | | |
| 76 | p-methoxycinnamaldehyde | | | * | | |
| 77 | toluene | | * | | | * |
| 78 | o-ethyltoluene | | * | | | |
| 79 | p-ethyltoluene | | * | | | |
| 80 | 2,6-di-T-butyl-4-hydroxytoluene | | | | * | |
| 81 | 2-amino-4-nitrotoluene | | | * | | |
| 82 | o-xylene | | * | | | |
| 83 | m-xylene | | * | | | |
| 84 | p-xylene | | * | | | |
| 85 | naphthalene | * | * | | | |
| 86 | 1-methylnaphthalene | * | * | | | |
| 87 | 2-methylnaphthalene | * | * | | | |
| 88 | 2-isopropylnaphthalene | | | * | | |
| 89 | 1,2-dimethylnaphthalene | | | * | | |
| 90 | 1,3-dimethylnaphthalene | | | * | | |
| 91 | 1,4-dimethylnaphthalene | * | | | | |
| 92 | 1,6-dimethylnaphthalene | * | | | | |
| 93 | 2,6-dimethylnaphthalene | * | | | | |
| 94 | 2,7-dimethylnaphthalene | * | | * | | |
| 95 | 1,4,5-trimethylnaphthalene | | | * | | |
| 96 | 1,3,8-trimethylnaphthalene | | | * | | |
| 97 | 1,4,6-trimethyl-1,2,3,4-tetrahydronaphthalene | | | * | | |
| 98 | napthol | | | * | | |
| 99 | biphenyl | * | * | * | | |
| 100 | diphenylmethane | | | * | | |
| 101 | diphenylamine | | | * | | |
| | ALCOHOLS | | | | | |
| 102 | methanol | | * | * | | |
| 103 | ethanol | * | * | | * | |
| 104 | propanol | * | | | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 105 | pentanol | | * | | * | |
| 106 | hexanol | | * | | | |
| 107 | heptanol | | * | * | | |
| 108 | octanol | | * | | | |
| 109 | dodecanol | | | * | | |
| 110 | hexadecanol | | | * | | |
| 111 | butan-2-ol | * | * | | * | |
| 112 | heptan-2-ol | | * | | | |
| 113 | octan-3-ol | | * | | | |
| 114 | 2-methylbutanol | | * | | | |
| 115 | 3-methylbutanol | | * | | * | |
| 116 | 3-methylpentanol | | | * | | |
| 117 | 2-methylpentan-2-ol | | | * | | |
| 118 | 3-isobutyloctanol | | | * | | |
| 119 | 2,4-dimethyl-3-pentanol | | | * | | |
| 120 | 3,6-dimethyl-3-octanol | | | * | | |
| 121 | 2-pentenol | | | * | | |
| 122 | 2-methyl-1-penten-3-ol | | | * | | |
| 123 | 4-methyl-3-penten-2-ol | | | * | | |
| 124 | 4-methyl-4-pentenol | | | * | | |
| 125 | 1-hexen-1-ol | potato plant foliage | | | | |
| 126 | 2-hexen-1-ol | | | | | |
| 127 | 3-hexen-1-ol | | | | | |
| 128 | 1-octen-3-ol | | * | * | | * |
| 129 | nerol | | * | | | |
| 130 | linalool | | * | | | |
| 131 | geraniol | | * | | | * |
| 132 | 2-nonenol | | * | | | |
| 133 | hexahydrofarnesol | | | * | | |
| 134 | α-terpineol | | * | | * | |
| 135 | cyclohexanol | | | * | | |

ALDEHYDES SATURATED

| | | | | | | |
|---|---|---|---|---|---|---|
| 136 | acetaldehyde | * | * | | * | * |
| 137 | propanal | * | * | | | * |
| 138 | butanal | | * | | * | * |
| 139 | pentanal | * | * | * | * | * |
| 140 | hexanal | * | * | * | * | * |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 141 | heptanal | * | * | * | * | * |
| 142 | octanal | * | * | | * | * |
| 143 | nonanal | | | * | * | * |
| 144 | decanal | | * | * | | |
| 145 | hexadecanal | | | * | | |
| 146 | octadecanal | | | * | | |
| 147 | 2-methylpropanal | | * | * | * | * |
| 148 | 2-methylbutanal | | * | * | * | * |
| 149 | 3-methylbutanal | | * | * | * | * |
| 150 | 2-ethylhexanal | | | * | | |
| | ALDEHYDES UNSATURATED | | | | | |
| 151 | propenal | | * | | * | |
| 152 | 2-butenal | | * | | | |
| 153 | 2-pentenal | | * | * | * | * |
| 154 | 2-hexenal | * | * | * | * | * |
| 155 | 3-hexenal | | | * | * | |
| 156 | 2-heptenal | | * | * | * | |
| 157 | 2-octenal | | * | * | * | * |
| 158 | 2-nonenal | | * | * | * | |
| 159 | 2-decenal | | | * | * | |
| 160 | 2-undecenal | | | * | * | |
| 161 | 2-methyl-2-propenal | | * | * | | |
| 162 | 3-methyl-1-butenal | | | * | | |
| 163 | 2-methyl-2-butenal | | | * | | |
| 164 | 3-methyl-2-butenal | | | * | | |
| 165 | 2-isopropyl-2-butenal | | | | * | |
| 166 | 2-phenyl-2-butenal | | | | * | |
| 167 | 4-methyl-2-pentenal | | | | * | |
| 168 | 4-methyl-2-hexenal | | | | * | |
| 169 | 5-methyl-2-hexenal | | | | * | |
| 170 | 2-phenyl-4-methyl-2-pentenal | | | * | * | |
| 171 | 2-phenyl-5-methyl-2-hexenal | | | * | * | |
| 172 | hepta-2,4-dienal | | * | * | * | |
| 173 | octa-2,4-dienal | | | | * | |
| 174 | nona-2,4-dienal | | | * | * | |
| 175 | deca-2,4-dienal | | * | * | * | |
| 176 | undeca-2,4-dienal | | | | * | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 177 | deca-2,4,7-trienal | | | | * | |
| | KETONES | | | | | |
| 178 | acetone | * | * | * | * | * |
| 179 | 2-butanone | * | | | * | |
| 180 | 2-pentanone | | | | * | |
| 181 | 2-hexanone | | * | * | * | |
| 182 | 2-heptanone | * | * | * | * | * |
| 183 | 2-octanone | * | | | * | |
| 184 | 2-nonanone | * | | | * | |
| 185 | 2-decanone | | * | | * | |
| 186 | 4-heptanone | | | * | | |
| 187 | 4-decanone | | | * | | |
| 188 | 2-methylpropanone | | * | | | |
| 189 | 3-hydroxy-2-butanone | * | | | | |
| 190 | 4-methyl-2-pentanone | | | * | | |
| 191 | 5-methyl-2-pentanone | | | * | | |
| 192 | cyclopentanone | | | * | * | |
| 193 | 2,5-dimethyl-2-cyclopentanone | | | * | | |
| 194 | 2-acetyl-3,3-dimethylcyclohexanone | | | * | | |
| 195 | 2-methyl-4-heptanone | | | * | | |
| 196 | 2,6-dimethyl-4-heptanone | | | * | | |
| 197 | pent-2-en-4-one | | | | * | |
| 198 | 1-octen-3-one | | * | | | |
| 199 | 3-octen-2-one | | | * | | |
| 200 | 2-nonen-4-one | | * | | * | |
| 201 | 4-methyl-3-penten-2-one | | | * | | |
| 202 | 2,6-dimethyl-3-penten-2-one | | | * | | |
| 203 | 2-methyl-hepten-6-one | | | * | | |
| 204 | 1-phenyl-1,2-propanedione | | | * | | |
| 205 | 2,3-butanedione | * | * | | * | |
| 206 | 2,3-pentanedione | | * | | * | |
| 207 | 2-methyl-hexa-4,5-dione | | | | * | |
| | ACIDS | | | | | |
| 208 | acetic | | | * | | |
| 209 | propanoic | | | * | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 210 | butanoic | | | * | * | |
| 211 | pentanoic | | * | * | * | |
| 212 | hexanoic | | * | * | * | |
| 213 | heptanoic | | | * | * | |
| 214 | octanoic | | | | * | |
| 215 | decanoic | | | | * | * |
| 216 | pentadecanoic | | | | | * |
| 217 | heptadecanoic | | | | | * |
| 218 | 2-methylpropanoic | | | * | * | |
| 219 | 2-methylbutanoic | | | | * | |
| 220 | 3-methylbutanoic | | | * | | |
| 221 | 2-ketoadipic | | | * | | |
| 222 | 2-methylpentanoic | | | * | | |
| 223 | 3-methylpentanoic | | | * | | |
| 224 | 4-methylpentanoic | | | * | | |
| 225 | 2-methylhexanoic | | | * | | |
| 226 | myristic | | | | | * |
| 227 | palmitic | | | | | * |
| 228 | palmitoleic | | * | | | * |
| 229 | stearic | | | | | * |
| 230 | oleic | | | | | * |
| 231 | linoleic | | * | | | * |
| 232 | linolenic | | * | | | * |
| 233 | arachidic | | | | | * |
| 234 | 2-phenylcrotonic | | | * | | |
| | ESTERS | | | | | |
| 235 | methyl acetate | * | | | | |
| 236 | ethyl acetate | * | | * | | |
| 237 | propyl acetate | | | | * | |
| 238 | 1-methylpropyl acetate | | | * | | |
| 239 | butyl acetate | | | * | * | |
| 240 | 2-methylbutyl acetate | | | * | | |
| 241 | pentyl acetate | | | * | | |
| 242 | hept-1-enyl acetate | | | * | | |
| 243 | methyl-2-methyl butanoate | | | * | | |
| 244 | methyl pentanoate | | | * | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 245 | methyl-2-methyl pentanoate | | | * | | |
| 246 | 2-methylbutyl pentanoate | | | * | | |
| 247 | methyl hexanoate | | | * | | |
| 248 | allyl hexanoate | | | * | | |
| 249 | methyl octanoate | | | * | | |
| 250 | methyl nonanoate | | | * | | |
| 251 | diisobutylphthalate | | | * | | |
| 252 | diethylphthalate | | | * | | |
| | ETHERS | | | | | |
| 253 | methyl ether | | | * | | |
| 254 | diethyl ether | | * | | | |
| 255 | ethylisopropyl ether | | | * | | |
| 256 | ethylpentyl ether | | | * | | |
| 257 | methylnonyl ether | | | * | | |
| 258 | 1-ethoxy-1-propoxyethane | | | * | | |
| 259 | 1,1-diethoxyethane | | | * | | |
| 260 | 1,1-diethoxyisopentane | | | * | | |
| 261 | diethyleneglycoldiethyl ether | | | * | | |
| 262 | diphenyl ether | | | * | | |
| | SULPHUR CONTAINING COMPOUNDS | | | | | |
| 263 | hydrogen sulphide | * | * | | | |
| 264 | dimethyl sulphide | * | * | | | |
| 265 | dimethyl disulphide | * | * | | * | |
| 266 | methylethyl sulphide | | * | | | |
| 267 | diethyl sulphide | | * | | | |
| 268 | methyl-n-propyl sulphide | | * | | | |
| 269 | methylisopropyl disulphide | | * | | | |
| 270 | methyl mercaptan | | * | | | |
| 271 | ethyl mercaptan | | * | | | |
| 272 | propyl mercaptan | * | * | | | |
| 273 | isopropyl mercaptan | | * | | | |
| 274 | T-butyl mercaptan | | * | | | |
| 275 | 2-methylmercaptoacetaldehyde | | | | * | |
| 276 | 2-methyl-mercaptomethyl-4-methyl pent-2-enal | | | | * | |
| 277 | 2-ethylhexylmercaptan | | | * | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 278 | methional | | * | * | * | * |
| 279 | thiophene | | | * | | |
| 280 | 2-formylthiophene | | | * | | |
| 281 | 2-butyl-5-ethylthiophene | | | * | | |
| 282 | benzothiophene | | * | | | |
| 283 | benzothiazole | * | * | | | |
| 284 | benzylthiobenzoate | | | | * | |
| 285 | 3,5-dimethyl-1,2,4-trithiolane | | * | | | |
| | PYRAZINES | | | | | |
| 286 | 2-methylpyrazine | | | * | | * |
| 287 | 2-ethylpyrazine | | | * | * | |
| 288 | 2,3-dimethylpyrazine | | | * | * | |
| 289 | 2,5-dimethylpyrazine | | | * | * | * |
| 290 | 2,6-dimethylpyrazine | | | * | * | |
| 291 | 2-methyl-5-vinylpyrazine | | | * | * | |
| 292 | 2-methyl-6-vinylpyrazine | | | | * | |
| 293 | 2-ethyl-3-methylpyrazine | | | * | * | |
| 294 | 2-ethyl-5-methylpyrazine | | | * | * | |
| 295 | 2-ethyl-6-methylpyrazine | | | * | * | |
| 296 | 2,3-diethylpyrazine | | | * | | |
| 297 | 2,5-diethylpyrazine | | | | * | |
| 298 | 2,6-diethylpyrazine | | | | * | |
| 299 | 2-ethyl-6-propylpyrazine | | | * | | |
| 300 | 2-ethyl-6-vinylpyrazine | | | * | | |
| 301 | 2-butyl-3-methylpyrazine | | | * | | |
| 302 | 2-butyl-6-methylpyrazine | | | * | | |
| 303 | 2-isobutyl-3-methylpyrazine | | | * | * | |
| 304 | 2-isobutyl-5-methylpyrazine | | | * | | |
| 305 | 2-isoamyl-5-methylpyrazine | | | | * | |
| 306 | 2-isobutenyl-3-methylpyrazine | | | | * | |
| 307 | 2-methoxy-3-ethylpyrazine | * | * | | | |
| 308 | 2-methoxy-3-isopropylpyrazine | | * | | | |
| 309 | 2,3,5-trimethylpyrazine | | | * | * | |
| 310 | 2,3-dimethyl-5-butylpyrazine | | | * | | |
| 311 | 2,5-dimethyl-3-butylpyrazine | | | * | | |
| 312 | 2,5-dimethyl-3-vinylpyrazine | | | | * | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 313 | 2,5-dimethyl-6-isopropylpyrazine | | | | * | |
| 314 | 2,6-dimethyl-3-butylpyrazine | | | * | | |
| 315 | 2-ethyl-3,5-dimethylpyrazine | | | * | * | |
| 316 | 2-ethyl-3,6-dimethylpyrazine | | | * | * | |
| 317 | 2,3-diethyl-5-methylpyrazine | | | * | * | |
| 318 | 2,5-diethyl-3-methylpyrazine | | | * | | |
| 319 | 2,6-diethyl-3-methylpyrazine | | | * | | |
| 320 | 3,5-diethyl-2-methylpyrazine | | | * | | |
| 321 | 2-isobutyl-3,6-dimethylpyrazine | | | * | * | |
| 322 | 2-isoamyl-3,6-dimethylpyrazine | | | | * | |
| 323 | 2-ethyl-3,5,6-trimethylpyrazine | | | * | | |
| 324 | 2-methyl-6,7-dihydro-5,H-cyclopentapyrazine | | | * | | |
| 325 | 5-methyl-6,7-dihydro-5,H-cyclopentapyrazine | | | * | | |
| 326 | 3,5-dimethyl-6,7-dihydro-5,H-cyclopentapyrazine | | | * | | |
| 327 | 2,3,6-trimethyl-5-hydroxy-cyclopentapyrazine | | | * | | |
| | PYRIDINES | | | | | |
| 328 | pyridine | | * | * | * | |
| 329 | 2-acetylpyridine | | | * | * | |
| 330 | 2-aminopyridine | | | * | | |
| | FURANS | | | | | |
| 331 | furan | | * | | | |
| 332 | 2-methylfuran | | * | | | |
| 333 | 3-methylfuran | | * | | | |
| 334 | 2-ethylfuran | | * | | | |
| 335 | 2-propionylfuran | | | * | | |
| 336 | 2-butylfuran | | * | | * | |
| 337 | 2-pentylfuran | | * | * | * | * |
| 338 | 2-acetylfuran | | | * | * | |
| 339 | Trans-2-(2-pentenyl)-furan | | | * | | |
| 340 | 2-hexylfuran | | * | | * | |
| 341 | 2,5-dimethyl-tetrahydrofuran | | | * | | |
| 342 | 2-methyl-tetrahydrofuran-3-one | | | * | | |
| 343 | furfural | | * | * | * | * |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 344 | 5-methyl-2-furaldehyde | | | * | * | * |
| 345 | methylfuroate | | | * | | |
| 346 | 2-methylketotetrahydrofuran | | | * | * | |
| 347 | 2-furfuryl alcohol | | | | * | |
| | OXAZOLES | | | | | |
| 348 | 2,4,5-trimethyloxazole | | | * | | |
| 349 | 5-acetyl-2,4-dimethyloxazole | | | * | | |
| | THIAZOLES | | | | | |
| 350 | 2,5-diethyl-4-methylthiazole | | | * | | |
| 351 | 2,5-dimethyl-4-ethylthiazole | | | * | | |
| 352 | 2,5-dimethyl-4-butylthiazole | | | * | | |
| | NITROGEN CONTAINING COMPOUNDS | | | | | |
| 353 | 2-acetylpyrrole | | | * | * | |
| 354 | N-methyl-2-formylpyrrole | | | * | | |
| 355 | N,N-diethylformamide | | | * | | |
| 356 | N,N-diethylacetamide | | | * | | |
| 357 | thymine | | | * | | |
| 358 | 2-isopropylbenzimidazole | | | * | | |
| | OXYGEN CONTAINING COMPOUNDS | | | | | |
| 359 | 2-propyl-1,3-dioxolane | | | * | | |
| 360 | phthalic anhydride | | | * | | |
| | HALOGEN CONTAINING COMPOUNDS | | | | | |
| 361 | chloroform | | | * | | |
| 362 | 1,1,1-trichloroethane | | | * | | |
| 363 | tetrachloroethane | | | * | | |
| 364 | 2-chloropropane | | | * | | |
| 365 | 1-chloro-2-methylbutane | | | * | | |
| 366 | 1-chloroheptane | | | * | | |
| 367 | 1,1-dichloroheptane | | | * | | |
| 368 | 1-chlorohexadecane | | | * | | |
| 369 | o-chloroaniline | | | * | | |

| | | R | Bl | Bk | Cr | Gr |
|---|---|---|---|---|---|---|
| 370 | p-chloroaniline | | | * | | |
| 371 | 2-chlorobiphenyl | | | * | | |
| 372 | trichloroacetic acid | | | * | | |
| 373 | 2-bromo-5-ethylnonane | | | * | | |
| 374 | 1-iodooctadecane | | | * | | |

TABLE 1

PART 2

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 1 | 17 |
| 2 | 17, 56 |
| 3 | 17 |
| 4 | 17, 97 |
| 5 | 17, 102 |
| 6 | 34, 60 |
| 7 | 60 |
| 8 | 34 |
| 9 | 34, 66, 60 |
| 10 | 34, 66 |
| 11 | 66 |
| 12 | 34, 66 |
| 13 | 29 |
| 14 | 29 |
| 15 | 29 |
| 16 | 29 |
| 17 | 29 |
| 18 | 29 |
| 19 | 29 |
| 20 | 29 |
| 21 | 29 |
| 22 | 29 |
| 23 | 29 |
| 24 | 29 |
| 25 | 29 |
| 26 | 29 |
| 27 | 37, 73, 30, 74, 67, 56 |
| 28 | 34 |
| 29 | 29 |
| 30 | 29 |
| 31 | 29 |
| 32 | 29 |
| 33 | 29 |
| 34 | 22 |
| 35 | 29 |
| 36 | 29 |
| 37 | 66, 29 |
| 38 | 34, 66, 29 |
| 39 | 34 |
| 40 | 29 |
| 41 | 29 |
| 42 | 89, 66 |
| 43 | 66, 28 |
| 44 | 34 |
| 45 | 89, 29, 60 |
| 46 | 34, (66) |
| 47 | 29 |
| 48 | 34 |
| 49 | 29 |
| 50 | 29 |
| 51 | 29 |
| 52 | 23 |
| 53 | 29 |
| 54 | 29 |
| 55 | 29 |
| 56 | 29 |
| 57 | 29 |
| 58 | 29 |
| 59 | 29 |
| 60 | 34, (66) |
| 61 | (66) |
| 62 | 89, (66) |
| 63 | 34 |
| 64 | 29 |
| 65 | 29 |
| 66 | 20, 29 |
| 67 | 29 |
| 68 | 29 |
| 69 | 20, 81, 22, 82, 25, 34, 66, 69, 29 |
| 70 | 29 |
| 71 | 29 |

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 72 | 20, 81, 22, 25, 34, 83, 66, 69, 29 |
| 73 | 29 |
| 74 | 29 |
| 75 | 20 |
| 76 | 29 |
| 77 | 66, 29, 60 |
| 78 | (66) |
| 79 | (66) |
| 80 | 34 |
| 81 | 29 |
| 82 | 89, 66, 60 |
| 83 | 66, 60 |
| 84 | 66 |
| 85 | 20, 60 |
| 86 | 60 |
| 87 | 20, 60 |
| 88 | 29 |
| 89 | 29 |
| 90 | 29 |
| 91 | 60 |
| 92 | 60 |
| 93 | 60 |
| 94 | 29, (60) |
| 95 | 29 |
| 96 | 29 |
| 97 | 29 |
| 98 | 29 |
| 99 | 60, 20, 29 |
| 100 | 29 |
| 101, | 29 |
| 102 | 88, 86, 89, 29 |
| 103 | 86, 34, 29, 74, 97 |
| 104 | 97, 53 |
| 105 | 89, 20, 22, 66 |
| 106 | 66 |
| 107 | 24, 29 |
| 108 | 24 |
| 109 | 29 |
| 110 | 29 |
| 111 | 22, 29, 97 |
| 112 | 66 |
| 113 | 24 |
| 114 | 20 |
| 115 | 20, 89, 22 |
| 116 | 29 |
| 117 | 29 |
| 118 | 29 |
| 119 | 29 |
| 120 | 29 |
| 121 | 29 |
| 122 | 29 |
| 123 | 29 |
| 124 | 29 |
| 125 | 100 |
| 126 | 100 |
| 127 | 100 |
| 128 | 20, 25, 66 |
| 129 | 20 |
| 130 | 20, 100 |
| 131 | 20, (66) |
| 132 | 24 |
| 133 | 29 |
| 134 | 20, 22 |
| 135 | 29 |
| 136 | 17, 87, 35, 88, 86, 89, 34, 54, 97 |
| 137 | 17, 88, 86, 89, 54 |
| 138 | 17, 35, (87), 34 |
| 139 | 17, 63, 53, 22, 34, 66, 102, 29, 54 |

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 140 | 17, 18, 7, 63, 89, 20, 80, 53, 54, 22, 82, 25, 34, 66, 102, 29 |
| 141 | 17, 35, 63, 20, 53, 22, 82, 23, 34, 66, 54, 29 |
| 142 | 17, 63, 22, 53, 54 |
| 143 | 17, 63, 22, 25, 29 |
| 144 | 25, 66, 60 |
| 145 | 29 |
| 146 | 29 |
| 147 | 17, 19, 87, 86, 89, 80, 22, 25, 29, 60 |
| 148 | 17, 18, 88, 86, 89, 80, 22, 25, 34, 93 |
| 149 | 17, 19, 86, 87, 89, 80, 22, 25, 83, 93 |
| 150 | 29 |
| 151 | 35, (87), 88 |
| 152 | 53 |
| 153 | (17), 35, (82), 66, 29 |
| 154 | (17), 35, 65, 53, 22, 82, 25, (66), 54, 100 |
| 155 | 34, 29 |
| 156 | 35, 63, 20, 22, 25, 34, 66 |
| 157 | (17), 63, 20, 22, 25, 34, 66 |
| 158 | 63, 20, 22, 25, 66 |
| 159 | 63, 22, 25 |
| 160 | 63, 22, 29 |
| 161 | 89, 29 |
| 162 | 29 |
| 163 | 29 |
| 164 | 29 |
| 165 | 23 |
| 166 | 23 |
| 167 | 23 |
| 168 | 23 |
| 169 | 22 |
| 170 | 23, 29 |
| 171 | 23, 29 |
| 172 | 23, 25, 34, 66 |
| 173 | 23 |
| 174 | 22, 23 |
| 175 | 63, 20, 22, 25, 34, 66 |
| 176 | 22 |
| 177 | (22) |
| 178 | 87, 88, 86, 89, 80, 29, 97, 35, 63, 53 |
| 179 | 63, 53, 22, 54, 97 |
| 180 | 63 |
| 181 | 63, 24, 25 |
| 182 | 63, 20, 53, 22, 82, 25, 66, 54, 29 |
| 183 | 63, 53, 22 |
| 184 | 63, 53, 54 |
| 185 | 20, 22 |
| 186 | 29 |
| 187 | 29 |
| 188 | 87 |
| 189 | 97 |
| 190 | 29 |
| 191 | 29 |
| 192 | 34, 29 |
| 193 | 29 |
| 194 | 29 |
| 195 | 29 |
| 196 | 29 |
| 197 | 22 |
| 198 | 20 |

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 199 | 29 |
| 200 | 20, 22 |
| 201 | 29 |
| 202 | 29 |
| 203 | 29 |
| 204 | 29 |
| 205 | 35, 86, 89, 53, 22 |
| 206 | 89, 22 |
| 207 | 23 |
| 208 | 29 |
| 209 | 29 |
| 210 | 29 |
| 211 | 34, 29 |
| 212 | 34, (66), 29 |
| 213 | 34, (66), 29 |
| 214 | 34, 29 |
| 215 | 34, (18) |
| 216 | (18) |
| 217 | 18 |
| 218 | (34), 29 |
| 219 | 34 |
| 220 | 29 |
| 221 | 29 |
| 222 | 29 |
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| 224 | 29 |
| 225 | 29 |
| 226 | (18) |
| 227 | 18 |
| 228 | 18, 66 |
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| 230 | 18 |
| 231 | 18, 66 |
| 232 | 18, 66 |
| 233 | 18 |
| 234 | 69 |
| 235 | 97 |
| 236 | 29, 97 |
| 237 | 34 |
| 238 | 29 |
| 239 | 34, 29 |
| 240 | 29 |
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| 249 | 29 |
| 250 | 29 |
| 251 | 29 |
| 252 | 29 |
| 253 | 29 |
| 254 | 89 |
| 255 | 29 |
| 256 | 29 |
| 257 | 29 |
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| 259 | 69 |
| 260 | 29 |
| 261 | 29 |
| 262 | 34 |
| 263 | 87, 88, 86, 89, 97 |
| 264 | 87, 88, 44, 89, 97 |
| 265 | 88, 86, 44, 89, 34, 97 |
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| 268 | 44 |
| 269 | 44 |
| 270 | 87, 88, 86, 44, 89 |

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 271 | 86, 87, 88, 44 |
| 272 | 88, 44, 97 |
| 273 | 44 |
| 274 | 44 |
| 275 | 22 |
| 276 | 23 |
| 277 | 29 |
| 278 | 20, 22, 25, 66 |
| 279 | 46, 29 |
| 280 | 46, 29 |
| 281 | 46, 29 |
| 282 | 60 |
| 283 | 20, 60 |
| 284 | 34 |
| 285 | 20 |
| 286 | 81, 25, 29 |
| 287 | 21, 25, 34, 29 |
| 288 | 21, 25, 29 |
| 289 | 32, 21, 81, 25, 34, 69, 29 |
| 290 | 21, 25, 34, 69, 29 |
| 291 | 21, 25 |
| 292 | (21) |
| 293 | 21, 25, 69, 29 |
| 294 | 21, 25, 34, 69, 29 |
| 295 | 21, 25, 69, 29 |
| 296 | 29 |
| 297 | 34 |
| 298 | 21 |
| 299 | 29 |
| 300 | 29 |
| 301 | 29 |
| 302 | 29 |
| 303 | 21, 25, 69, 29 |
| 304 | (25) |
| 305 | (21) |
| 306 | (21) |
| 307 | (20), 66, (60), (24) |
| 308 | 24, (60) |
| 309 | 21, 34, 29 |
| 310 | 29 |
| 311 | 29 |
| 312 | (21) |
| 313 | (21) |
| 314 | 29 |
| 315 | 21, 25, 29 |
| 316 | 21, 25, 34, 69, 29 |
| 317 | 21, 25, 69, 29 |
| 318 | 25 |
| 319 | 25 |
| 320 | 69, 29 |
| 321 | 21, 25, 69 |
| 322 | (21) |
| 323 | 69, 29 |
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| 325 | 29 |
| 326 | 29 |
| 327 | 29 |
| 328 | 20, 21, 25, 34 |
| 329 | 21, 29 |
| 330 | 29 |
| 331 | 89 |
| 332 | 60 |
| 333 | 60 |
| 334 | (66) |
| 335 | 46, 29 |
| 336 | 22, (60) |
| 337 | 20, 22, 82, 25, (66), 46, 29 |
| 338 | 22, 25, 46, 29, 34 |
| 339 | 46, 29 |

| COMPOUND NUMBER | REFERENCE NUMBER |
|---|---|
| 340 | 22, (60) |
| 341 | 46, 29 |
| 342 | 29 |
| 343 | 20, 81, 22, 34, 83 (66), 69, 46, 29 |
| 344 | 69, 46, 29, 81, 22 |
| 345 | 46, 29 |
| 346 | 22, 46 |
| 347 | 22 |
| 348 | 46, 29 |
| 349 | 46, 29 |
| 350 | 29 |
| 351 | 29 |
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| 353 | 22, 46, 29 |
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| 373 | 29, 47 |
| 374 | 29, 47 |

# CHAPTER 2

# POTATO VOLATILE ANALYSIS

## 2.1 INTRODUCTION

In Chapter 1 the identity and properties of potato volatiles were discussed with little reference to the methods that were used for their isolation. In this chapter the analytical techniques employed in the isolation and identification of potato volatiles are discussed. It should be emphasised that this chapter deals solely with the techniques that have been used for the analysis of potato volatiles. The range of techniques for volatile analysis outwith the field of potato volatile research are discussed in Chapter 3.

From the earliest attempts at the isolation of potato volatiles to the present day, the analytical techniques have become considerably more sophistocated. This is reflected in the large number of potato volatiles that are isolated and identified at the present time. In a recently published paper that dealt with baked potato volatiles, 228 compounds were identified (Coleman, E.C., et al., 1981). The main factor contributing to the increased range of potato volatiles currently being reported is the vast improvement in the separation and identification techniques used. In particular high resolution gas chromatography combined with computerised mass spectroscopy has enabled the detection and identification of many trace potato volatiles.

Throughout the work on potato volatiles there are three distinct stages to the analytical procedure, which are:- Collection, Separation and Identification. The methods used for the analysis of potato volatiles will be discussed in terms of these three stages.

## 2.2 POTATO VOLATILE COLLECTION

The most extensively used method for the isolation of potato volatiles is by steam distillation, which has found particular favour amongst the workers researching potato flavour volatiles. Some workers used a simple steam distillation of the potatoes: the steam distillate was then extracted with a suitable organic solvent, which was then concentrated and analysed (Buttery, R.G., 1961), (Dornseifer, T.P., Powers, J.J., 1963), (Sapers, G.M., et al., 1971), (Pareles, S.R., Chang., S.S., 1974).

A modification of the steam distillation method developed for the analysis of hop oils (Likens, S.T., Nickerson, G.B., 1964) has also been used to extract potato volatiles. This method enabled the simultaneous steam distillation/solvent extraction of potatoes. Essentially the technique involves the distillation of the potatoes and extraction solvent in two separate vessels which are linked to a cold finger condenser. Both the steam from the potatoes and the solvent vapour condense on the cold finger where the volatiles are extracted from the steam distillate by the solvent. Both steam and solvent condensates are then returned to their respective vessels to be redistilled, thereby producing a continuous steam distillation/solvent extraction process. This method has been used to isolate the volatiles from boiled potatoes (Buttery, R.G., et al., 1970), (Buttery, R.G., Ling L.C., 1973), (Nursten, H.E., Sheen, M.R., 1974), potato crisps (Buttery, R.G., et al., 1971), (Buttery, R.G., Ling, L.C., 1972), baked potatoes (Buttery, R.G., et al., 1973), and potato granules (Nursten, H.E., Sheen, M.R., 1974). The main factor contributing to the widespread use

of this technique is that the volatiles from a large amount of potatoes, (1 kg - 10 kg), can be extracted by and concentrated in a relatively small volume of solvent (10 cc - 100 cc), thereby providing a suitably concentrated extract for analysis. Steam distillation combined with functional group precipitation in lead acetate, mercuric chloride and mercuric cyanide traps has been used to collect and fractionate the sulphur containing volatiles produced by boiled potatoes. The potatoes were steam distilled and the distillate was swept with Nitrogen through the traps. The volatile sulphur compounds adsorbed in the traps were then regenerated for analysis by the addition of hydrochloric acid (Gumbmann, M.R., Burr, H.K., 1964).

Cryogenic techniques have been employed for the collection of potato volatiles in particular for the isolation of the volatiles produced by raw tubers (Burton, W.G., Meigh, D.F., 1971), (Creech, D.L., et al., 1973), (Meigh, D.F., et al., 1973), (Kahn, I., 1971), (Varns, J.L., Glynn, M.T., 1979). Cold trapping has also been used to isolate the volatiles produced by boiled potatoes (Self, R., et al., 1963), potato crisps (Deck, R.E., Chang, S.S., 1965), (Deck, R.E., et al., 1973), baked potatoes (Coleman, E.C., et al., 1961), and potato granules (Buttery, R.G., 1961). Most of these cold trapping techniques involved, sweeping with a gas stream or drawing under reduced pressure, the headspace surrounding the potatoes into a cold trap where the volatiles were concentrated. The volatiles were then regenerated from the cold trap by means of solvents (Coleman, E.C., et al., 1981), heat (Self, R., et al., 1963), or adsorption onto a porous polymer adsorbent (Varns, G.L., Glynn, M.T., 1974).

Direct headspace sampling using a gas tight syringe has been extensively used for the study of volatiles produced by potato granules (Buttery, R.G., 1961), Buttery, R.G., Teranishi, R., 1963), (Boggs, M.M., et al., 1964), (Sapers, G.M., 1970), (Sapers, G.M., et al., 1972), (Sullivan, J.F., et al., 1974). This method has found particular favour for the analysis of potato granule volatiles as it is quick and simple, and provided that the volatiles of interest are present in sufficient quantities, a headspace sample of a few cc above the granules provides sufficient volatiles for analysis. However, direct headspace sampling has not been used extensively for the collection of volatiles that occur at low concentrations from other potato products as it is not a concentrative technique.

## 2.3 POTATO VOLATILE SEPARATION

In contrast to the considerable variety of techniques involved in the isolation of potato volatiles, gas chromatography is the dominant technique used to separate the potato volatiles into their individual chemical constituents. The main factors contributing to its widespread use are as follows:-

1) Gas chromatography enables the efficient separation of a wide range of organic compounds of different polarity and volatility.
2) Flame ionisation detection enables the detection of individual volatiles at the levels of a few nanogrames.

3) When coupled to a mass spectrometer not only can a separation of the volatiles be achieved but also the complete identification of the individual volatiles as well.

4) By their volatile nature, potato volatiles are suited to this form of separation technique.

The column used for the separation of the potato volatiles into their individual components forms the core of the gas chromatographic system. Throughout the work, great emphasis has been placed by many workers on the resolution of the volatiles in distinct separate compounds to facilitate their identification and quantification. For this reason, capillary columns have been widely used, as in comparison to packed gas chromatographic columns, capillary columns provide superior resolution and sensitivity.

Initially, nylon capillary columns of lengths 15 m - 300 m were used to resolve the potato volatiles (Buttery, R.G., Teranishi, R., 1963), (Self, R., et al., 1963), (Gumbmann, M.R., Burr, H.K., 1964). However, they proved to be unpopular as the nylon irreversably adsorbed alcohols (Self, R., et al., 1963) and contributed to a high background bleed due to volatiles arising from the nylon itself when the column was heated during operation (Buttery, R.G., Teranishi, R., 1963). Stainless steel (Buttery, R.G., et al., 1970), and glass capillary columns (Self, R., 1968) of lengths 10 m - 30 m both wall and support-coated, replaced the nylon capillary columns in later work. Both types of columns exhibited less activity than the nylon capillary

columns, and are still the main form of column used at the present time.

However, there are exceptions to the widespread use of capillary columns, especially for the analysis of the off-flavour of potato granules where there are only a few compounds of interest. These off-flavour volatiles can be satisfactorily resolved on a 1 m - 3 m stainless steel packed column (Buttery, R.G., 1961), (Buttery, R.G., Teranishi, R., 1963), (Sapers, G.M., 1970), (Sapers, G.M., et al., 1971), (Sapers, G.M. et al., 1972).

A novel approach to the separation of a complex mixture of volatiles has recently been employed (Coleman, E.C., et al., 1981) for the analysis of baked potato volatiles. Instead of performing a separation of all the volatiles in one run on a high resolution capillary column, the baked potato volatiles were fractioned and successively subfractioned several times on a series of conventional 4 metre stainless steel columns: each fraction was collected at the effluent end of the column and rechromatographed until the individual volatiles were isolated.

This technique using conventional columns has the advantage over the use of capillary columns in that large amounts (1 mg - 2 mg) of volatiles can be isolated, providing sufficient amounts of an individual volatile for infra-red spectroscopic analysis. However, a complex and sophisticated fraction collector was required to collect the volatiles eluted from the chromatographic column (Deck, R.E., et al., 1965).

## 2.4 POTATO VOLATILE IDENTIFICATION

Just as gas chromatography (G.C.) is the dominant technique used for the separation of potato volatiles, mass spectroscopy (M.S.) is the dominant technique used for the identification of potato volatiles. A mass spectral cracking pattern from an unknown volatile when compared and matched to that of an authentic compound is generally accepted as a positive identification of the unknown, especially when confirmed by other data such as the comparison of retention time (RT) or retention index (RI) of the unknown volatile with an authentic compound on two gas chromatographic columns of different polarity.

Both G.C. and M.S. are closely linked techniques and usually both machines are interfaced so that the separated volatiles when leaving the G.C. column are led directly into the M.S. The combined G.C.M.S. unit is the most powerful analytical tool available to the potato volatile researcher, by virtue of the fact that a complex mixture of volatiles when introduced into a modern G.C.M.S. unit is resolved, characterised and identified at the end of the analytical run, even when the individual volatiles may only be present at the levels of a few ng. The subject of G.C.M.S. has been discussed in relation to potato volatiles by Self (Self, R., 1968).

Other means of identifying potato volatiles such as RT on a G.C. column is usually sufficient for simple mixtures of volatiles such as those encountered in the off-flavour of potato granules (Buttery, R.G., Teranishi, R., 1963), (Boggs, M.M., et al., 1964), (Sapers, G.M., et al., 1973).

However, RT is used in general to confirm mass spectroscopic identification of unknown volatiles (Buttery, R.G., et al., 1971), (Buttery, R.G., Ling, L.C., 1972), (Nursten, H.E., Sheen, M.R., 1974). Infra red spectroscopy (IR) is also used, as with RT, to confirm an identification by mass spectroscopy and is generally not used as the sole means of identification (Coleman, E.C., et al., 1981). The use of IR spectroscopy has the limitation, in that relatively large amounts of volatiles are required from the potato volatile mixture for analysis: the problem of both fractioning and obtaining sufficient amounts of the individual volatile dictates that this technique is not applicable to potato volatiles that occur in trace amounts.

## 2.5 CONCLUSIONS

Each analytical technique used for the isolation and identification of potato volatiles is suited to the individual demands of the particular potato product and the form of the potato volatiles. In the case of potato granule off-flavour volatiles a simple headspace sample can be sufficiently resolved on a conventional packed G.C. column, and the volatiles identified on an RT basis; whereas the volatiles from raw potato tubers require a concentration stage followed by separation on a capillary column and identification using mass spectroscopy. It was concluded that there is no standard method for the analysis of potato volatiles.

# INTRODUCTION TO THE ANALYTICAL CHAPTERS

The bulk of the work contained in this section of the thesis is concerned with the development of suitable analytical techniques for the collection and separation of potato headspace volatiles. Both aspects of the work involved the design, construction and assessment of equipment for this purpose. In the light of an assessment, improvements were then made in the equipment, therefore the progress of the work was in stages of refinement to the ultimate goal; the isolation of potato headspace volatiles, followed by their separation and identification.

However, advances in the design of the equipment used for the collection and separation of potato volatiles proceeded independently of one another. At certain stages of the work, the collection system was in advance of the separation system, in terms of sophistocation and efficiency and at other stages, the situation was reversed.

Therefore, it was decided that the best way of presenting the development of the analytical techniques used for the collection, separation and identification of potato volatiles would be to describe the collection and separation systems separately. A review of the available techniques and the development of the collection systems are described in Chapters 3, 4, 5 and 6; the development of the separation systems are described in Chapter 7. Reference will be made to the relevant separation system that was used with a particular collection system under discussion, thereby giving full details of the complete analysis at any particular stage of the work.

# CHAPTER 3

# VOLATILE ANALYSIS TECHNIQUES

## 3.1 INTRODUCTION

At the start of this project there were a number of methods that were considered for the collection of potato volatiles, these were in addition to the methods that had previously been used by workers researching potato volatiles, as described in Chapter 2. Collection systems for trace organic volatiles that have been employed in medical, food environmental research were drawn upon. The primary consideration was that the collection system for the potato volatiles was compatible with the aims of the project. These aims were to collect, separate and identify the volatiles present in the headspace surrounding raw potato tubers with reference to the question of potato tuber dormancy. With these aims in mind the collection systems were considered or rejected depending on their compatability with these aims and their practical feasibility.

## 3.2 STEAM DISTILLATION

Although a variety of steam distillation methods have been employed for the analysis of potato volatiles, this technique was not considered suitable for this project for two reasons.

A steam distillate of raw tubers was not expected to reflect the volatiles present in the headspace surrounding raw potato tubers.

The process of boiling the raw tubers to obtain a steam distillate meant that the volatiles obtained were attributable to boiled potatoes rather than raw tubers. Therefore, this technique was rejected.

## 3.3 GAS TIGHT SYRINGE

Although this method fulfilled the requirements of sampling, only the volatiles present in the headspace surrounding raw tubers so that the tubers themselves were unaffected by the sampling process, it was rejected on the grounds of practical feasibility. As it was not a concentrative technique, a sample of potato headspace volatiles taken using a gas tight syringe would be too dilute for the volatiles to be detected by a F.I.D. G.C. detector.

## 3.4 COLD TRAPPING

Cold trapping techniques were considered to be suitable for this project as they both fulfilled the requirements of the project in that only the headspace potato volatiles would be collected and the raw tubers would be unaffected by the collection method; it was also considered to be a practicable method as the potato headspace volatiles could be concentrated to detectable levels in the cold traps.

Cold trapping has been used for the collection of the volatiles produced by raw potato tubers (Burton, W.G., Meigh, D.F., 1971), (Creech, D.L., et al, 1973), (Meigh, D.F., et al, 1973), (Varns, J.L., Glynn, M.T., 1979) and was particularly suited, using liquid $N_2$ cooled traps, to the analysis of very volatile low molecular weight compounds such as ethylene (Creech, D.L., et al, 1973) which are of particular physiological interest. However, the major drawback of the cold trapping technique was the presence of large quantities of water in the headspace

surrounding actively respiring raw tubers. This water, along with the potato volatiles, would be frozen out in the cold traps and would make the subsequent analysis of the trapped volatiles by G.C. difficult.

## 3.5 POROUS POLYMER ADSORBENTS

Of the methods discussed so far these adsorbents are the most recently developed means of concentrating trace organic volatiles. They have had some limited use in the field of potato volatile research. The adsorbent Tenax G.C. (Akzo Research Labs) has been used to trap the volatiles produced by potatoes under disease stress (Varns, J.L., Glynn, M.T., 1979) and to study the headspace concentrations of commercially applied potato sprout suppressants in large scale potato stores (Filmer, A.A.E., Land, D.G., 1978). Apart from these two cases, porous polymer adsorbents have not made a great impact on potato volatile research. However, they have been widely used in environmental, food and medical research for the concentration of trace organic compounds from dilute media, (Bertuccioli, M., Montedoro, G., 1974), (Micketts, R.J., Lindsay, R.C., 1974), (Cole, R.A., 1980), (Williams, A.A., et al, 1978), (Simpson, R.F., 1980), (Ismail, H.H., et al, 1980), (Pellizzari, E.D., et al, 1975), (Pellizzari, E.D., et al, 1976), (Zlatkis, A., et al, 1973).

In use, these adsorbents provide a convenient and simple method of sampling headspace volatiles. Usually the adsorbent is contained in a glass tube, referred to as a precolumn, through which the headspace volatiles are

flushed. The volatiles are then trapped and concentrated on the adsorbent while the water is rejected as these adsorbents have little or no affinity for water. This provided a very attractive means of collecting raw potato volatiles. The use of porous polymer adsorbents would be compatible with the aims of this project in that only the headspace volatiles from raw potato tubers would be trapped, and the tubers themselves would be unaffected by the sampling procedure. The characteristics and use of porous polymer adsorbents have been well defined in the literature.

There are several commercially available porous polymer adsorbents, the most widely used being the Porapak series (Waters Associates), the Chromosorb Century series (Johns Manville Products Corp.), and Tenax G.C. (Akzo Research Labs). These adsorbents have been the subject of intensive study as to their suitability for concentrating trace organic volatiles from dilute media (Bunch, J.E., Pellizzari, E.D., 1979), (Daemen, J.M.H., et al, 1975), (Tanaka, T., 1978), (Butler, L.D., Burke, M.F., 1976), (Pellizzari, E.D., et al, 1975), (Gearhart, G.L., Burke, M.F., 1976), (Kuo, P.P.K., et al, 1977), (Brown, R.H., Purnell, C.J., 1979), (Sakodynskii, K., et al, 1974), (Supina, W.R., Rooe, L.P., 1969).

The conclusions of these studies were that no single adsorbent is best for every sampling application: the adsorbent must be chosen to suit the particular sampling problem (Butler, L.D., Burke, M.F., 1976). Of the

adsorbents investigated, Tenax G.C. was considered to be the best available adsorbent for general use because of its wide range of applicability (Pellizzari, E.D., et al, 1975), its thermal stability and low retentive index for water (Sakodynskii, R., et al, 1974). It has also been found to compare favourably with other adsorbents in terms of collection efficiency and breakthrough volume for the collection of a range of organic vapours from ambient air (Pellizzari, E.D., et al, 1976).

As the headspace potato volatiles that would be encountered in this project ranged from gasses (e.g. ethylene) to high molecular weight compounds (e.g. dimethylnaphthalene) it was decided that Tenax G.C. would be the most suitable single adsorbent to meet the demands of collecting and concentrating potato headspace volatiles.

Tenax G.C. is a porous polymer based on 2,6-diphenyl-p-phenylene oxide, its properties and characteristics are as follows:

1. Tenax G.C. after conditioning has a high thermal stability (Pellizzari, E.D., et al, 1975), (Russel, J.W., 1975), (Holzer, G., et al, 1977), it may be heated up to 360°C with little background bleeding (Zlatkis, A., et al, 1973), (Daemen, J.M.H., et al, 1975). This point is important to purifying/conditioning the Tenax G.C. adsorbent precolumns before use and for the subsequent desorption of the adsorbed volatiles from the precolumn for separation and identification.

2. Breakthrough volumes or collection efficiencies of Tenax G.C. when sampling headspace volatiles are unaffected by a range of humidity conditions or by compounds of secondary interest (Pellizzari, E.D., et al, 1976), (Janak, J., et al, 1974).
3. Tenax G.C. has no affinity for water (Russel, J.W., 1975), this is a dinstinct advantage as water would complicate the subsequent analysis of the volatiles by G.C. (Pellizzari, E.D., et al, 1976).
4. Tenax G.C. after being recycled up to fifteen times showed no significant decrease in collection efficiency (Pellizzari, E.D., et al, 1976).
5. Sampled precolumns packed with Tenax G.C. could be stored for up to 3 wk with no significant loss of adsorbed compounds (Pellizzari, E.D., et al, 1976).
6. Tenax G.C. has small breakthrough volumes, relative to other adsorbents, for light hydrocarbons and low molecular weight polar compounds, therefore limiting sample volumes if these compounds are to be trapped quantitatively (Black, M.S., et al, 1977).

Tenax G.C. is generally used to trap headspace volatiles packed in a precolumn. These precolumns are lengths of glass or stainless steel tubing 100 mm - 200 mm long, 4 - 6 mm in diameter in which 50 mg - 200 mg of Tenax G.C. is packed and held in place with glass wool plugs or scintered glass discs (Mickets, R.J., Lindsay, R.C., 1974), (Cole, R.A., 1980).

If the trapped volatiles are to be removed from the Tenax

G.C. by thermal desorption for analysis by G.C., the dimensions of the precolumn are such that it is easily connected to the gas chromatograph inlet port for the introduction of the volatiles onto the gas chromatographic column (Pellizzari, E.D., et al, 1975), (Tsugita, T., et al, 1979).

Thermal desorption was chosen as the best means of removing volatiles from Tenax G.C. for their subsequent analysis by G.C. This method provides greater sensitivity and is more convenient than solvent extraction (Zlatkis, A., et al, 1973), (Pellizzari, E.D., et al, 1975). Thermal desorption was also chosen as the best means of conditioning/purifying the Tenax G.C. before sampling.

## 3.6 CONCLUSIONS

On consideration of the more widely used methods of collecting potato volatiles such as steam distillation and direct headspace sampling, they were rejected in favour of the porous polymer adsorbent technique using Tenax G.C. The most significant factor in favour of the use of Tenax G.C. in this project was the fact that a direct measure of the headspace of raw potato tubers could be obtained, and that these volatiles could be concentrated to detectable levels in the absence of water. Although cold trapping was considered as a means of concentrating the headspace volatiles from raw potato tubers, it was not favoured because of the fact that the volatiles would be diluted by a considerable excess of water and $CO_2$ in the

traps, which would then involve further separation and concentration stages.

Having decided upon the Tenax G.C. precolumn for the collection of potato volatiles, the next considerations were the sample containment and G.C. analysis systems.

# CHAPTER 4

# TANK COLLECTION SYSTEM

## 4.1 INTRODUCTION

It has been estimated that raw potato tubers produce volatiles at the rate of 1 ng $kg^{-1}$ $hr^{-1}$ (Burton, W.G., Meigh, D.F., 1971). This rate of production is extremely low and therefore to obtain sufficient amounts of volatiles for analysis, long sampling times or large samples of potatoes would have to be employed. It was decided that it was preferable to sample the volatiles produced by a large sample of potatoes, 100 kg - 150 kg, as long sampling times would not be consistent with the use of Tenax G.C., which has a low breakthrough volume for low molecular weight compounds (Black, M.S. et al, 1977). Having decided upon Tenax G.C. precolumns for the concentration of potato volatiles, the next consideration was the development of a containment system to hold the potatoes during sampling.

The principle of the system was similar to systems that had previously been employed for the collection of headspace volatiles from large samples of fruit and vegetables (Ismail, H.H., et al, 1980), (Cole, R.A., 1980), (Heydanek, M.G., McGorrin, R.J., 1981). The potato tubers were contained in a large aluminium tank specially constructed for the purpose; the headspace volatiles were swept from the tubers onto the precolumns with an air stream.

## 4.2 EXPERIMENTAL

The initial experiments assessed the levels of headspace volatiles that the system itself produced, these background volatiles being trapped by operating the system under the conditions that were normally employed for the collection of potato volatiles, with no potatoes in the tank.

### Materials

Precolumns - Ten Tenax G.C. adsorbent precolumns were assembled as follows.

1. Ten lengths of borosilicate glass tubing 110 mm x ¼ inch O.D. (Jencons (Scientific) Ltd, Leighton Buzzard, England) were placed in concentrated hydrochloric acid (Analar, Analar Standards Ltd, Poole, England) for 24 hr.
2. The tubing was thoroughly rinsed with deionised water followed by acetone (Analar), and then heated in an oven at 220°C for 2 hr.
3. Each length of tubing was packed to 100 mm of its length with 100 mg - 150 mg of Tenax G.C. (Phase Separations Ltd, Queensferry, Wales) which was held in place with silanised glass wool plugs.
4. The assembled precolumns were purified by heating under a $N_2$ gas flow (B.O.C. Glasgow Ltd) of 20 cc - 30 cc $min^{-1}$ at 320°C for 2 hr. At the end of this period the precolumns were cooled and sealed with PTFE caps.

This method of assembling Tenax G.C. adsorbent precolumns, was used throughout the project.

Step 4. was repeated as necessary for the purification of the precolumns before sampling, this is referred to as conditioning. The apparatus used for conditioning precolumns is explained in greater detail in Chapter 7.

An aluminium tank 250 $dm^3$ in volume was constructed for the purpose of containing the potato tubers. The specifications of the tank are as follows:

1. Dimensions; height 1000 mm, length 500 mm, width 500 mm.
2. The tank had a removable lid into which five ¼ inch couplings (Dralim, Phase Separations Ltd) were screwed and tapped. The precolumns were inserted through the couplings for the purpose of sampling the headspace volatiles from the tank.
3. The lid was sealed onto the body of the tank with a silicone rubber gasket (Esco (Rubber) Ltd, Teddington, England).
4. The air supply used to sweep the headspace volatiles from the tank onto the precolumns was introduced at the bottom of the tank with a 400 mm length of ¼ inch O.D. copper tubing (Alltech Associates, Carnforth, England) perforated along its length.
5. An aluminium base plate 490 mm x 490 mm, 10 mm deep was placed in the bottom of the tank to raise the potatoes above the gas inlet.

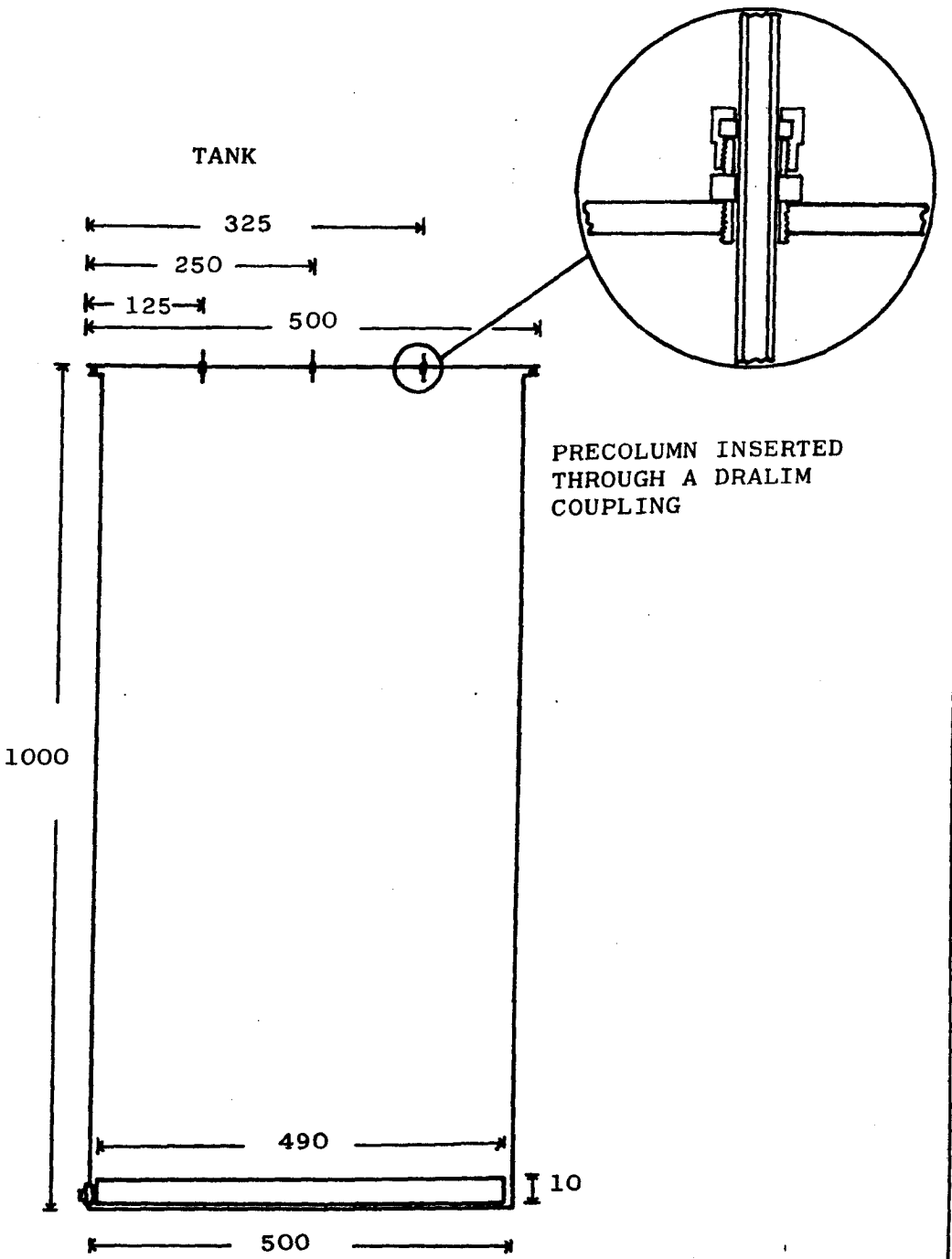
The aluminium tank is shown in Plate 1 and illustrated in Figure 4.1.

The air used to sweep the volatiles onto the precolumns was supplied from a pressurised air cylinder (B.O.C. Glasgow Ltd); which was fitted with a pressure regulator (C.S. Milne Ltd, Glasgow). The air flow to the tank was controlled with a needle valve flow controller (Model 8744, Brooks Instrument Division), Stockport, England). All the connections between the air supply and the tank were made with $^1/8$ inch O.D. PTFE tubing (Phase Separations Ltd).

FIGURE 4.1

DIAGRAM OF ALUMINIUM TANK

ALL DIMENSIONS IN MILLIMETRES



# PLATE 1

Gas Chromatography

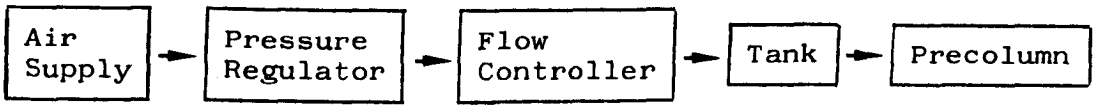
The sampled precolumns were analysed using System 1 described in Chapter 7.

| | |
|---|---|
| G.C.: | Pye 104 (Pye Unicam Ltd, Cambridge, England). |
| Column: | C.W.20M., 50m, 0.2 mm I.D., 0.32 mm O.D., 0.2um film thickness (SGE UK Ltd, Milton Keynes, England). |
| Carrier Gas: | $H_2$ u : 30 cm $sec^{-1}$. |
| Temperature:- | |
| Column, Initial | 50°C 10 mins |
| Rise | 5°C $min^{-1}$ |
| Hold | 190°C. |
| Detector Gases: | $H_2$ 20 cc $min^{-1}$ |
| | $O_2$ 200 cc $min^{-1}$ |
| | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | 5 x $10^{-10}$ A. |
| Chart Speed: | 5 mm $min^{-1}$. |

N.B.
All chromatograms in this thesis are direct reproductions of the origional trace and were run at an attenuation of X500

4.2.1 Tank System - Version 1

The first version of the system that was assessed is illustrated schematically below.




Before use the tank, seals, lid and connections were thoroughly cleaned with water and allowed to dry for 48 hr. The system was assembled and purged with air at 500 cc $min^{-1}$ (B.O.C. Glasgow Ltd) for 12 hr to flush any residual laboratory air from the tank.

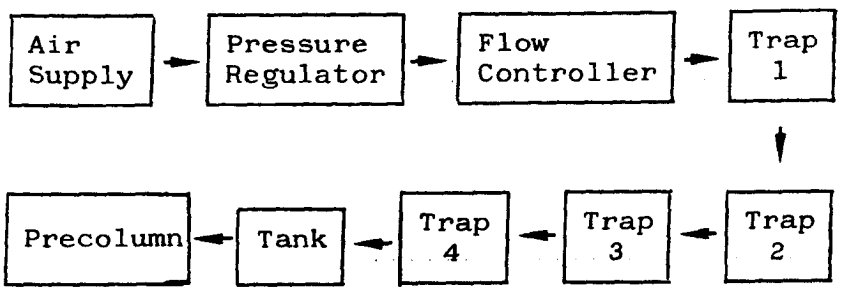
The air supply was then set at 50 cc $min^{-1}$ and the background volatiles from the tank were sampled over a 2 wk period. These volatiles were sampled at a flow rate of 50 cc $min^{-1}$, for sample volumes of 300 cc - 600 cc. It was found that the levels of background volatiles from the tank would have exceeded the levels of volatiles that were expected to be produced by 100 kg - 150 kg of potatoes. The background volatiles remained consistently high over the 2 wk sampling period. Several samples of the air supply were taken at the point just before the air entered the base of the tank. These air samples were taken at a flow rate of 50 cc $min^{-1}$ and for sample volumes of 300 cc - 600 cc. It was found that the volatiles present in the air supply matched the levels of background volatiles that were sampled from the tank itself.

It was concluded that the background volatiles contributed by the system were unacceptably high, as they would have exceeded the levels of potato volatiles that were expected

to be produced by the potatoes themselves. As the background volatiles were derived from volatiles present in the air supply, the system was modified to include a series of adsorbent traps to reduce these volatiles to an acceptable level. These modifications are described in Version 2 of this system.

4.2.2 Tank System - Version 2

The second version of the system that was assessed is illustrated schematically below. The traps were gas purifying tubes (Puritube, Phase Separations Ltd) and each trap was filled with 200g of activated carbon (Chrompack U.K. Ltd, London).



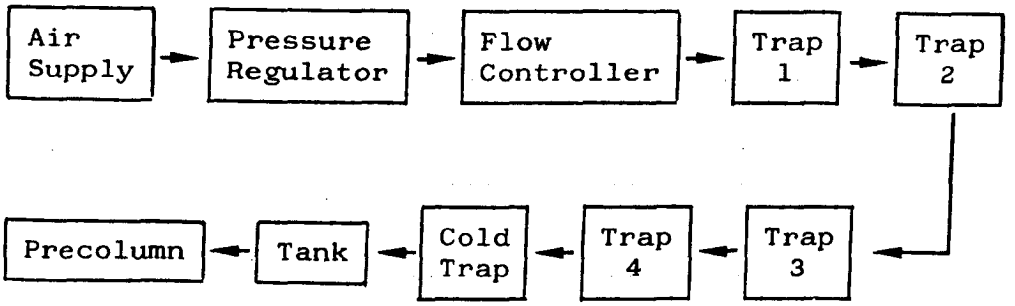

Before use the tank, seals, lid and connections were thoroughly cleaned with water and allowed to dry for 48 hr. The system was assembled and purged with air at 500 cc $min^{-1}$ for 12 hr. The air supply flow rate was then set at 50 cc $min^{-1}$ and the background volatiles from the tank were sampled over a 3 wk period. These volatiles were sampled at a flow rate of 50 cc $min^{-1}$, for sample volumes of 300 cc - 600 cc $min^{-1}$. The levels of background volatiles were decreased by the inclusion of four activated traps in the air supply.

In particular, the background volatiles that eluted after n-hexadecane, under the gas chromatographic conditions used, were substantially decreased. The background volatiles remained at a constant level throughout the 3 wk sampling period. Several samples of the air supply were taken before the air entered the tank.

These samples were taken at a flow rate of 50 cc $min^{-1}$ for sample volumes of 300 cc - 600 cc, and were compared with the samples of the background volatiles present in the tank. As with Version 1 of the tank system it was found that the background volatiles in the tank were primarily due to the volatiles present in the air supply. It was thought that the background volatiles were still too high to merit an analysis of potato volatiles without further reducing these volatiles to an acceptable level. Therefore the system was modified further to include a cryogenic trap in addition to the four activated carbon adsorbent traps.

### 4.2.3 Tank System - Version 3

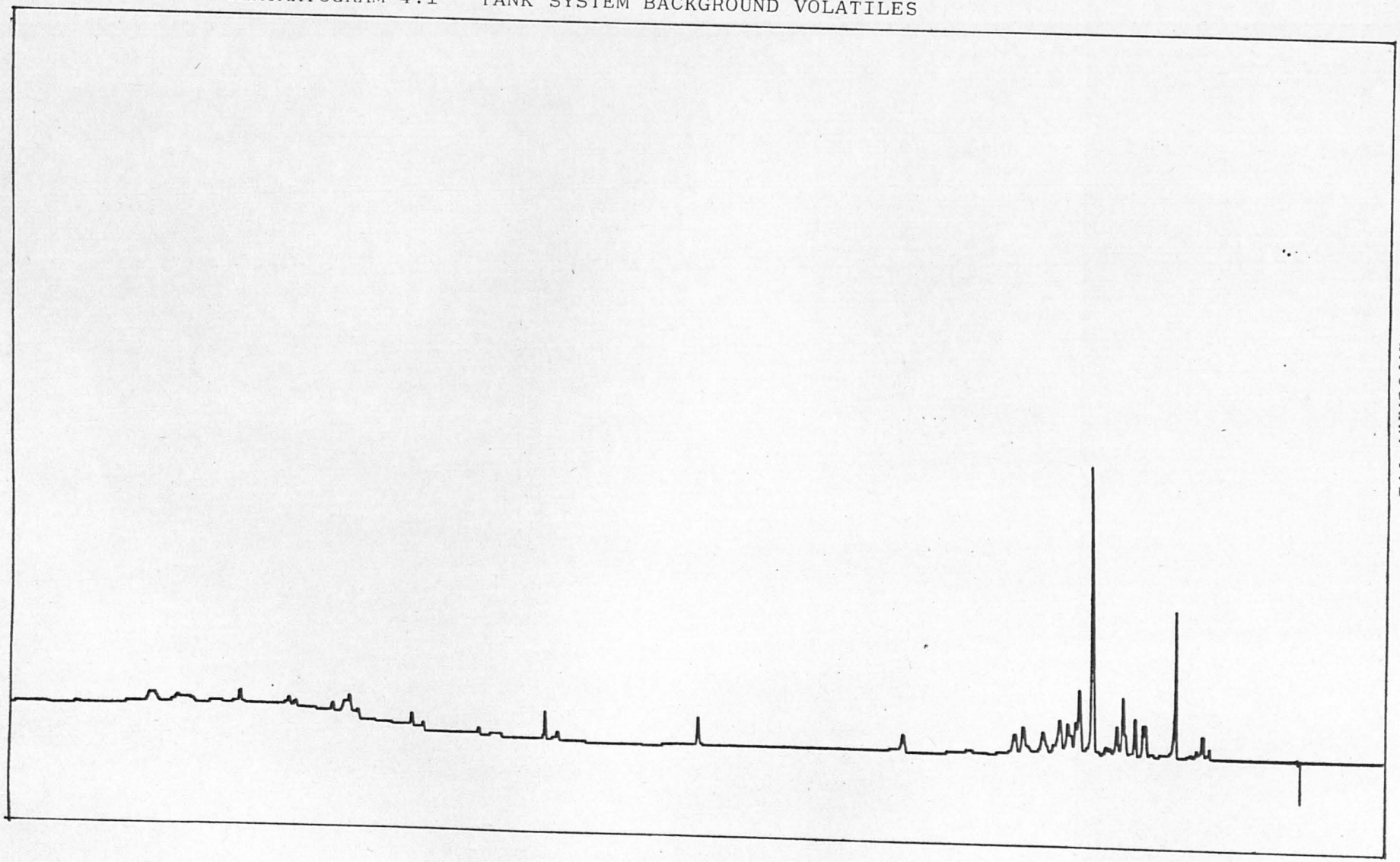
The third version of the system that was assessed is illustrated schematically below. Traps 1 - 4 were the same as used in the second version of the system, the traps were filled with fresh activated carbon. The cold trap was constructed from a 20m coil of $^{3}/_{8}$ inch O.D. copper tubing (Alltech Associates, Carnforth, England) which was placed in a dewar flask packed with dry ice.




Before use the tank, seals, lid and connections were thoroughly cleaned with water and allowed to dry for 48 hr; the cold trap copper tubing was rinsed with acetone (Analar) and heated at 220°C for 48 hr. The system was assembled, the cold trap placed in the dewar flask and packed with dry ice, and the system was purged with air at 500 cc $min^{-1}$ for 12 hr. The air supply flow rate was then set at 50 cc $min^{-1}$ and the background volatiles were sampled over a 3 wk period. The background volatiles were sampled at a flow rate of 50 cc $min^{-1}$, for sample volumes of 300 cc - 600 cc $min^{-1}$. Several samples of the air supply were taken before it entered the tank. These air samples were taken at a flow rate of 50 cc $min^{-1}$ for sample volumes of 300 cc - 600 cc. A representative chromatogram, Chromatogram 4.1, shows the levels of volatiles present in a 400 cc sample of the tank headspace, Chromatogram 4.2 is a representative chromatogram of a 400 cc sample of the volatiles present in the air supply.

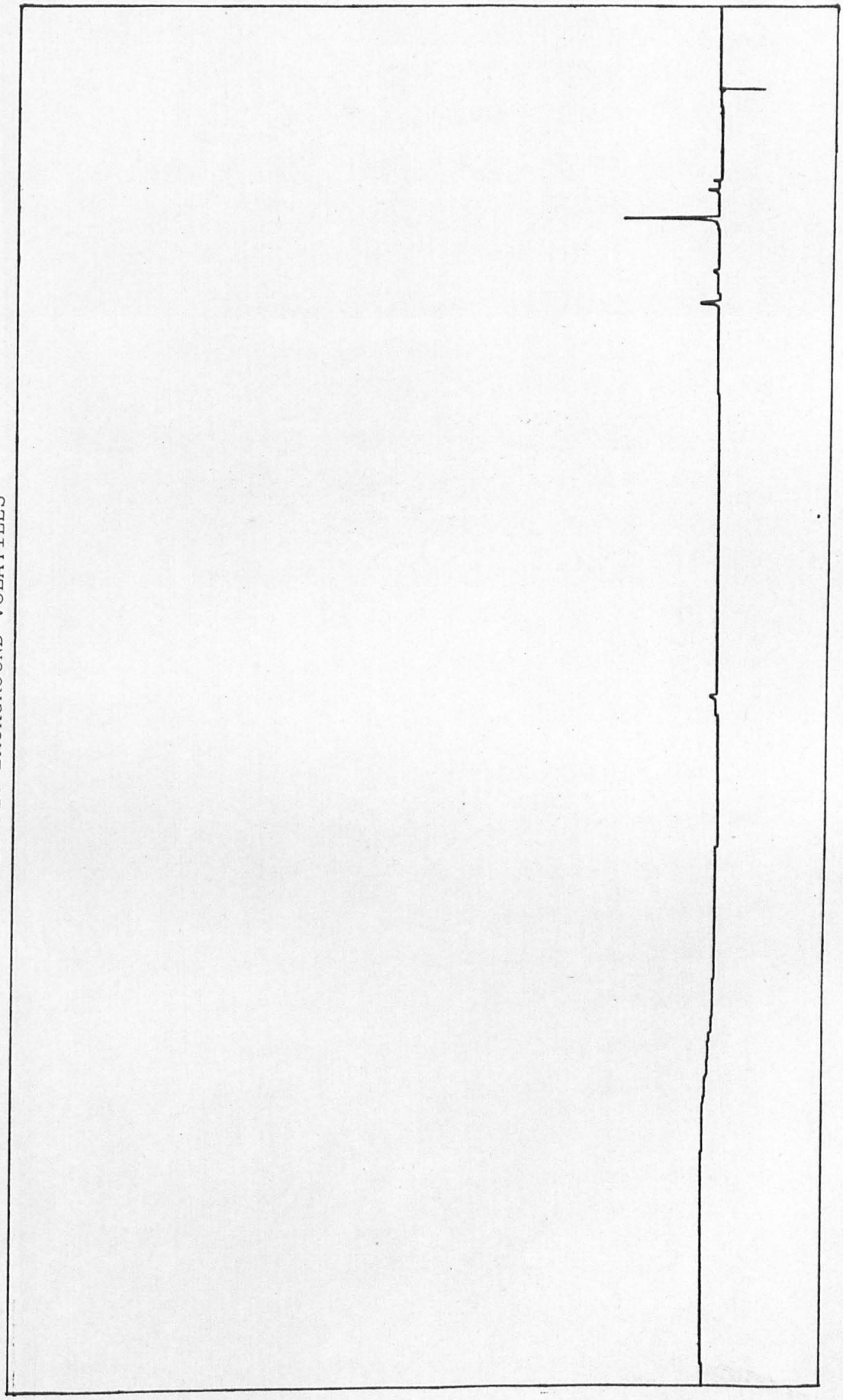
A comparison of both chromatograms showed that, at the sample volumes used, the air supply to the tank was free from volatile impurities, and the background volatiles present in the tank headspace were due to volatiles derived from the tank itself.

CHROMATOGRAM 4.1 TANK SYSTEM BACKGROUND VOLATILES



CHROMATOGRAM 4.1

CHROMATOGRAM 4.2



CHROMATOGRAM 4.2 AIR SUPPLY BACKGROUND VOLATILES

Although not fully satisfied with the levels of volatiles present in the tank headspace, it was decided that it would be worthwhile to attempt some preliminary experiments at collecting potato volatiles, using this version, Version 3, of the tank system.

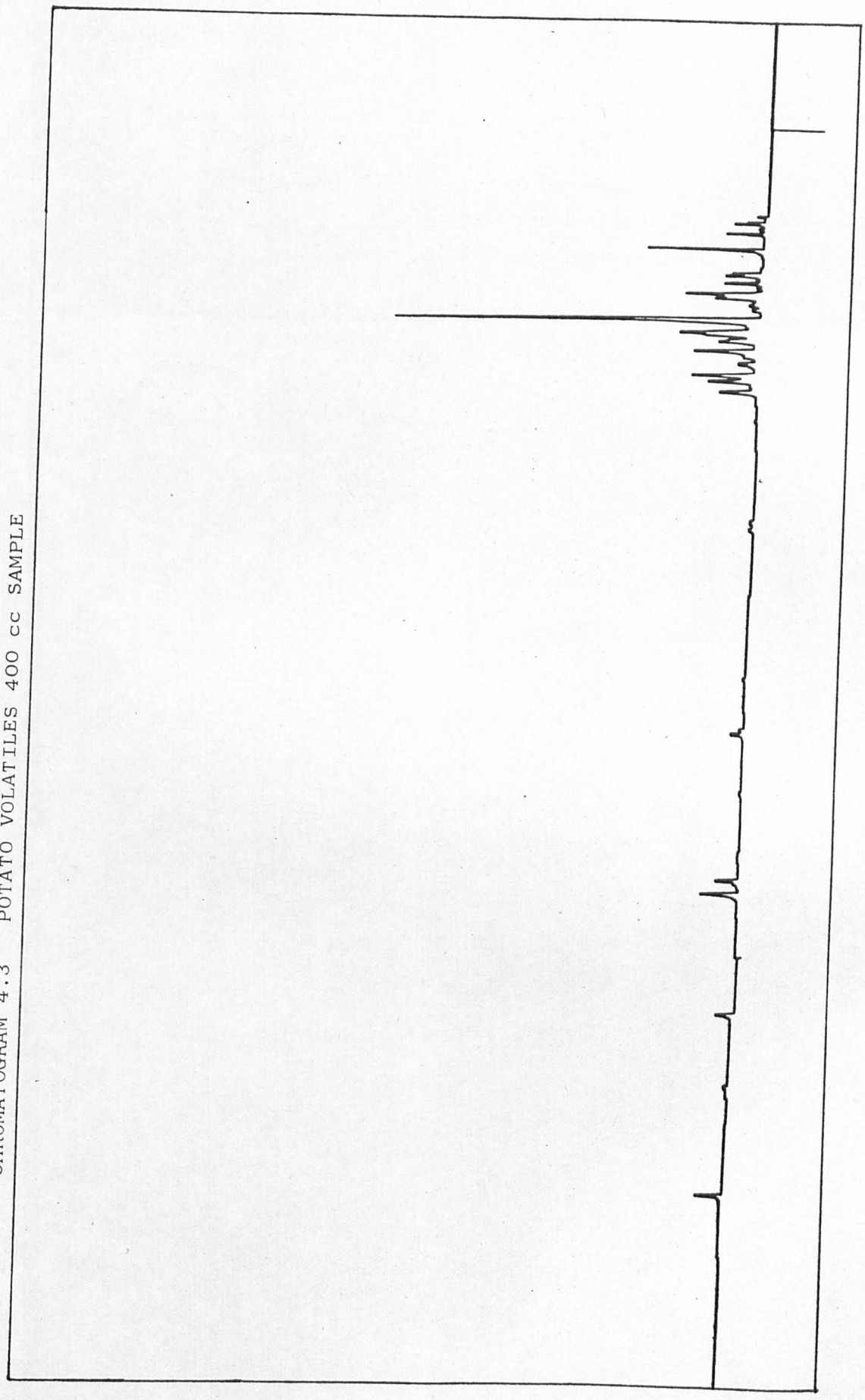
4.2.4 Potato Volatile Collection

Potatoes: 150 kg of dormant potato tubers, c.v.: Pentland Dell, were obtained from commercial sources. The potatoes were disease-free and showed no major signs of damage and had not been treated with any chemicals post-harvest. The tubers were washed to remove any adhering soil and then allowed to dry for 48 hr. The tubers were then placed in boxes and stored in the dark at 10°C for 3 wk before use.

The tank was filled with 120 kg of the dormant tubers, and the system was then assembled and purged as described for Version 3 of the system. The air supply flow rate was then set at 50 cc $min^{-1}$, and the headspace of the tank was sampled over a 2 wk period, for sample volumes of 300 cc - 400 cc. A representative sample of the tank headspace is shown in Chromatogram 4.3. It was found that the levels of volatiles present in the tank headspace with the tank filled with 120 kg of tubers were the same as the levels of volatiles present in the headspace of the tank when it was empty, Chromatogram 4.1. It was concluded that the rate of volatile production by the dormant potato tubers was very low, and that at the sample volumes used only background volatiles from the system itself were being

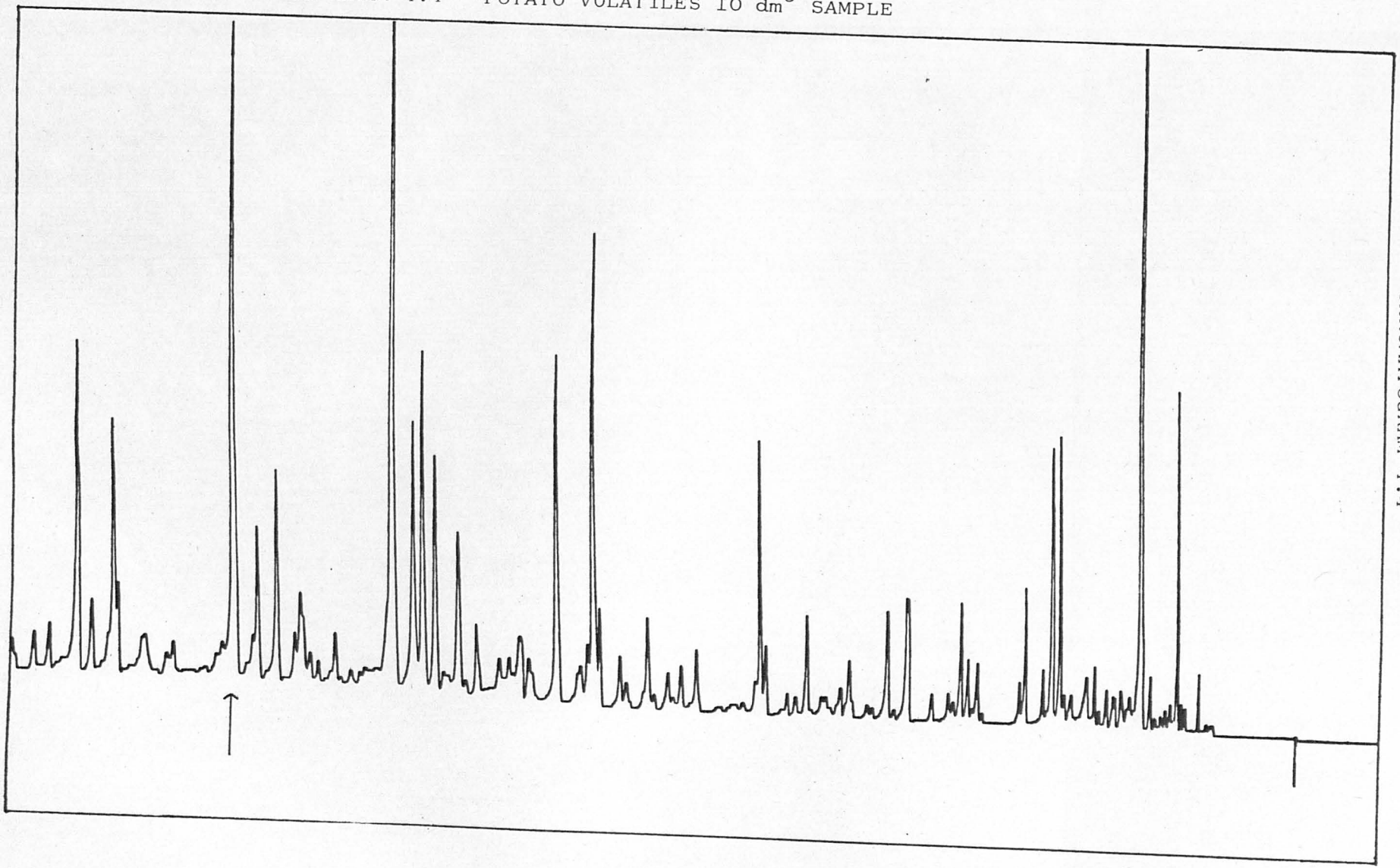
sampled. Therefore several samples of the tank headspace were taken at sample volumes of 10 $dm^3$, a representative sample is shown in Chromatogram 4.4, which was run at a temperature programme rate of 2°C $min^{-1}$, all other chromatographic conditions were the same as stated at the start of this chapter. The levels of volatiles present in the larger headspace samples were, as expected, greater than the previous 300 cc - 400 cc headspace samples. However it was not known whether the peaks in Chromatogram 4.4, were due to the background volatiles, potato volatiles or, as was thought most probable, due to a mixture of both.

After the sampling of the volatiles from the tank was completed, the tank was opened and the potatoes examined. During the 2 wk period that the tubers had been stored in the tank, their condition had deteriorated; there was evidence of soft rot infections in the tubers and condensation on the sides of the tank. The reason for the deterioration in the tuber health was the storage conditions and low ventilation rates used, 50 cc $min^{-1}$ of air, which was too low for the ventilation of 150 kg of respiring potato tubers. Under these storage conditions, the tubers were prone to the development of soft rot infections (Varns, J.L., Glynn, M.T., 1979). Similar problems of sample deterioration due to low ventilation rates have been encountered in the headspace analysis of soft fruit volatiles (Ismail, H.H., et al. 1980).



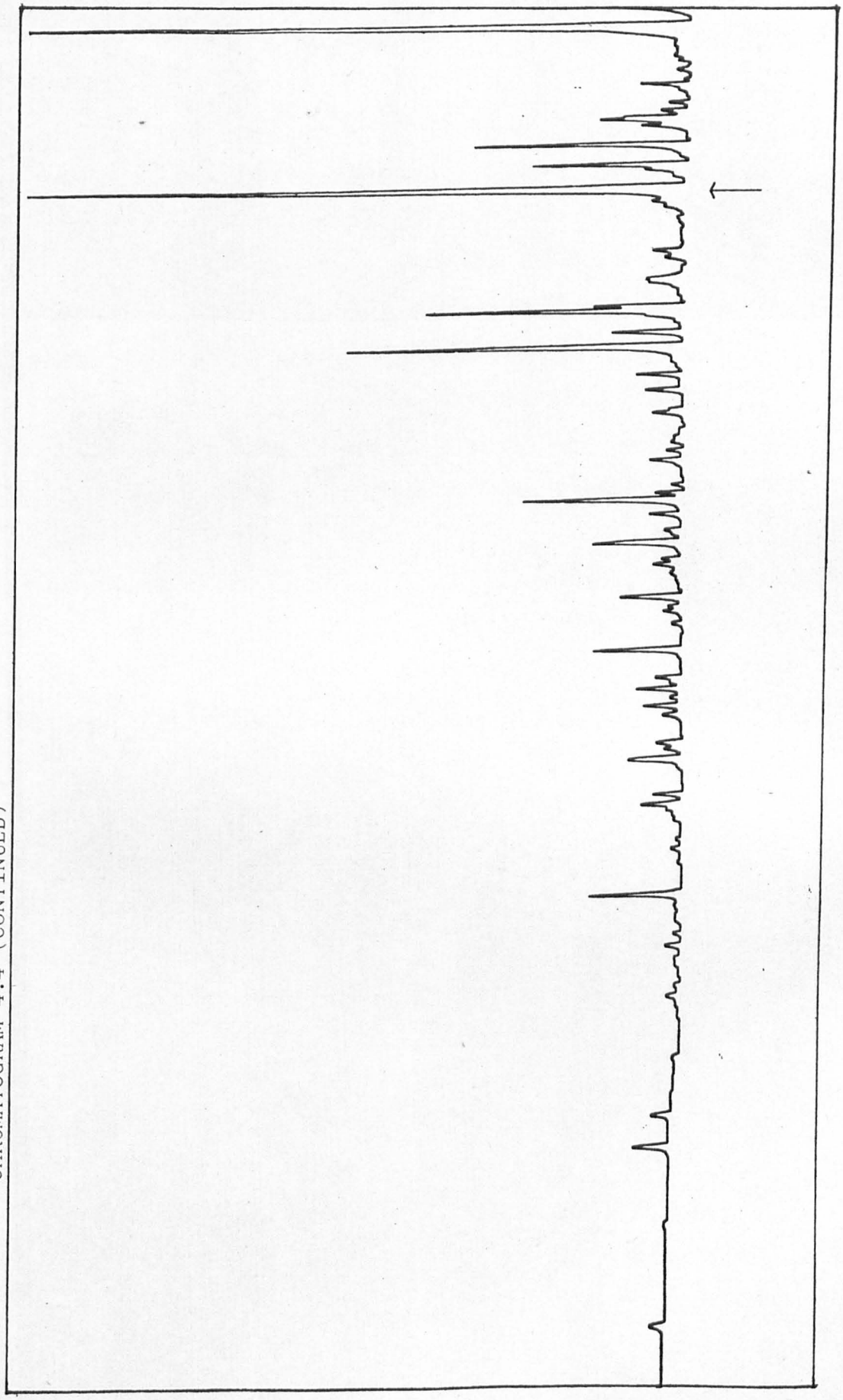
CHROMATOGRAM 4.3 POTATO VOLATILES 400 cc SAMPLE

CHROMATOGRAM 4.4 POTATO VOLATILES 10 $dm^3$ SAMPLE



CHROMATOGRAM 4.4

CHROMATOGRAM 4.4 (CONTINUED)



CHROMATOGRAM 4.4 (CONTINUED)

## 4.3 CONCLUSIONS

In view of the fact that the potato tubers were not sufficiently ventilated under the systems operating conditions, and that the headspace volatiles collected could not be attributed with any certainty to the volatiles produced by potatoes. The tank system was abandoned in favour of a smaller system where a smaller sample of potatoes could be ventilated at flow rates compatible with the use of Tenax G.C. precolumns, and where the materials used to construct the system contributed the minimum level of background volatiles.

# CHAPTER 5

# ALL GLASS COLLECTION SYSTEM

## 5.1 INTRODUCTION

In view of the problems of high levels of background volatiles and insufficient ventilation of the potato sample with the Tank system. The second system developed for the collection of potato volatiles, the All Glass system, was designed where certain design criteria were given priority. These criteria are as follows:

1. The system was constructed entirely from glass and PTFE, both highly inert materials that could be easily and efficiently cleaned, and that contributed the minimum level of background volatiles to the headspace in the system.
2. The air supply used to sweep the headspace volatiles onto the precolumns and to ventilate the potatoes was obtained by mixing Oxygen and Nitrogen in the appropriate proportions from two separate pressurised cylinders. It was found that the purity of Oxygen and Nitrogen obtained from two separate pressurised cylinders was far higher than for air obtained from a pressurised air cylinder (B.O.C., manufacturers specifications).
3. The Oxygen/Nitrogen mixture was cryogenicaly purified by three large volume cold traps, to remove background volatiles from the Oxygen/Nitrogen mixture before it reached the potato headspace volatiles.
4. The sample of potatoes was sufficiently large to provide enough potato headspace volatiles for analysis, and could also be ventilated efficiently at flow rates compatible with the use of Tenax G.C. precolumns.

It was thought that the criteria outlined above for the design of the All Glass system would eliminate the problems of background volatiles and insufficient ventilation of the potato sample, while still providing sufficient amounts of potato headspace volatiles for analysis.

## 5.2 EXPERIMENTAL

The initial experiments assessed the levels of headspace volatiles that the system itself produced, these background volatiles were trapped by operating the system under the conditions that were normally employed for the collection of potato volatiles, with no potatoes in the system.

### Materials:

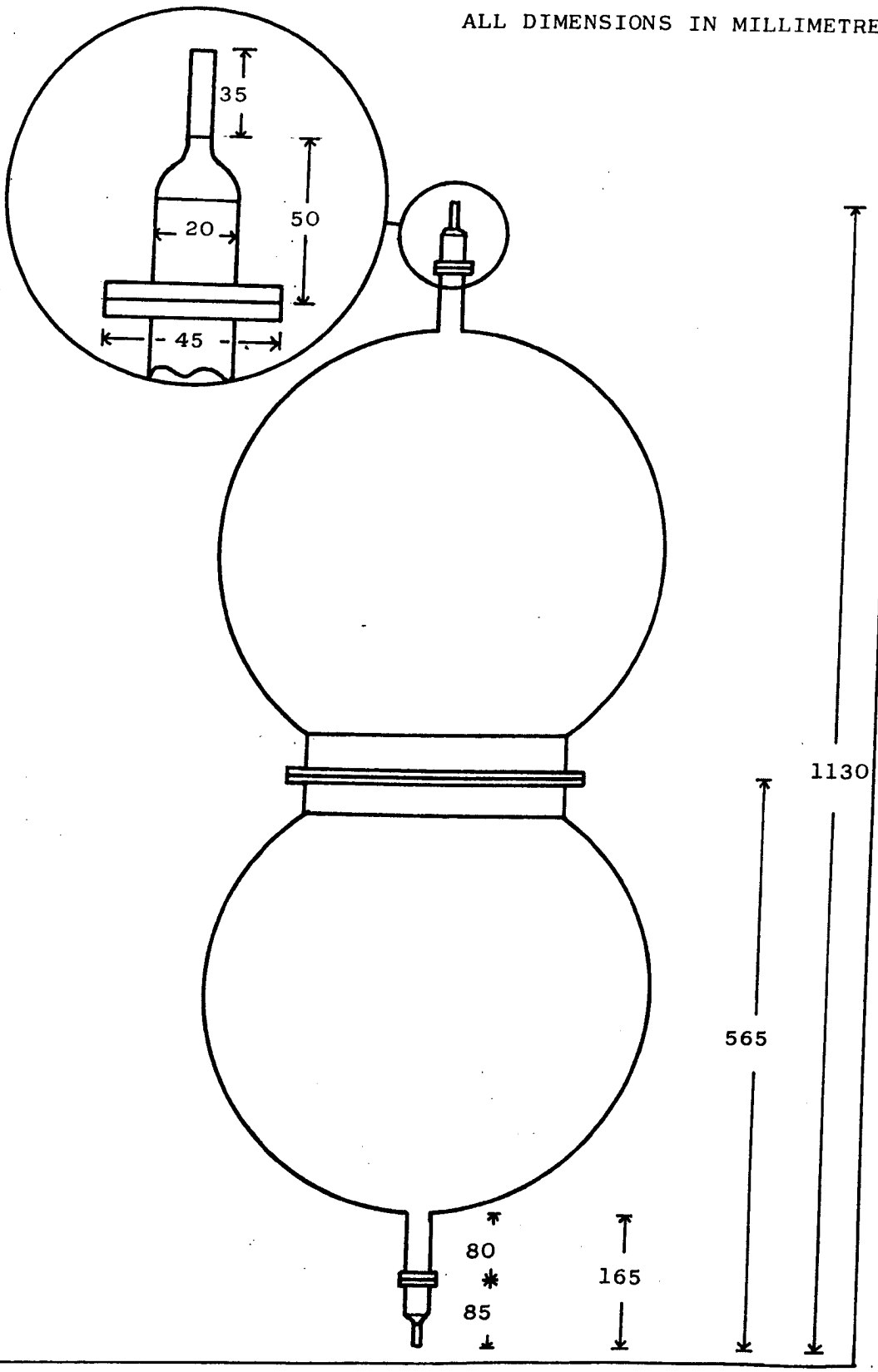
### Containment Vessel

The vessel used to contain the potatoes was constructed from two modified 10 $dm^3$ reaction vessels (Corning Ltd, Laboratory Division, Stone, England). To the base of each of the reaction vessels an 80 mm length of 20 mm O.D. glass tubing terminated with a ground glass 45 mm flange (Corning Ltd), was attached. Two 50 mm lengths of 20 mm O.D. glass tubing terminated at one end with a 45 mm ground glass flange and at the other end with 35 mm of ¼ inch O.D. glass tubing (Jencons (Scientific) Ltd, Leighton Buzzard, England) were constructed. These lengths of tubing were used to connect the reaction vessels to the rest of the apparatus. The complete unit consisting of the two reaction vessels and connection tubing is referred to as the containment vessel and is illustrated in Figure 5.1 and shown in Plate 2. The seals between the two reaction

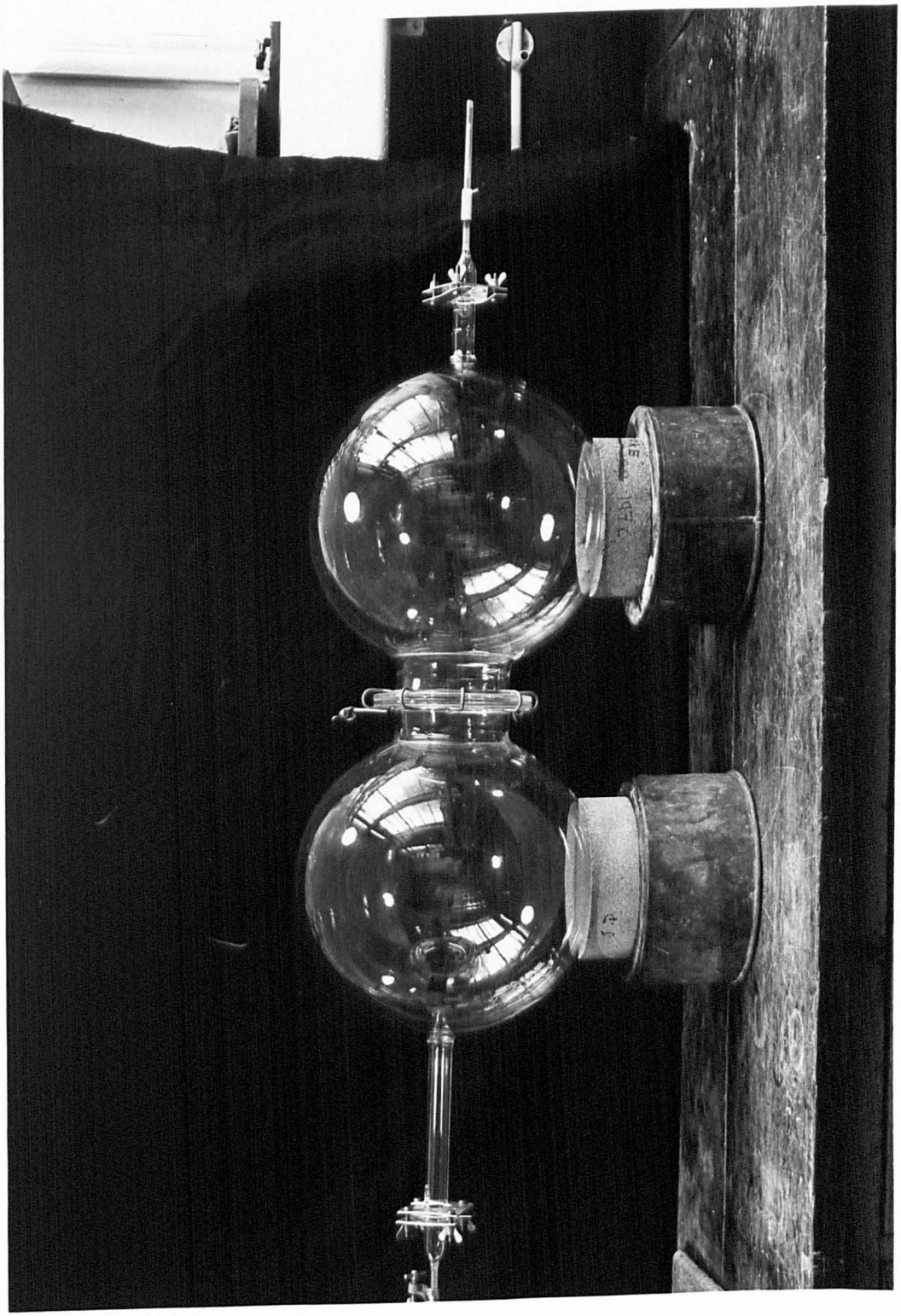
FIGURE 5.1

CONTAINMENT VESSEL

ALL DIMENSIONS IN MILLIMETRES



# PLATE 2



vessels, the reaction vessels and the connection tubing, were made with PTFE O rings, 0.25 mm thick, which were held in place with metal clips. The total volume of the containment vessel was 25 $dm^3$.

Cold Traps

A series of three 500 cc all glass cold traps were used to cryogenicaly scrub the volatile impurities from the $O_2/N_2$ mix. All three cold traps were constructed according to the specifications shown in Figure 5.2. The inlet and outlet arms of the cold traps were terminated with 35 mm lengths of ¼ inch O.D. glass tubing (Jencons (Scientific) Ltd). The cold traps were linked with drilled out ¼ inch couplings ((Dralim), Phase Separations Ltd) fitted with PTFE O ring seals, the couplings were drilled out so that the inlet and outlet arms of the cold traps could be butted together providing an all glass flow path for the $O_2/N_2$ mix. The cold traps were coupled to the containment vessel with a drilled out ¼ inch coupling ((Dralim), Phase Separations Ltd). The cold traps were cooled by placing them in dewar flasks packed with dry ice. The cold trap assembly is shown in Plate 3.

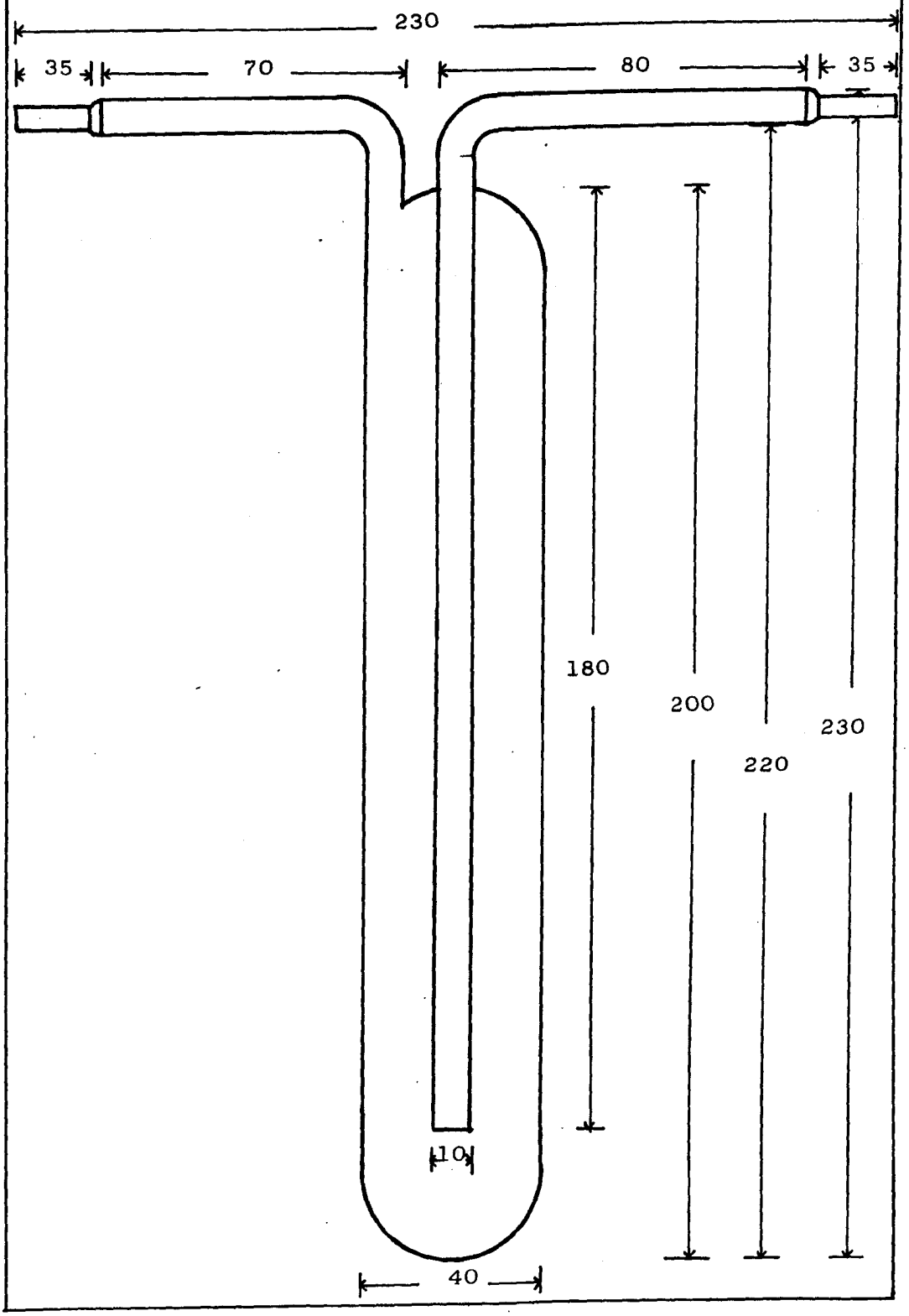
Air Supply

The air supply used to sweep the headspace volatiles from the containment vessel onto the precolumn was obtained by mixing $O_2$ and $N_2$ from pressurised cylinders (B.O.C. Glasgow Ltd) in the $O_2/N_2$ proportions of 2/5. Both cylinders were fitted with a pressure regulator (C.S. Milne Ltd, Glasgow) which was in turn connected to a needle valve flow controller (Model 8744, Brooks Instrument Division, Stockport, England). The outlets from the flow controllers were

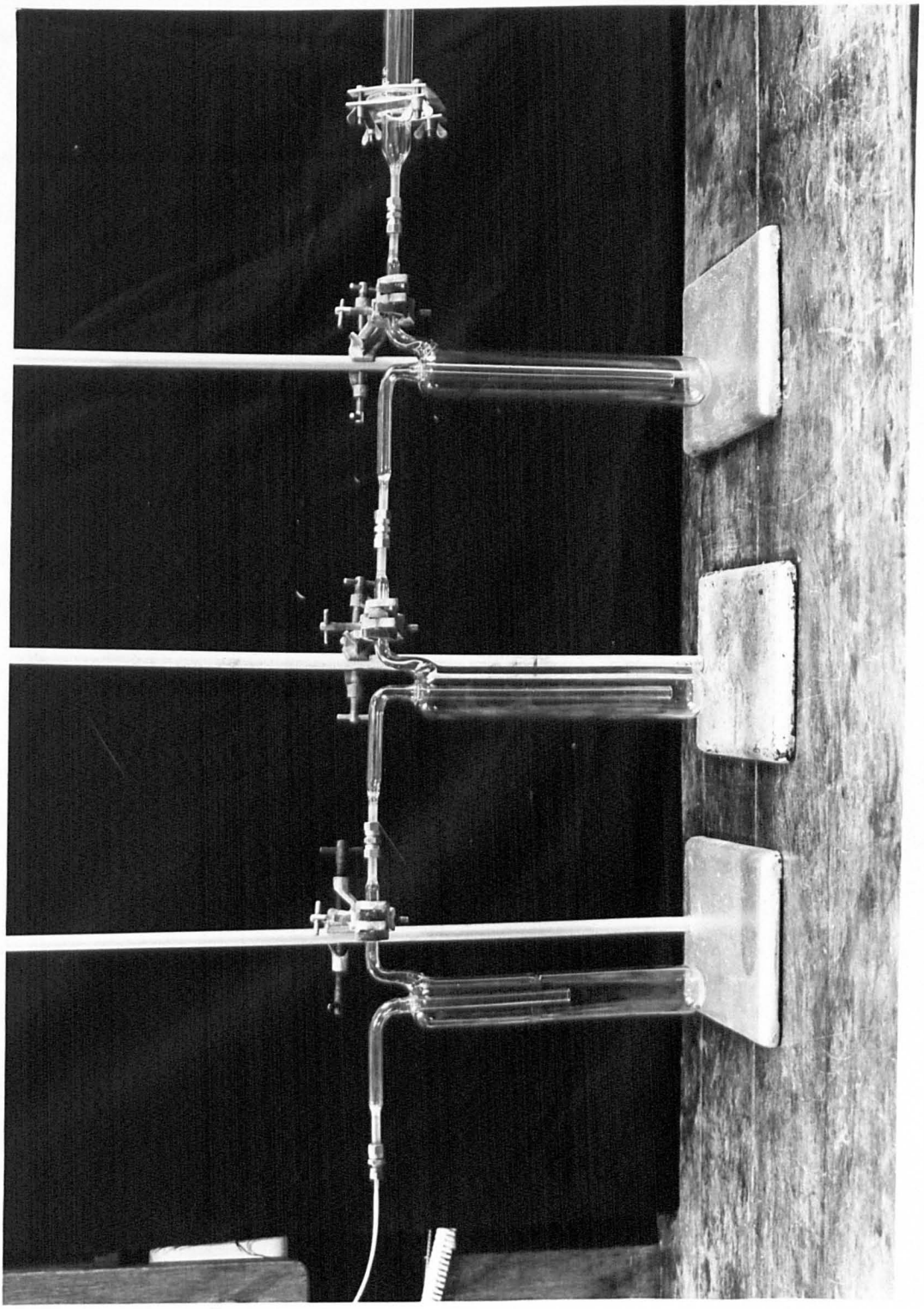
FIGURE 5.2

ALL GLASS COLD TRAP

ALL DIMENSIONS IN MILLIMETRES



# PLATE 3



connected to an $^{1}/_{8}$ inch brass T piece ((Swagelok), Glasgow Valve and Fitting Co. Ltd, Glasgow) with short lengths of $^{1}/_{8}$ inch O.D. PTFE tubing (Phase Separations Ltd). The brass T piece was used to mix the $O_2/N_2$ supplies, and was connected to the cold traps with a short length of $^{1}/_{8}$ inch O.D. PTFE tubing (Phase Separations Ltd).

The complete All Glass system is shown in Plate 4 and is illustrated schematically in Figure 5.3.

Gas Chromatography

| | |
|---|---|
| G.C.: | Pye 104 (Pye Unicam UK Ltd). |
| Column: | C.W.20M S.C.O.T. 43m, 0.5 mm I.D., 1 mm O.D. (S.G.E. UK Ltd). |
| Carrier Gas: | $H_2$ u 20 cm $sec^{-1}$. |
| Temperature: | |
| Column, Initial | 50°C 10 mins |
| Rise | 5°C $min^{-1}$ |
| Hold | 185°C. |
| Detector F.I.D.: | 270°C. |
| Detector Gasses: | $H_2$ 20 cc $min^{-1}$ |
| | $O_2$ 200 cc $min^{-1}$ |
| | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | 5 x $10^{-10}$ A |
| Chart Speed: | 5 mm $min^{-1}$. |

Assembly

Before use the All Glass system was set up as follows:

1. The containment vessel, cold traps, seals, couplings, brass T piece and PTFE tubing were thoroughly washed with hot water and detergent, rinsed with deionised

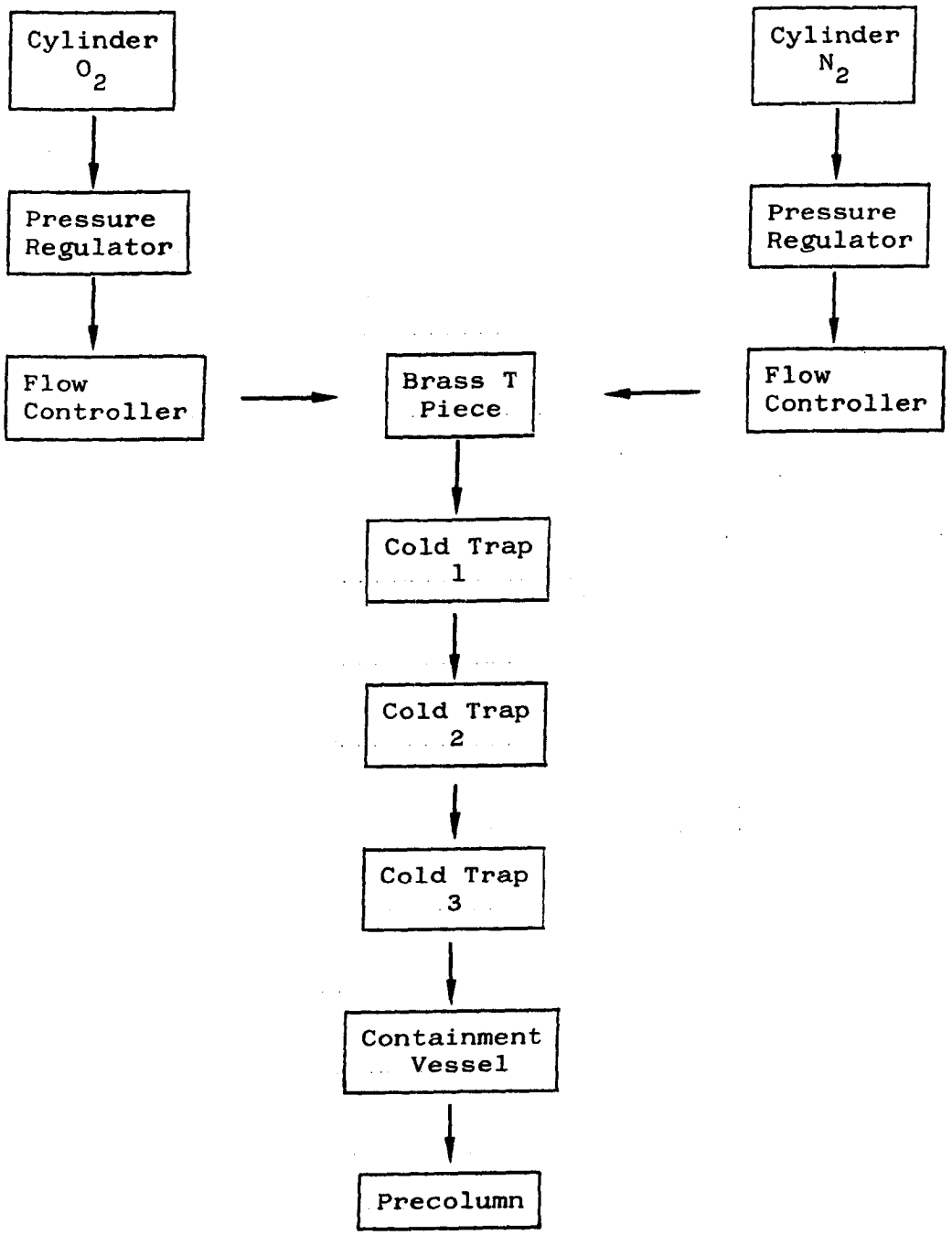
water, followed by acetone (Analar) and then heated at 220°C for 24 hr.

2. The system was assembled and sealed.
3. The cold traps were placed in dewar flasks packed with dry ice. It was found that the traps could be maintained at -70°C when the traps were topped up with dry ice daily.
4. The $O_2$ and $N_2$ flow rates were set at a 2 : 5 ratio with a total flow rate of 200 cc $min^{-1}$.
5. The system was then flushed with the $O_2/N_2$ air mix, henceforth referred to as the air mix, for 2 hr before sampling commenced.

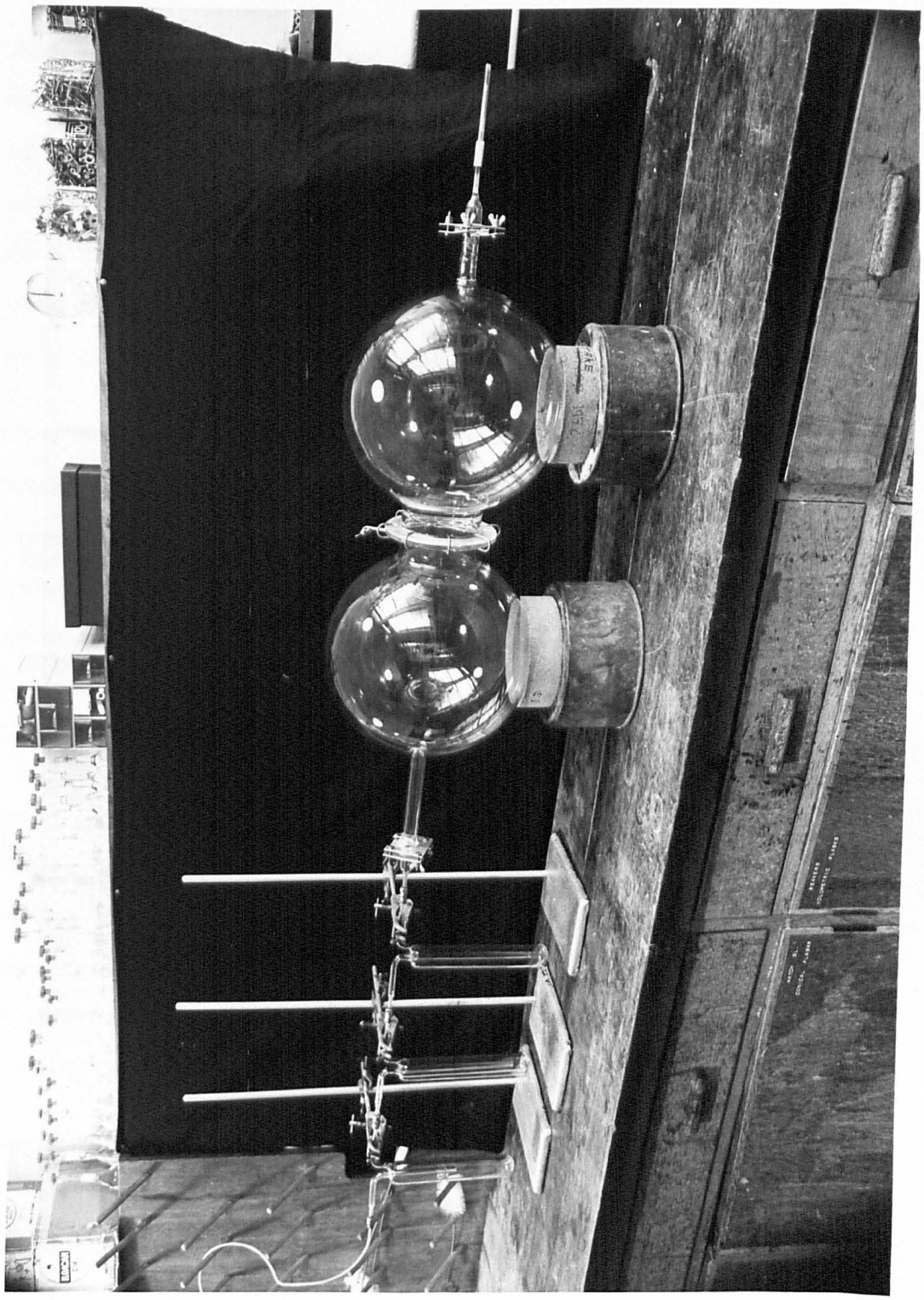
The headspace samples from the containment vessel were taken using the precolumns described in Chapter 4. The precolumns were connected to the effluent end of the containment vessel with a 30 mm length of PTFE rod (Jencons (Scientific) Ltd) $^3/8$ inch O.D. that had been drilled out with a 6 mm drill. This provided a convenient and inert gas tight seal.

FIGURE 5.3

Schematic Diagram of All Glass System



# PLATE 4



5.2.1 Run 1

This experiment assessed the levels of background volatiles contributed by the containment vessel, the air mix and the precolumn.

The All Glass system was set up as described in a room maintained at 15°C, the air mix was set at a flow rate of 200 cc $min^{-1}$. Several headspace samples of the air mix from the containment vessel and the air mix from the cold traps just before it entered the containment vessel, were taken. These headspace samples were taken at a flow rate of 200 cc $min^{-1}$ for sample volumes of 20 $dm^3$.

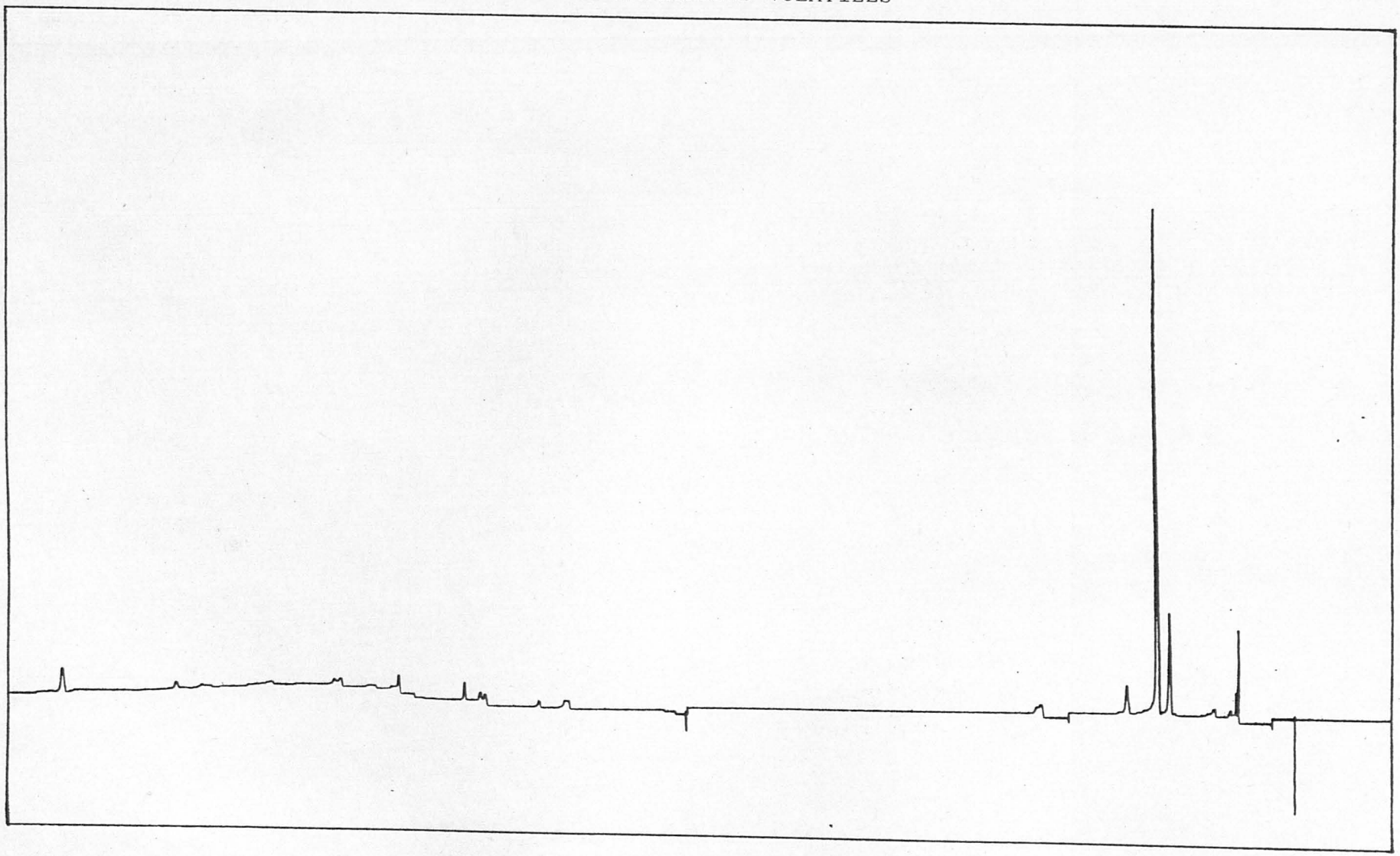
Chromatogram 5.1 shows the levels of background volatiles present in the headspace of the containment vessel, Chromatogram 5.2 shows the levels of background volatiles present in the air supply, and Chromatogram 5.3 shows the background volatiles that were desorbed from a conditioned, unsampled precolumn that had been sealed with PTFE caps (Jencons (Scientific) Ltd) and stored at 15°C for 24 hr.

Apart from the air peak, the conditioned, unsampled and sealed precolumn had not adsorbed any background volatiles during the 24 hr storage period. Normally the precolumns were used for sampling within 4 hr after conditioning, it was concluded that the precolumn itself did not contribute any background volatiles.

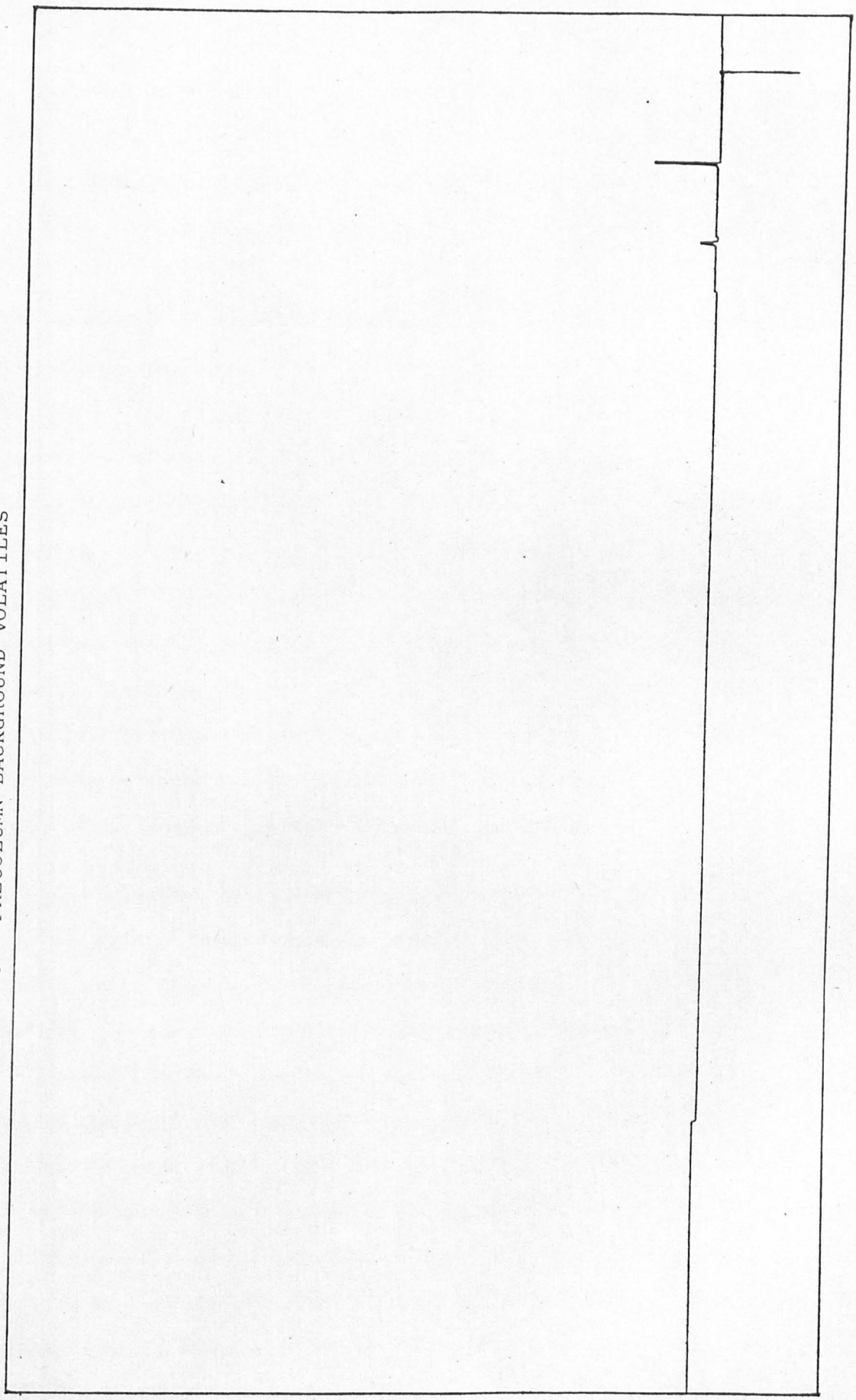
The chromatograms of the containment vessel headspace sample and the air supply headspace sample show that apart from one major peak with an RT of 5 min, there were no significant

CHROMATOGRAM 5.1 ALL GLASS SYSTEM BACKGROUND VOLATILES

CHROMATOGRAM 5.2 AIR SUPPLY BACKGROUND VOLATILES



CHROMATOGRAM 5.2



CHROMATOGRAM 5.3 PRECOLUMN BACKGROUND VOLATILES

levels of background volatiles present in either sample. The headspace sample from the containment vessel had trace amounts of longer R.T. background volatiles, however, they were not thought to be significant.

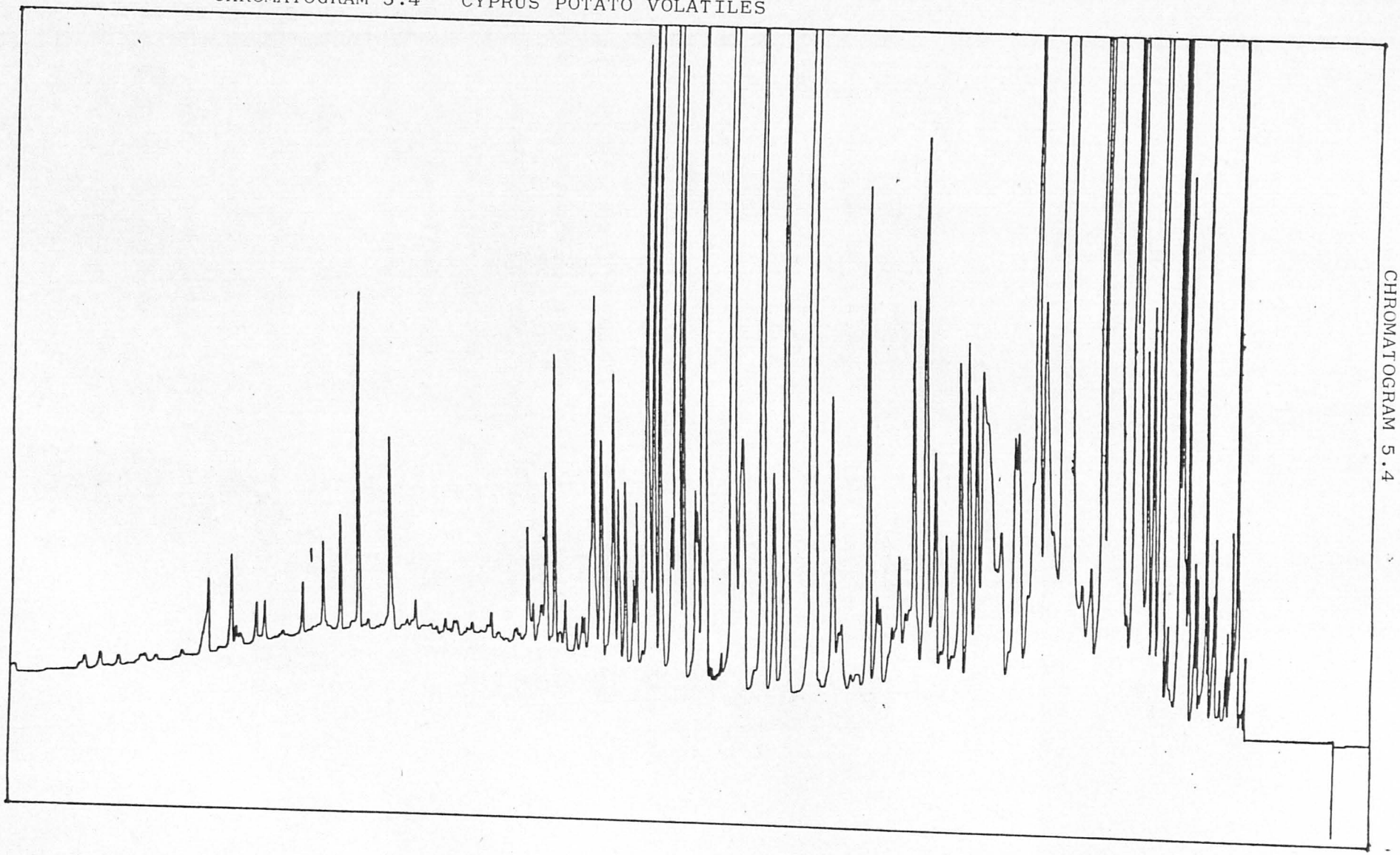
5.2.2 Run 2

Following the results of the assessment of the background volatiles contributed by the system, it was decided that it would be reasonable to use the All Glass system as it stood with no futher modifications for some preliminary attempts at collecting potato headspace volatiles.

Potatoes: 10 kg of Cyprus potatoes c.v. (unknown), were obtained from commercial sources. All the tubers had broken dormancy and had sprouts 5 mm - 10 mm long, the tubers were disease free and showed no sign of damage. The tubers were washed to remove any adhering soil and were then allowed to dry for 48 hr before use.

The containment vessel was filled with 8.5 kg of the Cyprus potato tubers and the system was assembled as described. The air mix flow rate was set at 200 cc $min^{-1}$ and the potato headspace volatiles were sampled over a 10 day period for sample volumes of 20 $dm^3$. Throughout the experiment the containment vessel was covered with a box to exclude light from the potatoes. Chromatogram 5.4 shows a representative sample of the potato headspace volatiles obtained from the potatoes. The levels of volatiles produced by the potatoes far exceeded the levels of background volatiles produced by the system. The tubers were kept in the containment vessel and ventilated

CHROMATOGRAM 5.4 CYPRUS POTATO VOLATILES



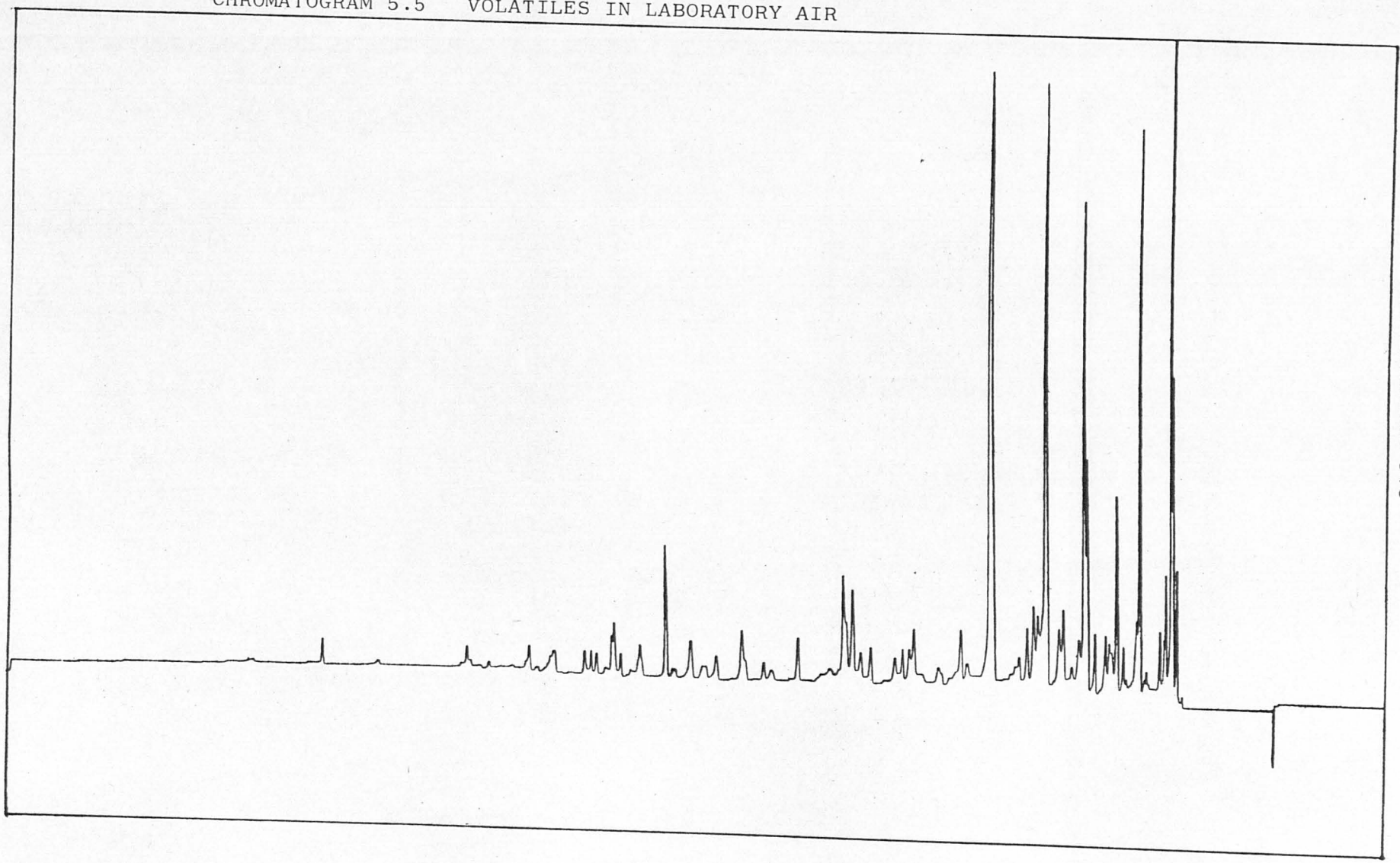
CHROMATOGRAM 5.4

with the air mix at 200 cc $min^{-1}$ for a period of 4 wk, the tuber health remained good throughout the 4 wk period. It was thought that headspace volatiles present in the laboratory atmosphere may have contributed to the background volatiles that were isolated in the initial assessment of this system, Run 1. A sample of the headspace volatiles present in the room in which the All Glass system was set up, was taken. The sample was taken by drawing a 1.0 $dm^3$ sample of the laboratory atmosphere at 100 cc $min^{-1}$, through a precolumn, using a small diaphragm air pump ((Capex Mk 2) Charles Austen Pumps Ltd, Weybridge, England).

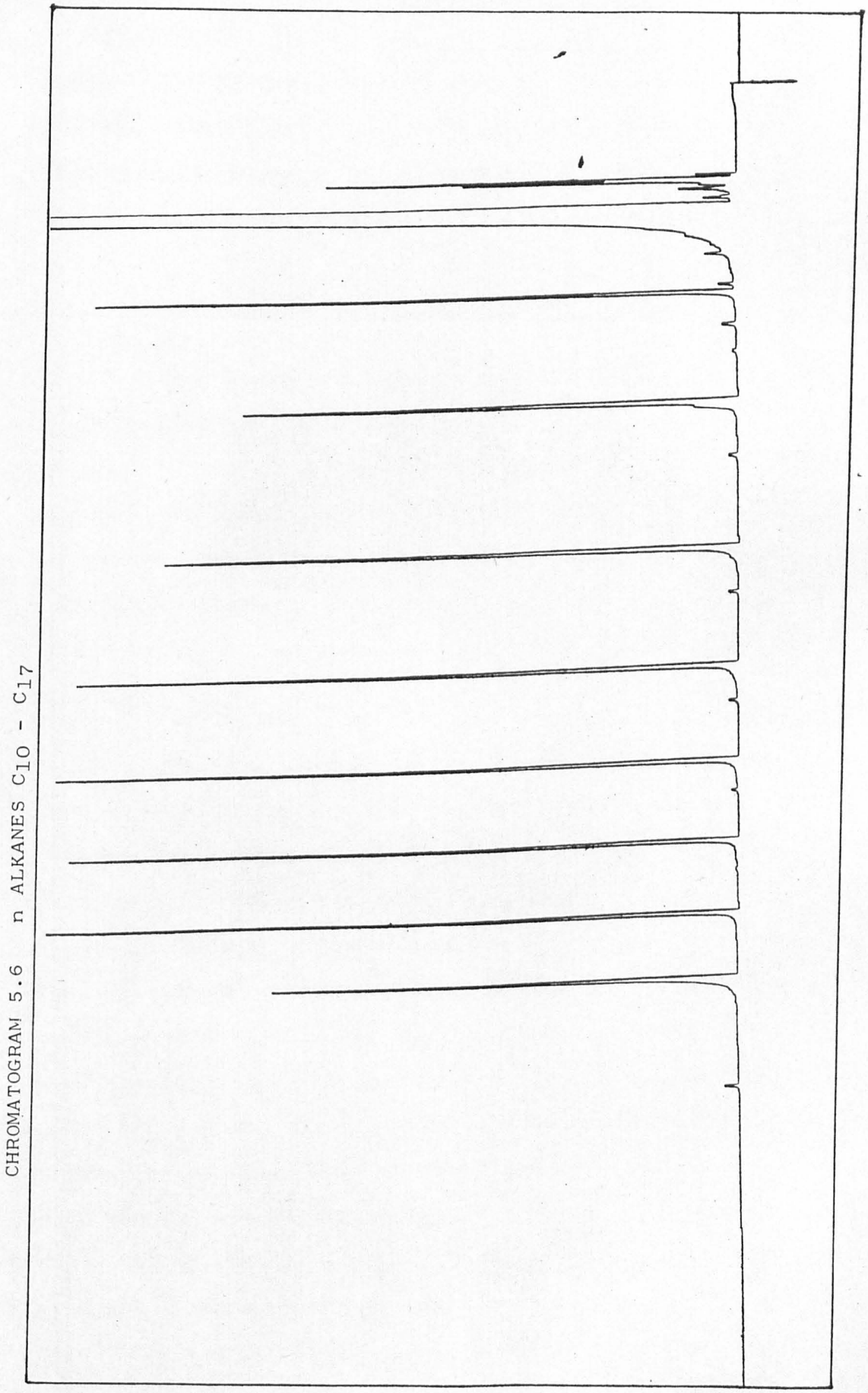
A series of n-alkanes, $nC_{10}$ - $nC_{17}$ (Alltech Associates, Carnforth, England) were run under the same chromatographic conditions used for the analysis of the headspace samples. The n-alkanes were made up in glass distilled hexane (Rathburn Chemicals Ltd, Walkerburn, Scotland), each n-alkane at a concentration of 500 ug $cc^{-1}$, a 0.1 $mm^3$ aliquot of the n-alkane standard solution was injected with a 1 $mm^3$ syringe ((700 series) Hamilton, Phase Separations Ltd), onto the precolumn and desorbed as described.

The chromatogram of the headspace sample of the laboratory atmosphere shows that there are substantial amounts of volatiles present in the 1.0 $dm^3$ sample, Chromatogram 5.5. A comparison of Chromatogram 5.5 with the chromatogram of the n-alkanes, Chromatogram 5.6, shows that the major headspace volatiles present in the laboratory atmosphere eluted before n-$C_{11}$. It was thought that these more volatile

CHROMATOGRAM 5.5 VOLATILES IN LABORATORY AIR



CHROMATOGRAM 5.5



CHROMATOGRAM 5.6 n ALKANES $C_{10}$ - $C_{17}$

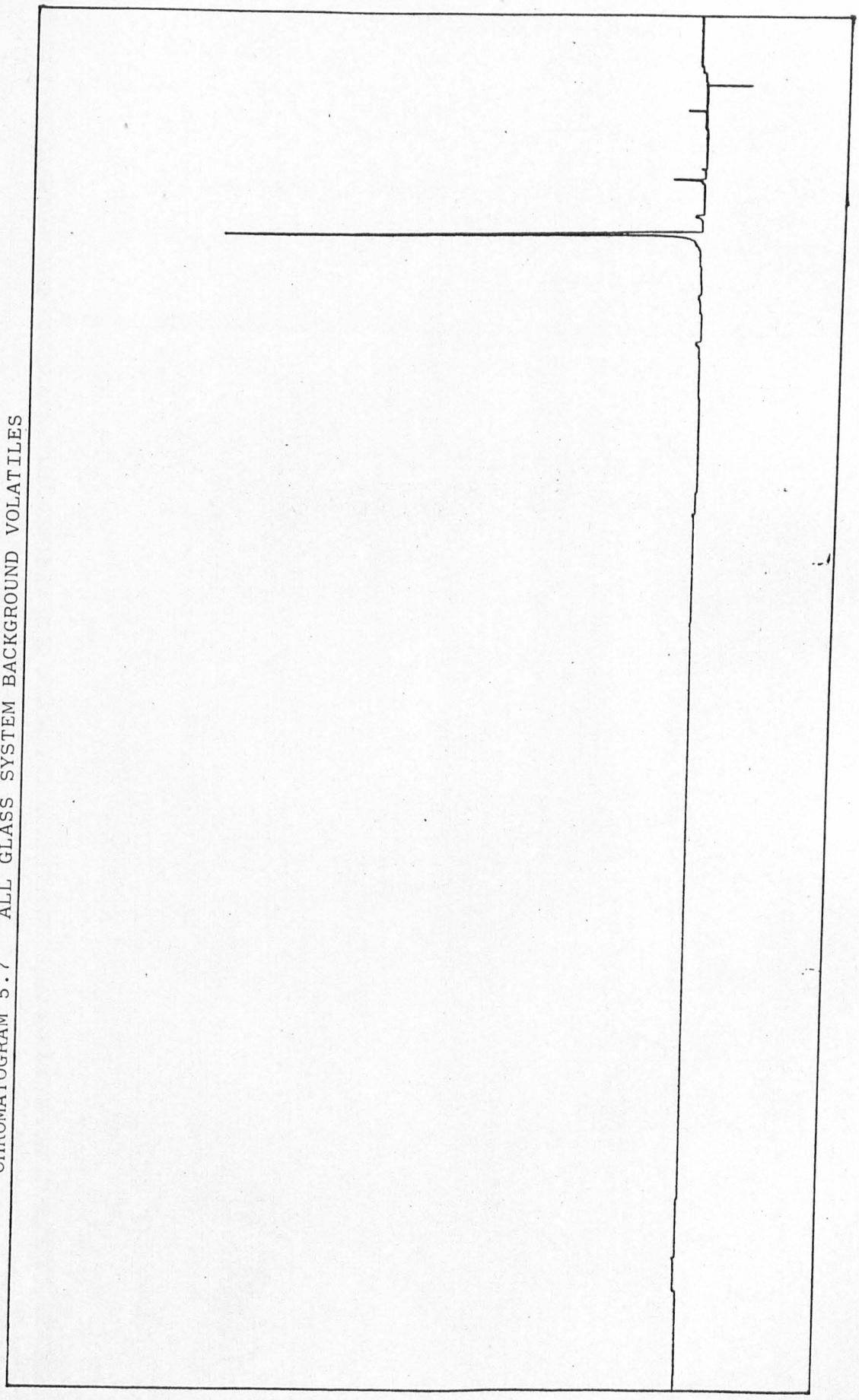
headspace components could be attributed to volatile organic solvents commonly used in the laboratory, and that they might have contributed to the background volatiles sampled from the containment vessel.

5.2.3 Run 3

The All Glass system was used to sample the headspace potato volatiles produced by dormant potato tubers.

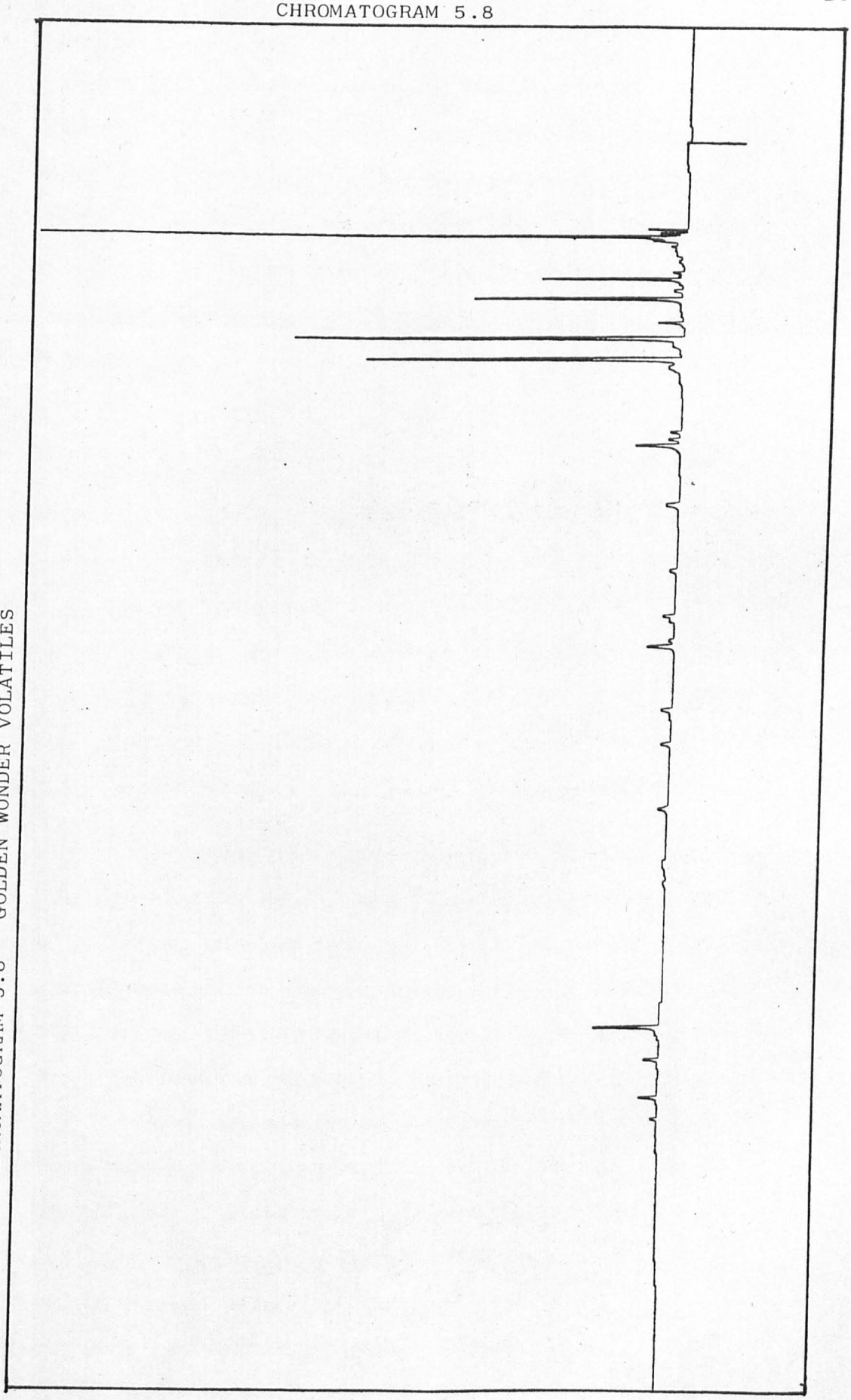
Potatoes: 15 kg of dormant potato tubers c.v. Golden Wonder were obtained commercially. All the tubers were dormant, disease free and showed no sign of damage. The tubers were washed to remove any adhering soil and were allowed to dry for 48 hr. They were then stored in the dark at 10°C before use.

Initially the levels of background volatiles present in the headspace of the containment vessel were assessed. The apparatus was set up as described in a room at 10°C, the air mix flow rate was set at 200 cc $min^{-1}$, the background volatiles were sampled at 200 cc $min^{-1}$ for sample volumes of 20 $dm^3$ over a period of 5 days. The levels of background volatiles in the headspace of the containment vessel were very low, Chromatogram 5.7, only one compound with an RT of 6 min was trapped. The containment vessel was then filled with 10 kg of the dormant potato tubers, the system was resealed and flushed with the air mix for 16 hr. After the 16 hr period, several samples of the potato volatiles were taken over a 7 day period, at 200 cc $min^{-1}$ for sample volumes of 20 $dm^3$. A representative sample is shown in Chromatogram 5.8. The levels



CHROMATOGRAM 5.7 ALL GLASS SYSTEM BACKGROUND VOLATILES

CHROMATOGRAM 5.8



CHROMATOGRAM 5.8 GOLDEN WONDER VOLATILES

of potato volatiles produced by the dormant Golden Wonder tubers at 10°C were substantially lower than levels of volatiles produced by the Cyprus tubers at 15°C that had broken dormancy. It was thought that this difference was due to the difference in variety, origin and dormancy of the tubers and also to the tuber temperature during sampling.

## 5.3 CONCLUSIONS

It was concluded that the All Glass system was a satisfactory means of containing potato tubers for headspace sampling. The levels of background volatiles contributed by the system were acceptable, the tuber health remained good throughout the sampling periods used and in the case of the Cyprus tubers, sufficient amounts of potato headspace volatiles were isolated from the samples of the potatoes used.

However, the levels of volatiles that were isolated from the dormant Golden Wonder tubers gave some cause for concern. It was thought that the levels of some of the trace volatiles present in the headspace sample would not be sufficient for identification by mass spectroscopy. Therefore it was decided that a second system for the collection of potato volatiles should be developed. This second system would be supplementary to the All Glass system, in that it would provide greater amounts of potato volatiles for analysis, should the situation arise that the levels of potato volatiles isolated by the All Glass system were not sufficient for identification.

The development of the second system for the collection of potato volatiles is described in the next chapter.

# CHAPTER 6

# VACUUM DISTILLATION COLLECTION SYSTEM

## 6.1 INTRODUCTION

The levels of potato headspace volatiles isolated from the Golden Wonder potato tubers in the latter experiments of the All Glass system were considered to be too low for their subsequent identification by mass spectrometry.

The Vacuum Distillation system was developed for the collection of larger samples of volatiles. As with the All Glass system the Vacuum Distillation system was constructed entirely from glass and PTFE to reduce the levels of background volatiles contributed by the system to a minimum. The ventilation of the tuber sample was not a major consideration with the Vacuum Distillation system, as the volatiles were obtained from the tubers under anaerobic conditions, also the tubers were not subjected to long term storage periods in the apparatus, e.g. 1 wk - 4 wk.

The principle of the Vacuum Distillation system was to draw volatiles from potato tubers under reduced pressure, the volatiles were then concentrated in a liquid $N_2$ cooled cold trap. The volatiles present in the cold trap condensate were then flushed from the cold trap onto a precolumn using an $N_2$ gas flow and heat.

It was thought that volatiles isolated using the Vacuum Distillation system would be qualitatively similar to those isolated using the All Glass system, the only major difference being in the relative amounts of volatiles isolated by each system. This it was hoped would be confirmed by a G.C.M.S. analysis of potato volatiles from

the same batch of potatoes, where volatiles were collected using both systems.

Similar vacuum distillation techniques have been applied to the collection of potato volatiles from potato skins (Meigh, D.F., et al, 1973) and potato crisps (Deck, R.E., et al, 1965), in these systems the potato volatiles were drawn under vacuum into a liquid $N_2$ cooled cold trap. The volatiles were removed from the cold trap either by concentration in a small volume cold trap or by solvent extraction.

## 6.2 EXPERIMENTAL

The vacuum distillation apparatus was constructed from a reaction vessel fitted with a removable head to which a cold trap was connected. A 5 $dm^3$ reaction vessel (Corning Ltd) was used to contain the potato tubers. The reaction vessel head was fitted with two PTFE stopcocks ((Rotaflo) Corning Ltd) each terminated with a 35 mm length of ¼ inch O.D. glass tubing (Jencons (Scientific) Ltd). The seal between the reaction vessel and the head was made with a PTFE O ring, 110 mm O.D., 0.5 mm thick. The dimensions of the reaction vessel and the head are shown in Figure 6.1 and are illustrated in Plate 5.

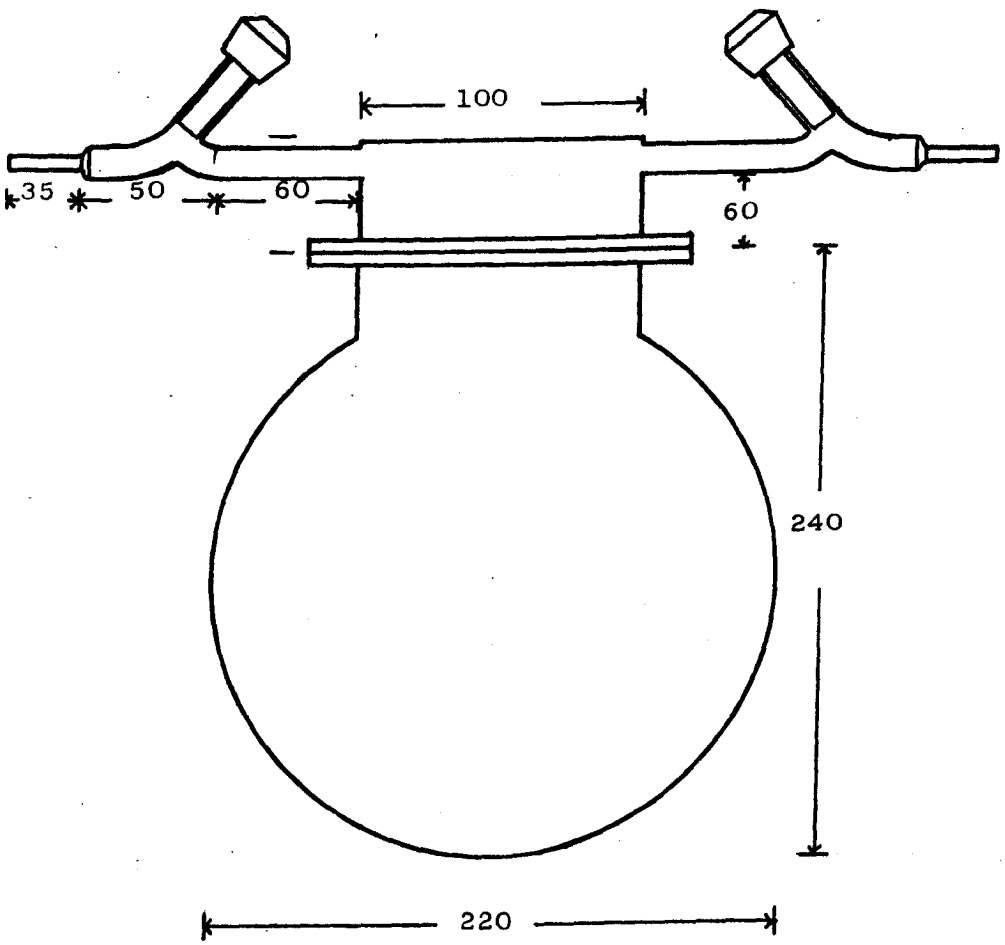
A 400 cc glass cold trap fitted with three PTFE stopcocks ((Rotaflo) Corning Ltd) each terminated with a 35 mm length of ¼ inch O.D. glass tubing was used to trap the potato volatiles. The dimensions of the cold trap are given in Figure 6.2 and it is illustrated in Plate 6. The complete apparatus when assembled is shown schematically in Figure 6.3 and illustrated in Plate 7.

FIGURE 6.1

VACUUM DISTILLATION SYSTEM

VESSEL AND HEAD

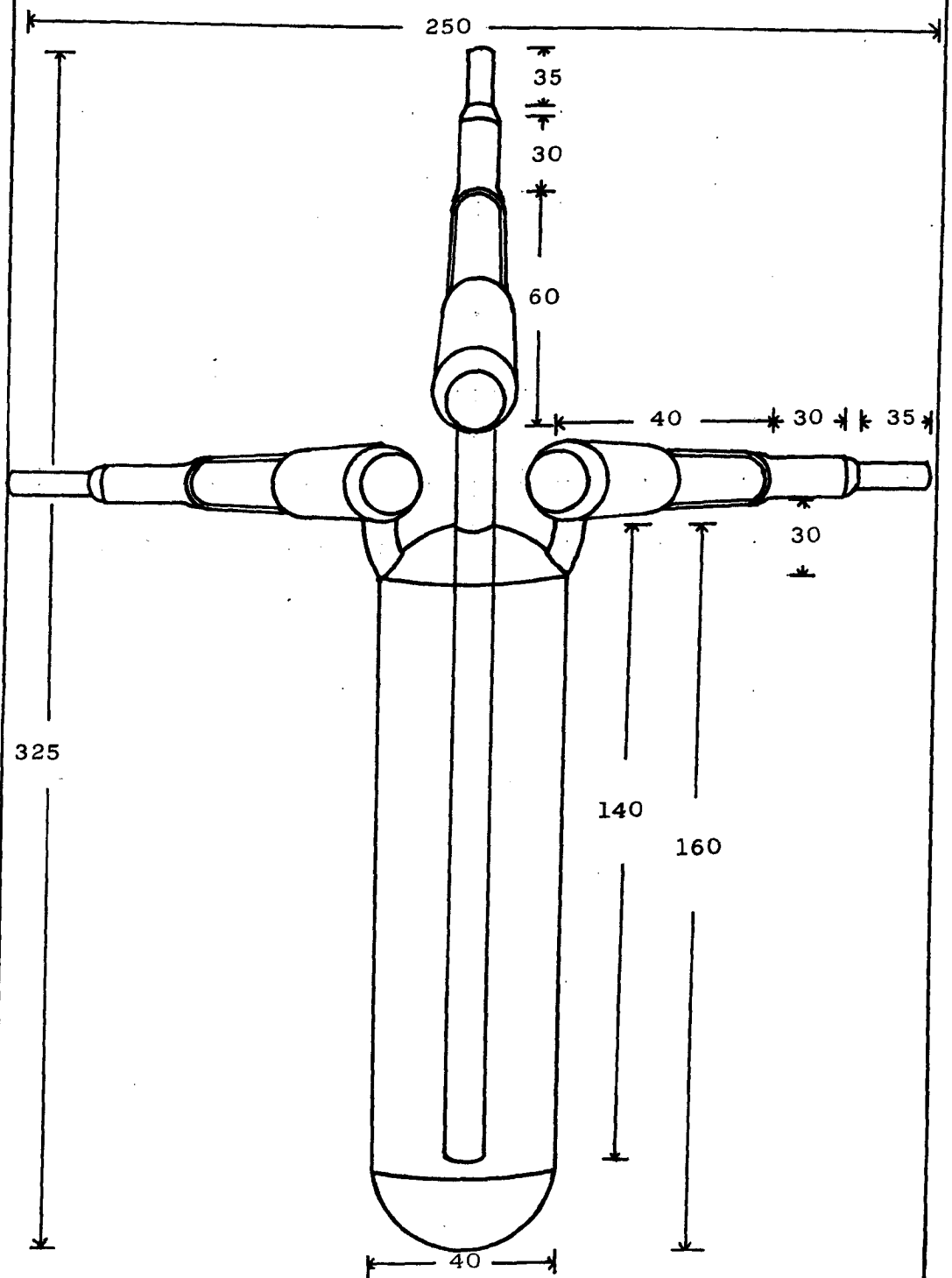
ALL DIMENSIONS IN MILLIMETRES



# PLATE 5

FIGURE 6.2
VACUUM DISTILLATION SYSTEM
COLD TRAP

ALL DIMENSIONS IN MILLIMETRES



# PLATE 6

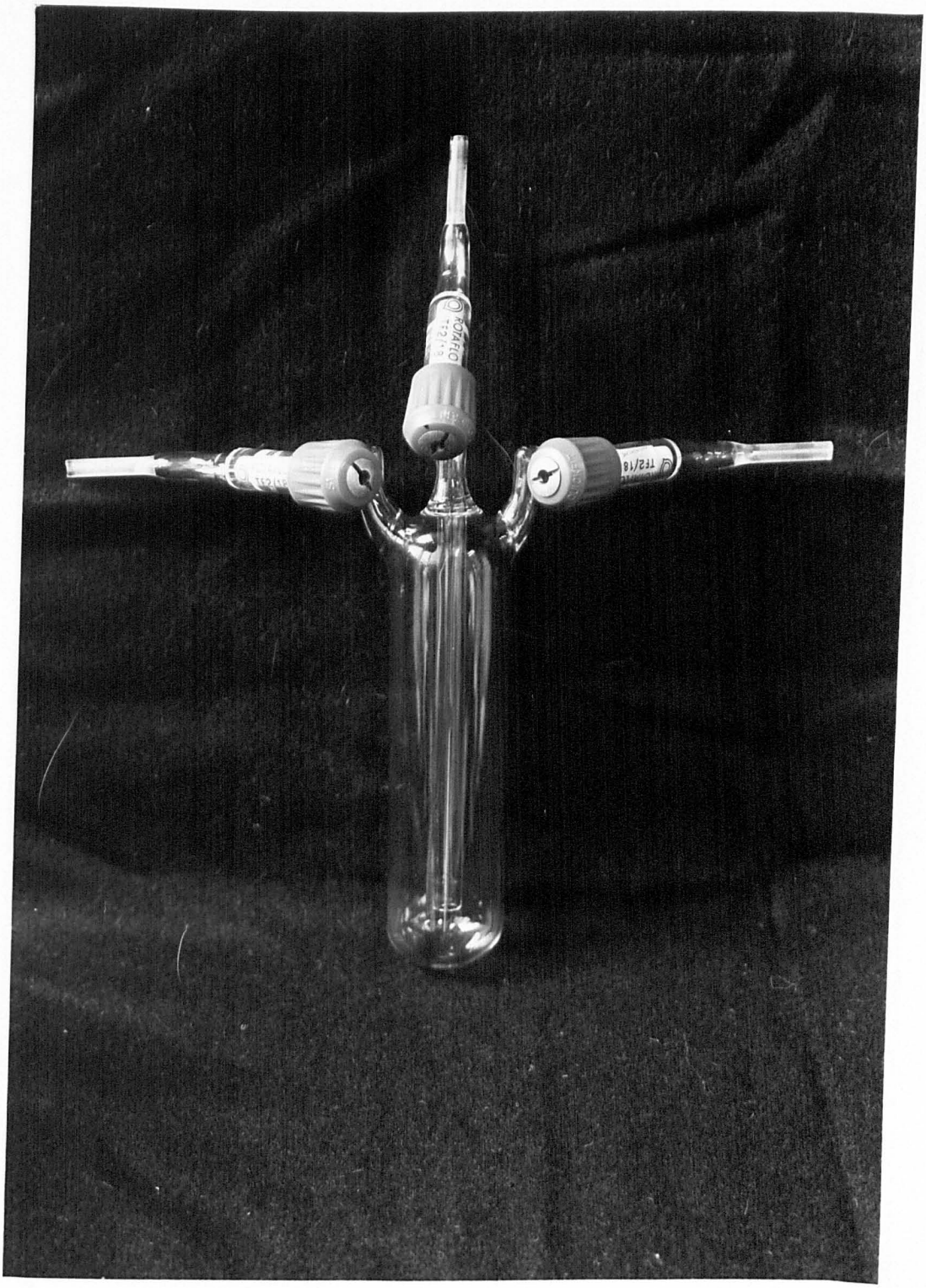
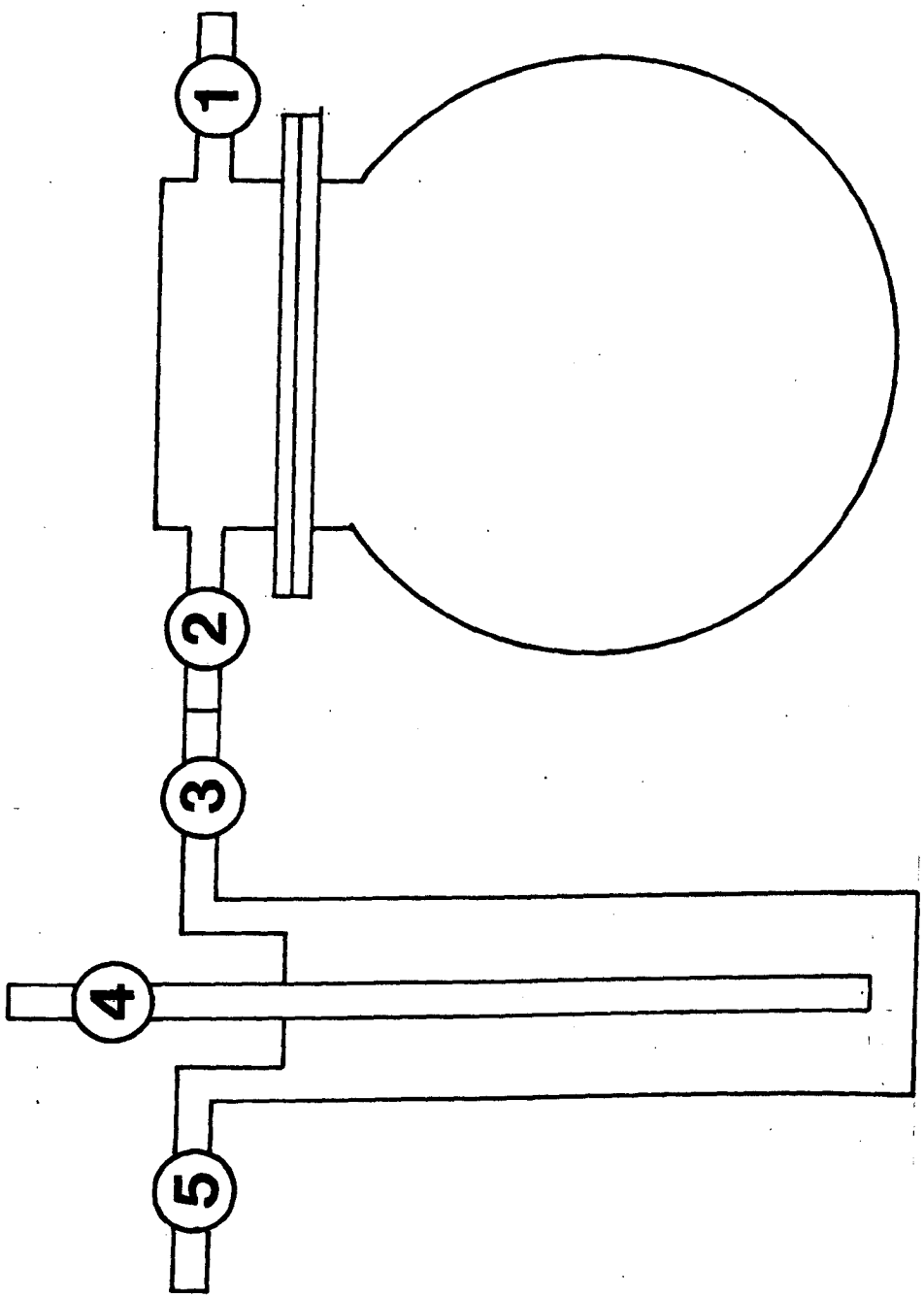


FIGURE 6.3

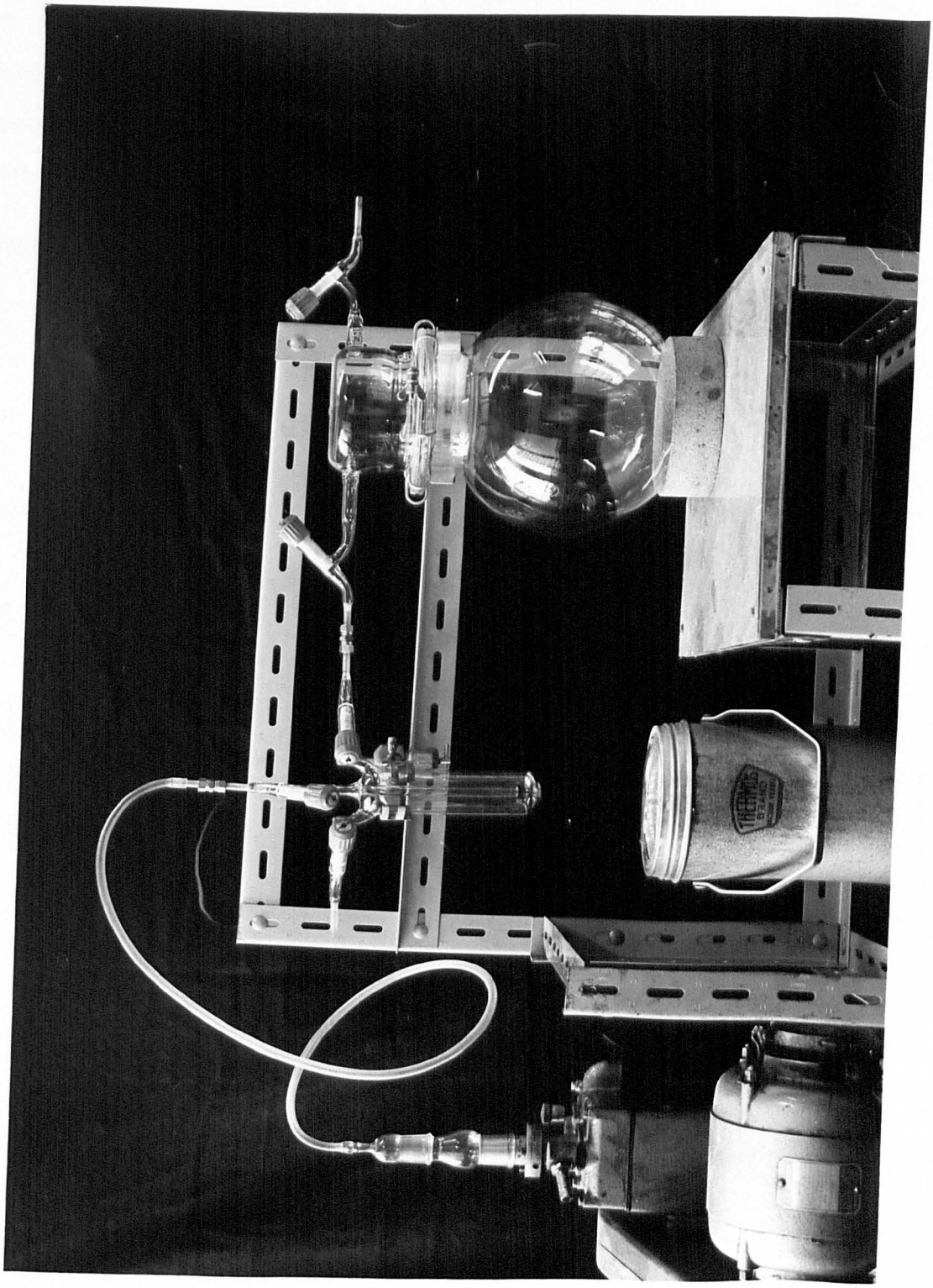
VACUUM DISTILLATION SYSTEM

SCHEMATIC DIAGRAM

1 - 5 = STOPCOCK NUMBER IN THE TEXT



# PLATE 7



The connections between the vacuum pump (Edwards High Vacuum, Crawley, England) and the cold trap; the head and the cold trap were made with a drilled out ¼ inch coupling ((Dralim) Phase Separations Ltd) fitted with PTFE O ring seals (Glasgow Valve and Fitting Co. Ltd). This enabled the ¼ inch glass tubing to be butted together providing a glass and PTFE flowpath for the volatiles in the system.

The volatiles were collected as follows:

1. The apparatus was thoroughly cleaned in hot water and detergent and then rinsed with deionised water followed by acetone and then heated at 220°C for 24 hr before use.
2. The apparatus was assembled, all the seals were made gas tight.
3. Stopcocks 1, 2, 3, 4 and 5 were opened and a $N_2$ supply (B.O.C. Glasgow Ltd) was connected to the inlet at stopcock 1. The apparatus was flushed with $N_2$ at 500 cc $min^{-1}$ for 15 min.
4. Stopcocks 5, 4, 3 and 2 and then stopcock 1 were closed. The $N_2$ supply was disconnected from the inlet at stopcock 1.
5. The liquid $N_2$ coolant was then put on the cold trap and the trap was allowed to equilibrate for 5 min.
6. The vacuum pump was coupled to the outlet at stopcock 4. The vacuum pump was then switched on.
7. Stopcock 4 was opened and the cold trap evacuated for 2 min.

8. Stopcocks 3 then 2 were opened and the reaction vessel was evacuated for 4 hr - 5 hr into the liquid $N_2$ cooled trap.
9. After the 4 hr - 5 hr sampling period, stopcocks 2 then 3 then 4 were closed and then the vacuum was disconnected.
10. The cold trap was disconnected from the reaction vessel and removed from the liquid $N_2$ coolant and allowed to equilibrate to room temperature.

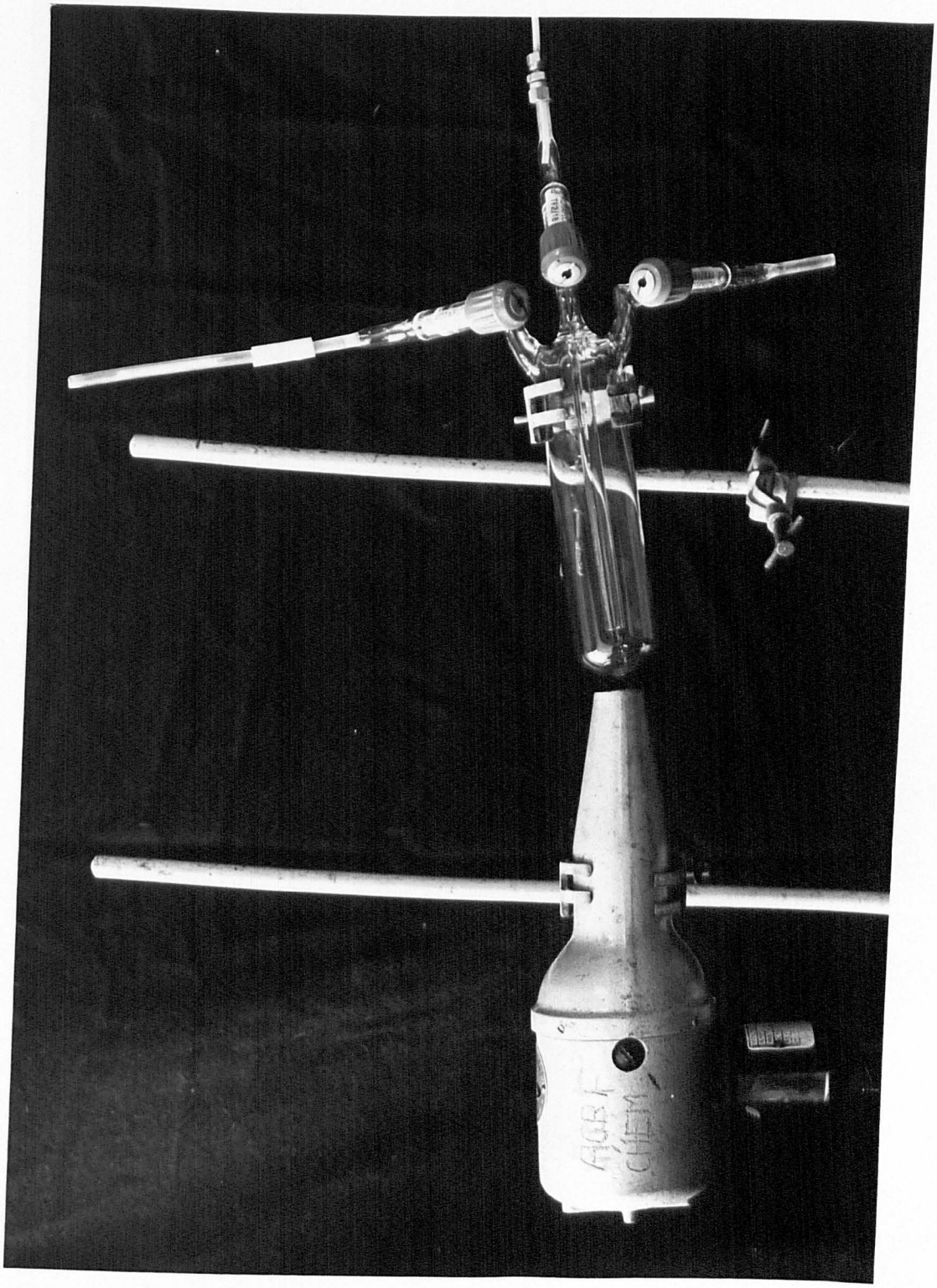
The volatiles in the cold trap condensate were flushed from the cold trap onto the precolumn as follows. The apparatus used is shown in Plate 8.

1. The cold trap was held at an angle to provide a greater surface area of the condensate in the bottom of the trap.
2. A $N_2$ supply was connected to the inlet at stopcock 4, a hot air stream was used to reheat the volatiles in the cold trap to 65°C - 75°C.
3. Stopcock 4 was opened and $N_2$ at 20 cc $min^{-1}$ was flushed into the cold trap for 2 min.
4. The precolumn was connected to the outlet at stopcock 5 using a drilled out length of PTFE rod 30 mm long (Jencons (Scientific) Ltd).
5. Stopcock 5 was opened and the volatiles were flushed from the cold trap onto the precolumn at 20 cc $min^{-1}$ for 40 min, giving a sample volume of 1.0 $dm^3$.

Gas Chromatography

The sampled precolumns were analysed using System 3 described in Chapter 7.

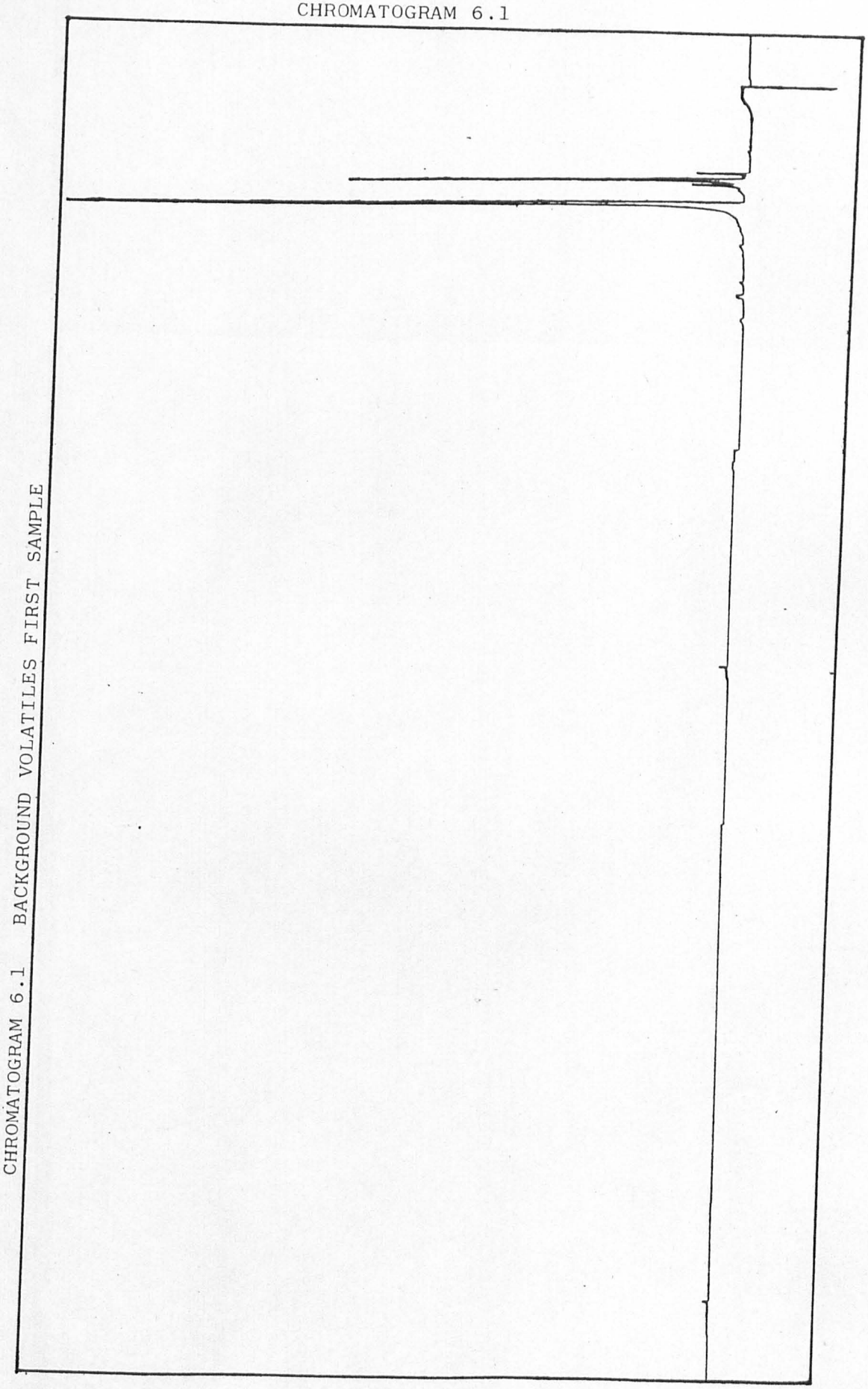
# PLATE 8



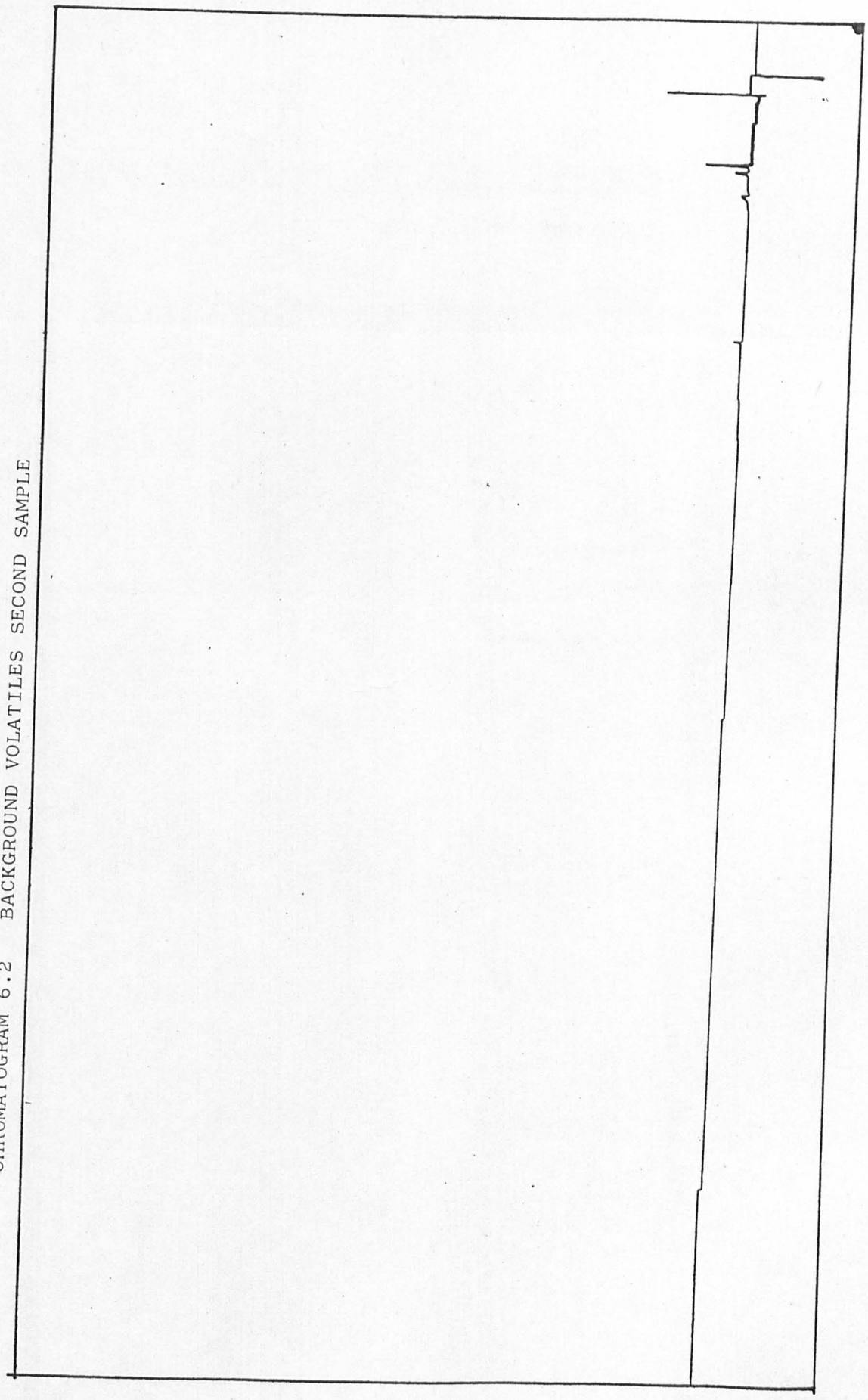
| | |
|---|---|
| G.C.: | Pye 104 (Pye Unicam Ltd). |
| Column: | C.W.20M, S.C.O.T. 43 metre, 0.5 mm I.D., 1 mm O.D. (S.G.E. UK Ltd). |
| Carrier Gas: | $H_2$ u : 20 cm $sec^{-1}$. |
| Temperature: | |
| Programme A - Initial | 50°C 10 mins |
| Rise | 5°C $min^{-1}$ |
| Hold | 185°C. |
| Programme B - Initial | 50°C 10 mins |
| Rise | 10°C $min^{-1}$ |
| Hold | 185°C. |
| Detector: F.I.D. | 270°C. |
| Detector Gases: | $H_2$ 20 cc $min^{-1}$ |
| | $O_2$ 200 cc $min^{-1}$ |
| | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | 5 x $10^{-10}$ A. |
| Chart Speed: | 5 mm $min^{-1}$. |

As the previous systems, the vacuum distillation system was assessed for background volatiles.

The system was assembled empty and a sample taken using the procedure described. Two consecutive samples of the background volatiles from the cold trap were taken and run using programme A, Chromatogram 6.1 and Chromatogram 6.2, respectively. Chromatogram 6.1 has two large peaks at the start and Chromatogram 6.2 has just the air peak. Both chromatograms showed that the levels of background volatiles were low and that they were trapped on the first precolumn.

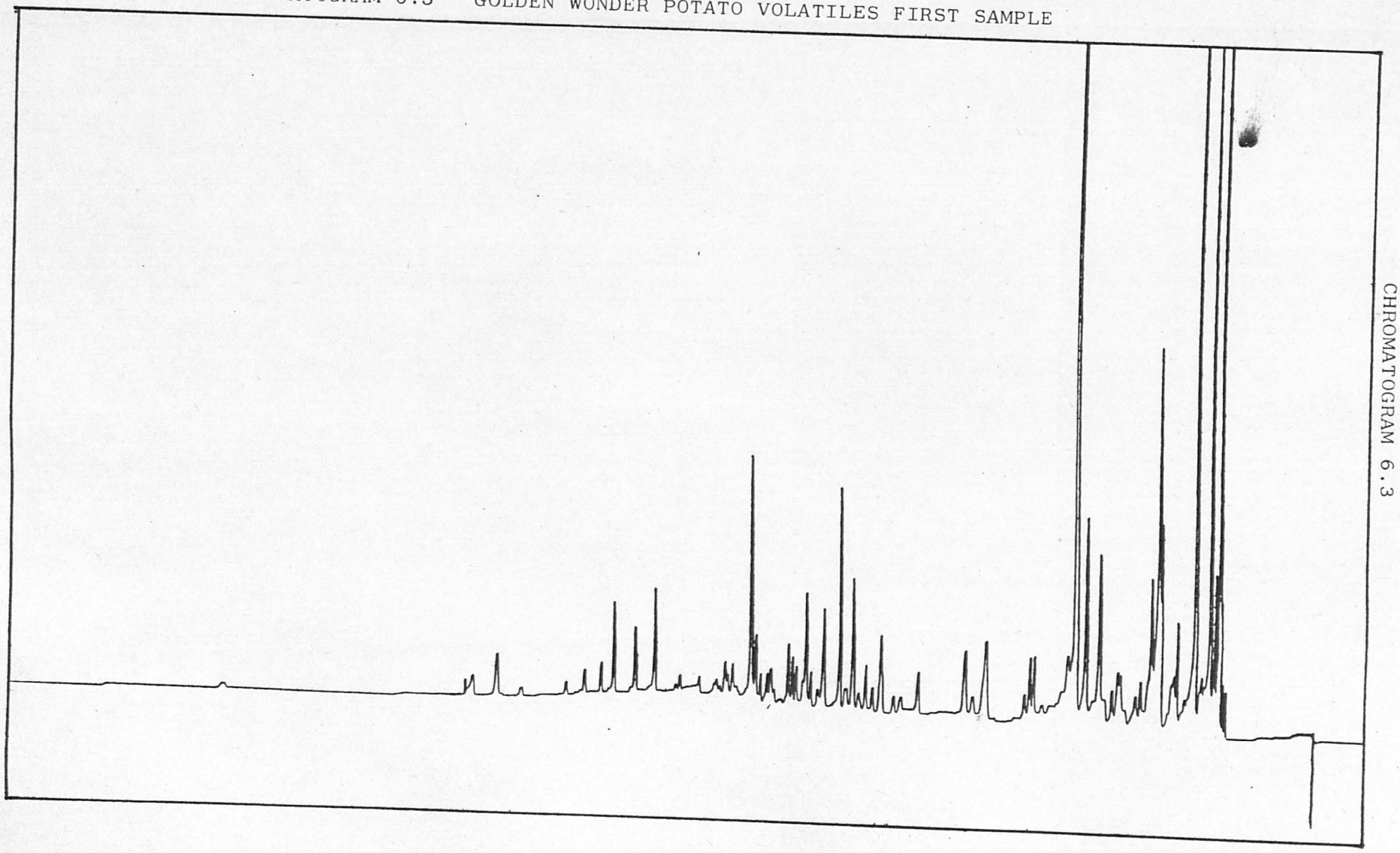


CHROMATOGRAM 6.1 BACKGROUND VOLATILES FIRST SAMPLE

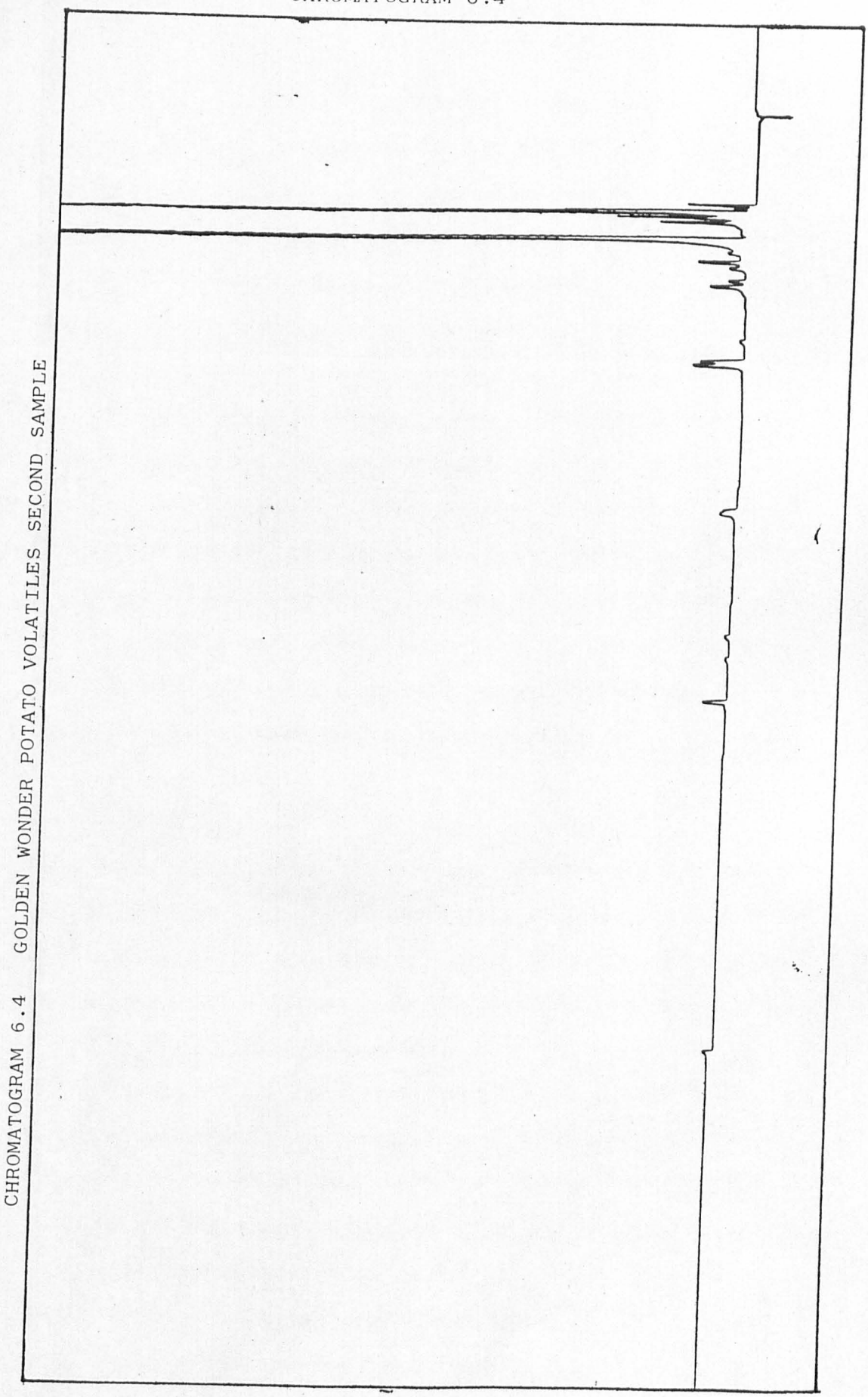


CHROMATOGRAM 6.2 BACKGROUND VOLATILES SECOND SAMPLE

CHROMATOGRAM 6.3 GOLDEN WONDER POTATO VOLATILES FIRST SAMPLE



CHROMATOGRAM 6.3



CHROMATOGRAM 6.4 GOLDEN WONDER POTATO VOLATILES SECOND SAMPLE

A sample of the volatiles from the Golden Wonder tubers used in the last assessment of the All Glass system, Chapter 5, was taken. The apparatus was filled with 2.8 kg of Golden Wonder tubers, and the volatiles collected in the cold trap condensate as described. Two consecutive samples of the potato volatiles from the cold trap condensate were taken and run using programme B, Chromatogram 6.3, and Chromatogram 6.4, respectively. The first sample, Chromatogram 6.3, trapped the majority of volatiles, only a few short RT volatiles were present in the second sample, Chromatogram 6.4. A comparison of Chromatogram 6.3 with Chromatogram 5.8, Chapter 5, of the All Glass system where the same tubers were used, shows that the Vacuum Distillation system collected a greater number and amount of potato volatiles than the All Glass system.

## 6.3 CONCLUSIONS

The Vacuum Distillation system was considered to be satisfactory in that a artefact free sample of potato volatiles was isolated and that the relative levels of potato volatiles were greater than those isolated by the All Glass system. However, the main consideration with this system was whether the volatiles in the cold trap condensate reflected the volatiles present in the headspace of the tubers. The volatiles in the cold trap condensate would probably contain a portion of the tuber headspace volatiles which were present in the reaction vessel prior to evacuation. A further portion of the volatiles could be attributed to the intracellular volatiles present in the tubers and possibly to

volatiles produced by cell damage due to the applied vacuum. Bearing these considerations in mind it was hoped that further investigations of potato volatiles using the All Glass and Vacuum Distillation systems would show whether the same volatiles were isolated by each technique, the major difference being in the relative proportions of the individual volatiles, or whether the Vacuum Distillation system in addition isolated artefact volatiles produced by the extreme sampling conditions. However, although the precise nature of the volatiles isolated by the Vacuum Distillation system can be questioned, the origin of these volatiles can be attributed to the potato tubers. Therefore, the Vacuum Distillation system was regarded as being supplementary to the All Glass system as opposed to being a replacement.

# CHAPTER 7

# GAS CHROMATOGRAPHIC ANALYSIS SYSTEM

## 7.1 INTRODUCTION

In Chapters 4, 5 and 6 the development of the systems for the collection of potato volatiles were described. This chapter is concerned with the development of the analytical techniques used for separation and identification of the potato volatiles.

The potato volatiles having been trapped on a Tenax G.C. precolumn were to be removed from the precolumn, introduced into the G.C. in a narrow band and then resolved into their individual components. The methods employed for the introduction of the potato volatiles into the G.C. column comprise the bulk of the work in this chapter. From the outset capillary columns were used for the resolution of the potato volatiles. It has been clearly established that in terms of separation efficiencies, speed of analysis, sensitivity and inertness, capillary columns are vastly superior to their packed column counterparts. The work on the use of capillary columns in trace analysis is extensive, the books of Jennings (Jennings, W.G., 1980) and Freeman (Freeman, R.R. 1979) provide excellent coverage.

The methods for the transfer of volatiles from a porous polymer adsorbent onto a capillary column are well documented. The two techniques used are solvent elution or thermal desorption. Solvent elution has been used for the extraction of plum volatiles from large adsorbent traps, the eluted volatiles in the solvent were concentrated to a small volume and injected into the G.C. column (Ismail, H.H., et al, 1980). However, solvent elution has not found widespread use for the removal of volatiles from a porous polymer adsorbent,

as the solvent dilutes the volatiles to a large extent, therefore reducing sensitivity, in addition the solvent obscures the early eluting volatiles. The low levels of potato headspace volatiles encountered in this project ruled out the use of any method that diluted the volatiles and made their subsequent detection difficult. Therefore, solvent elution was not considered to be a suitable method for this project.

Thermal desorption was chosen as the most appropriate method for the transferal of the potato volatiles from the precolumn, as this method did not involve dilution of the volatiles. Thermal desorption techniques have been extensively used for the analysis of trace organic volatiles, the basis of most of the desorption techniques has been to rapidly heat the adsorbent trap and flush the desorbed volatiles from the precolumn into the G.C. column using a sealed system and in the minimum volume of carrier gas.

The details of the procedures are varied, depending upon the individual needs of the analytical problem. Some researchers rapidly heated the precolumn and flushed the desorbed volatiles onto the G.C. column either by directly coupling the precolumn to the top of the column (Tsugita, T., et al, 1979), or through a switching valve (Barnes, R.D., et al, 1981), (Ryan, J.P., Fritz, J.S., 1978). Thermal desorption in conjunction with on-column cold trapping to thermally focus the volatiles onto the top of the analytical column has also been used (Jennings, W.G., 1981), (Heydanek, M.G., McGorrin, R.J., 1981), (Ott, U., Liardon, R., 1981), (Adam, S., 1983), (Kalman, D., et al, 1980). The on-column

cold trap usually consisted of a sleeve round the top of the column through which cold $N_2$ gas was passed as the coolant and then a hot air stream to vapourise the sample for the start of the analysis (Jennings, W., 1981), (Adams, S., 1983), (Ott, U., Liardon, R., 1981), sometimes the whole G.C. oven was cooled and reheated (Heydanek, M.G., McGorrin, R.J., 1981). Thermal focussing of desorbed volatiles in small volume cold traps external to the G.C. oven has also been used (Schultz, T.H., et al, 1971), (Cole, R.A., 1980), (Williams, A.A., et al, 1978), (Pellizzari, E.D., et al, 1975), (Pellizzari, E.D., et al, 1976). These systems were the most sophisticated involving heated switching valves and minimum dead volume flow lines and inert, non-catalytic surfaces for transferring the volatiles to the analytical column.

On the basis of the systems that had previously been used to transfer desorbed volatiles onto a G.C. column, the systems that were assessed and adopted in this project were based upon the following criteria.

1. The desorbed volatiles were introduced onto the capillary column in a small sample volume. This involved on-column cold trapping of the desorbed volatiles to concentrate them into a small "plug" before separation, or as used in the final system adopted, cold trapping in a small volume cold trap external to the G.C. oven.
2. The connection and/or disconnection of the precolumn to the G.C. system did not interrupt the carrier gas flow to the column. This was achieved using a heated switching valve.

3. The flow path of the volatiles to the capillary column was of minimum volume and dead volume, the materials that came into contact with the volatiles were as far as possible physically and chemically inert. The flow path was heated from the point of desorption to the capillary column, to prevent condensation of the volatiles on the flow path surfaces, which would have resulted in non-quantitative transfer and "memory" effects.

## 7.2 EXPERIMENTAL

Three systems were developed and assessed for the transfer of potato volatiles from a Tenax G.C. adsorbent precolumn into a capillary column. All three systems were based upon the principle of the thermal desorption of the potato volatiles from the precolumn into a small volume cold trap to thermally focus the volatiles into a small volume, before their subsequent separation on a capillary column. The major difference between the three systems developed was in the form of the cold trap used.

However, two fundamental pieces of equipment were utilised in all three systems, they were: a desorption block which was used to heat the precolumn for thermal desorption and a heated switching valve which was used to direct the volatiles onto the cold trap and to maintain a carrier gas flow to the capillary column. The heating block and heated switching valve were constructed as follows.

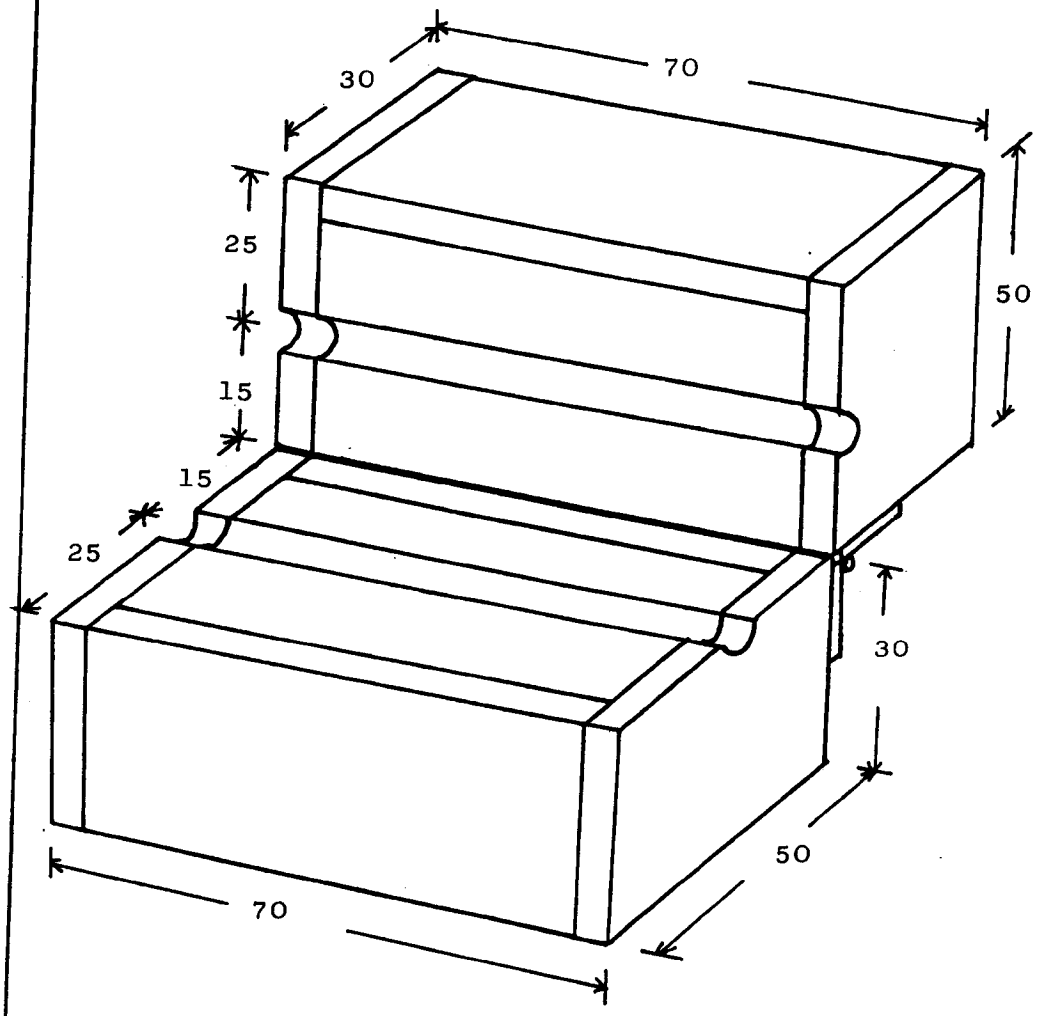
### Heating Block

The heating block was constructed from two blocks of aluminium which were joined together with a hinge, so that it could be clamped round the precolumn without interrupting the gas flow through the precolumn. The inner surface of both sections of the heating block were grooved so that when it was clamped round the precolumn, the entire surfaces of the precolumn was heated. The dimensions of the heating block are given in Figure 7.1. Both sections of the block were heated by a 150W cartridge heater (Hedin Ltd, London) 60 mm x 10 mm O.D. The outer surfaces of the heating block were covered with ¼ inch maranite which served as a thermal

FIGURE 7.1

HEATING BLOCK

ALL DIMENSIONS IN MILLIMETRES



insulant. During operation the heating block was maintained at 260°C.

Heated Switching Valve

A six port gas chromatographic switching valve ((Calve Model 2021), Techmation, Edgeware, England) was used to switch the gas flows. The valve was placed in a heated enclosure which also contained the inlet gas flow from the precolumn and outlet gas flow line to the cold trap. The heated valve enclosure is illustrated in Figure 7.2. The valve enclosure was constructed from $^{3}/_{8}$ inch maranite, and was heated by two aluminium blocks placed at either end of the enclosure. Each aluminium block was heated by two 150W cartridge heaters (Hedin Ltd, London) 60 mm x 10 mm O.D. The valve was placed in the middle of the enclosure. The precolumn was coupled to a $^{1}/_{4}$ inch/$^{1}/_{16}$ inch reducing union (Pye Unicam Ltd) which was fitted to the side of the enclosure, this was in turn connected to the inlet of the valve with a short length of $^{1}/_{16}$ inch O.D. 0.4 mm I.D. glass lined stainless steel tubing (S.G.E., U.K. Ltd). The outlet flow line from the valve was made with a short length of $^{1}/_{16}$ inch O.D. 0.4 mm I.D. glass lined stainless steel tubing (S.G.E., U.K. Ltd). The outlet gas flow line connected the valve to the cold trap. During operation the valve enclosure was maintained at 230°C.

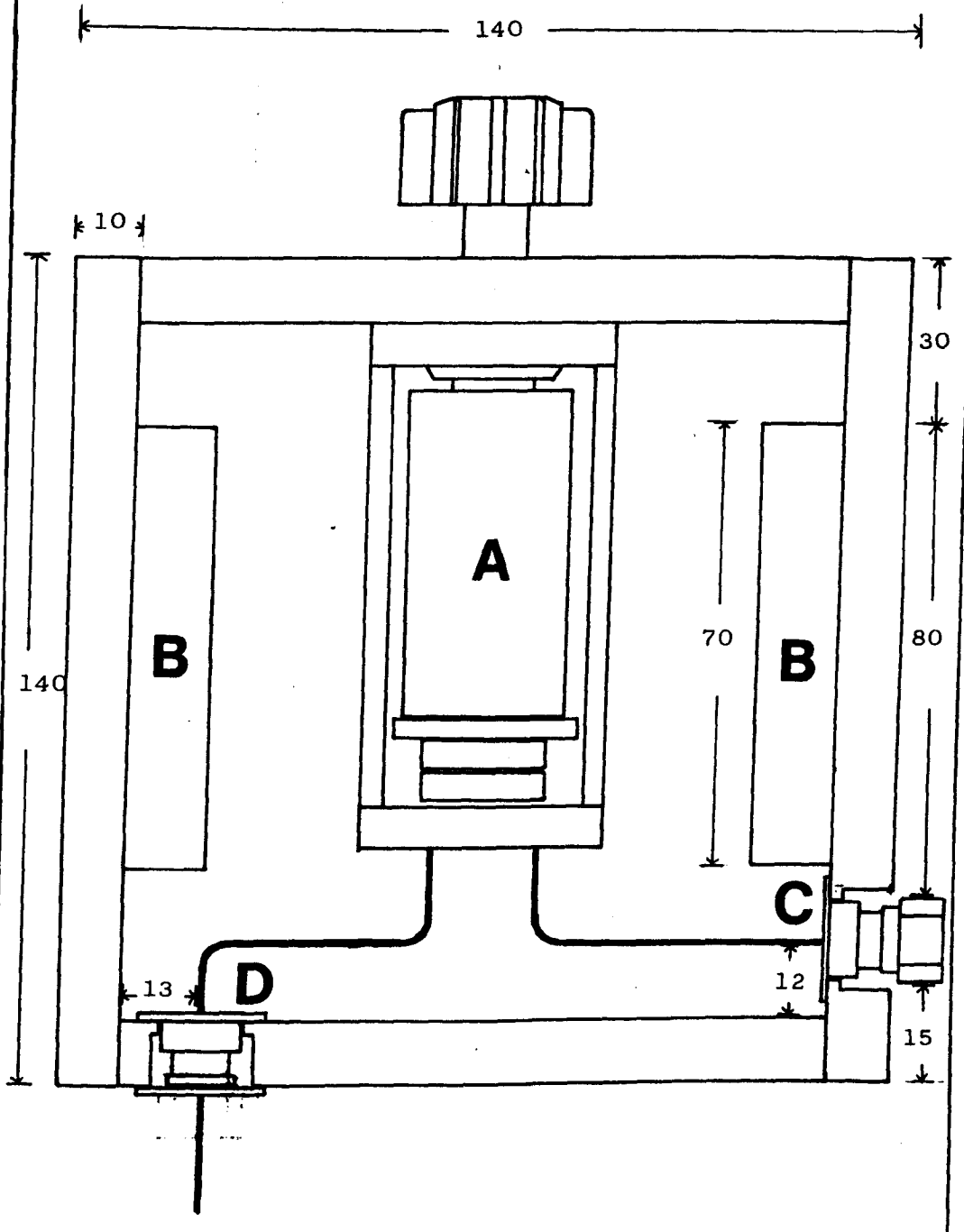
The heated valve enclosure was mounted on top of the G.C. oven with the outlet flow line led through the injection port into the G.C. oven, in the case of the first and second systems assessed. In the third system assessed, the valve enclosure

FIGURE 7.2

HEATED SWITCHING VALVE

A SWITCHING VALVE
B ALUMINIUM HEATING BLOCK
C INLET COUPLING
D OUTLET TO CAPILLARY COLUMN

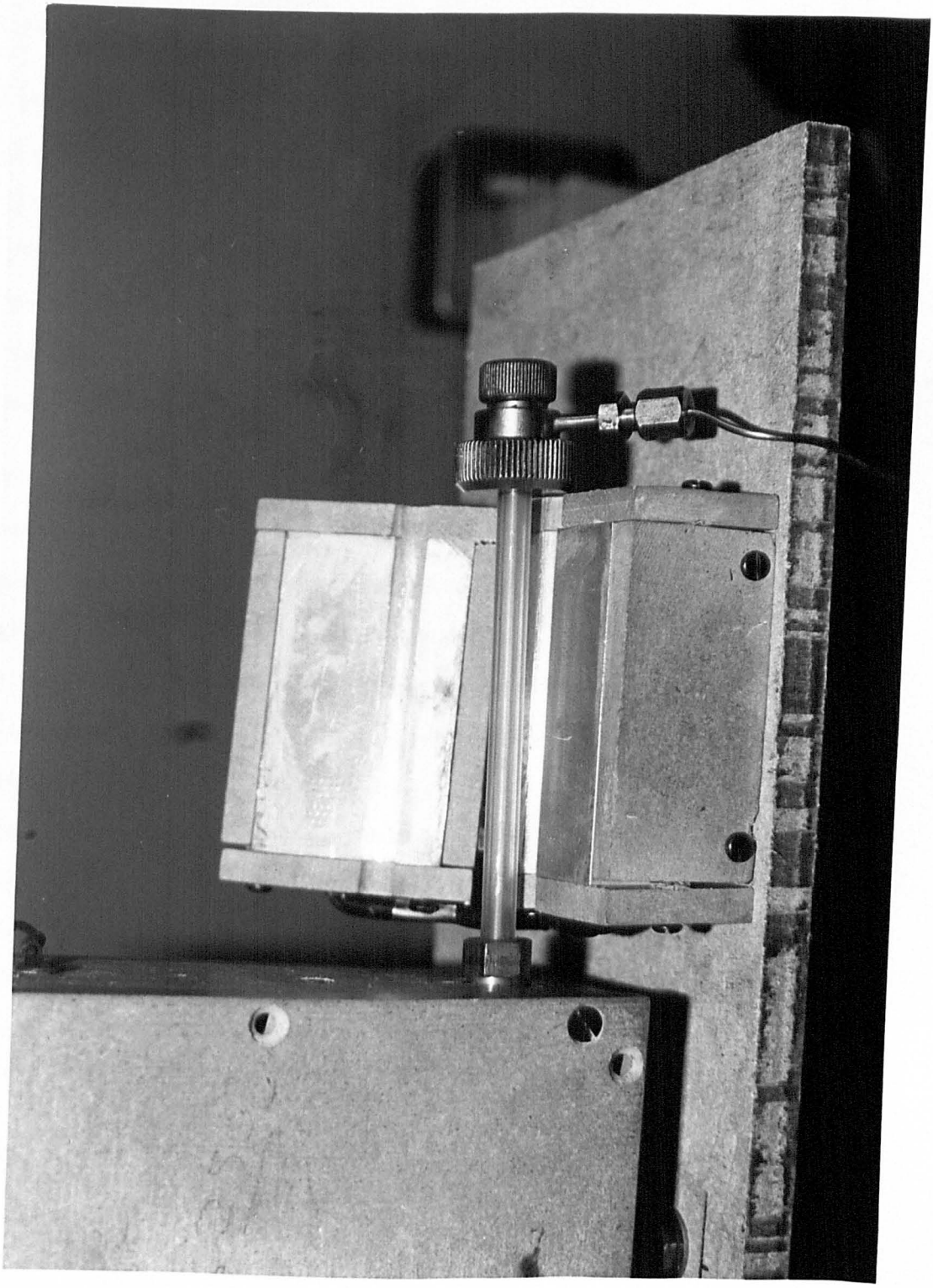
ALL DIMENSIONS IN MILLIMETRES



was mounted at the side of the G.C. oven. The heating block was placed adjacent to the valve enclosure. The general orientation of the valve enclosure and the heating block was that the distance between the precolumn and the capillary column was kept to a minimum.

A precolumn coupled to the side of the heated valve enclosure and the heating block are shown in Plate 9.

# PLATE 9



7.2.1 System 1

The first system that was assessed for the transfer of potato volatiles from a precolumn onto a capillary column employed the technique of on-column cold trapping of the volatiles.

The system consisted of the heating block and heated switching valve as described. The cold trap was formed by placing a short loop of the capillary column in liquid $N_2$. The capillary column used in this system was made from flexible silica tubing which was ideally suited to this technique as the column tubing could be looped, cooled and manipulated without breakage.

A schematic diagram of System 1 is illustrated in Figure 7.3, where the carrier gas flow paths are shown for both valve positions. The flow controllers were laminar flow, flow controllers((Porter VCD 1000), S.G.E., U.K. Ltd), the connections between the flow controllers, the inlet to the switching valve and the injection port head (Pye Unicam Ltd) were made with $^1/16$ inch O.D. stainless steel tubing (Phase Separations Ltd). The outlet flow line from the heated switching valve was a 200 mm length of $^1/16$ inch O.D., 0.4 mm I.D. glass lined stainless steel tubing (S.G.E., U.K. Ltd), which was coupled to the capillary column with a $^1/16$ inch zero dead volume coupling fitted with graphite ferrules (S.G.E., U.K. Ltd).

The system was operated as follows:

1. The valve was set to Position 1.
2. The precolumn was connected to the side of the valve

enclosure with the $^{1}/_{4}/^{1}/_{16}$ inch reducing union, the injection port head was connected to the other end of the precolumn. At this stage, the precolumn was connected via the $^{1}/_{4}/^{1}/_{16}$ inch reducing union to the inlet of the switching valve, and the carrier gas flowing through the precolumn was vented. The precolumn was flushed with carrier gas, to vent, for 5 min to remove any residual air from the precolumn.

3. A length of the capillary column 300 mm - 400 mm at the injection port end of the column was placed in liquid $N_2$.
4. The heated switching valve was turned to Position 2 and the heating block clamped round the precolumn. The precolumn was heated to 260°C for 10 min - 15 min, during this time the volatiles were desorbed from the precolumn, routed through the heated switching valve and trapped on the cooled portion of the capillary column. The gas flow rate passing through the precolumn was 2 cc $min^{-1}$ - 4 cc $min^{-1}$ during desorption.
5. After desorption, the valve was switched to Postion 1, the cooled capillary loop was removed from the liquid $N_2$ and allowed to heat to the oven temperature. The temperature programmed separation of the volatiles was then commenced.

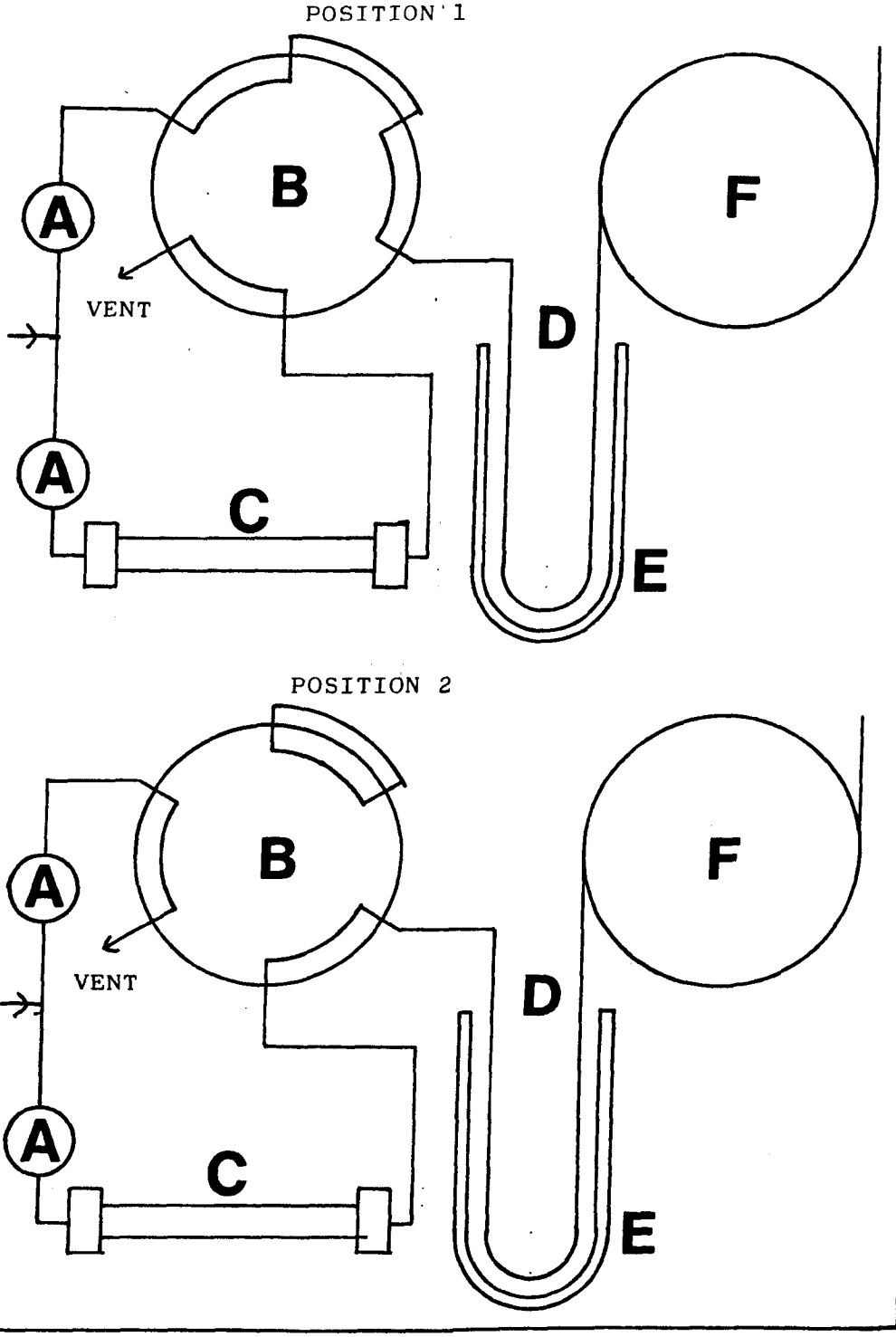
Gas Chromatography

| | |
|---|---|
| G.C.: | Pye 104 (Pye Unicam U.K. Ltd). |
| Column: | C.W.20M, W.C.O.T. fused silica 50m 0.2 mm I.D., 0.32 mm O.D., 0.2 um film thickness. |
| Carrier Gas: | $H_2$ u = 30 cm $sec^{-1}$. |

FIGURE 7.3

FLOW DIAGRAM SYSTEM 1

| | | | |
|---|---|---|---|
| A | FLOW CONTROLLER | D | CAPILLARY LOOP |
| B | SWITCHING VALVE | E | DEWAR FLASK |
| C | PRECOLUMN | F | CAPILLARY COLUMN |



Temperature:

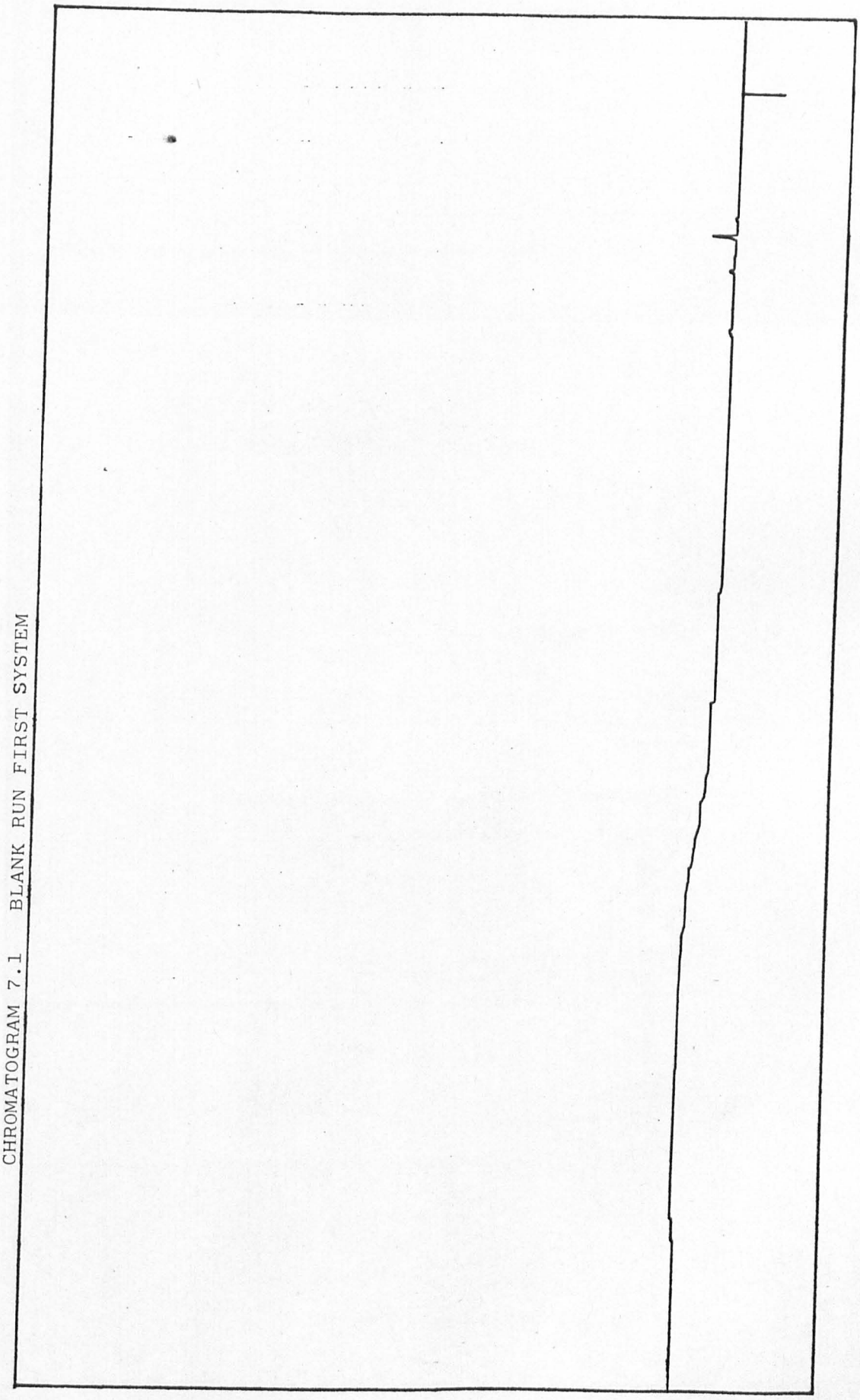
| | | |
|---|---|---|
| Programme A - | Initial | 50°C 10 min. |
| | Rise | 2°C $min^{-1}$. |
| | Hold | 185°C. |
| Programme B - | Initial | 50°C 10 min. |
| | Rise | 5°C $min^{-1}$. |
| | Hold | 185°C. |
| Detector: F.I.D. | | $H_2$ 20 cc $min^{-1}$. |
| | | $O_2$ 200 cc $min^{-1}$. |
| | | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | | $5 \times 10^{-10}$ A. |
| Chart Speed: | | 5 mm $min^{-1}$. |

This desorption system was used for the analysis of potato volatiles collected using the Tank system described in Chapter 4. A blank run was performed on the desorption system, a freshly conditioned precolumn was desorbed, as described, and the background volatiles chromatographed using programme B, Chromatogram 7.1. The blank run showed that there were no volatiles contributed by the desorption system or the precolumn. Duplicate samples of potato volatiles were taken from the Pentland Dell potatoes used in Run 4 of the Tank system assessment, Chapter 4. Both samples were 10 $dm^3$ in volume and taken at a flow rate of 50 cc $min^{-1}$ - 60 cc $min^{-1}$. The first sample was desorbed as described and chromatographed using temperature programme A, Chromatogram 7.2. The second sample was desorbed as described except step 3., the on-column cold trapping step was ommited, the volatiles were desorbed directly onto the

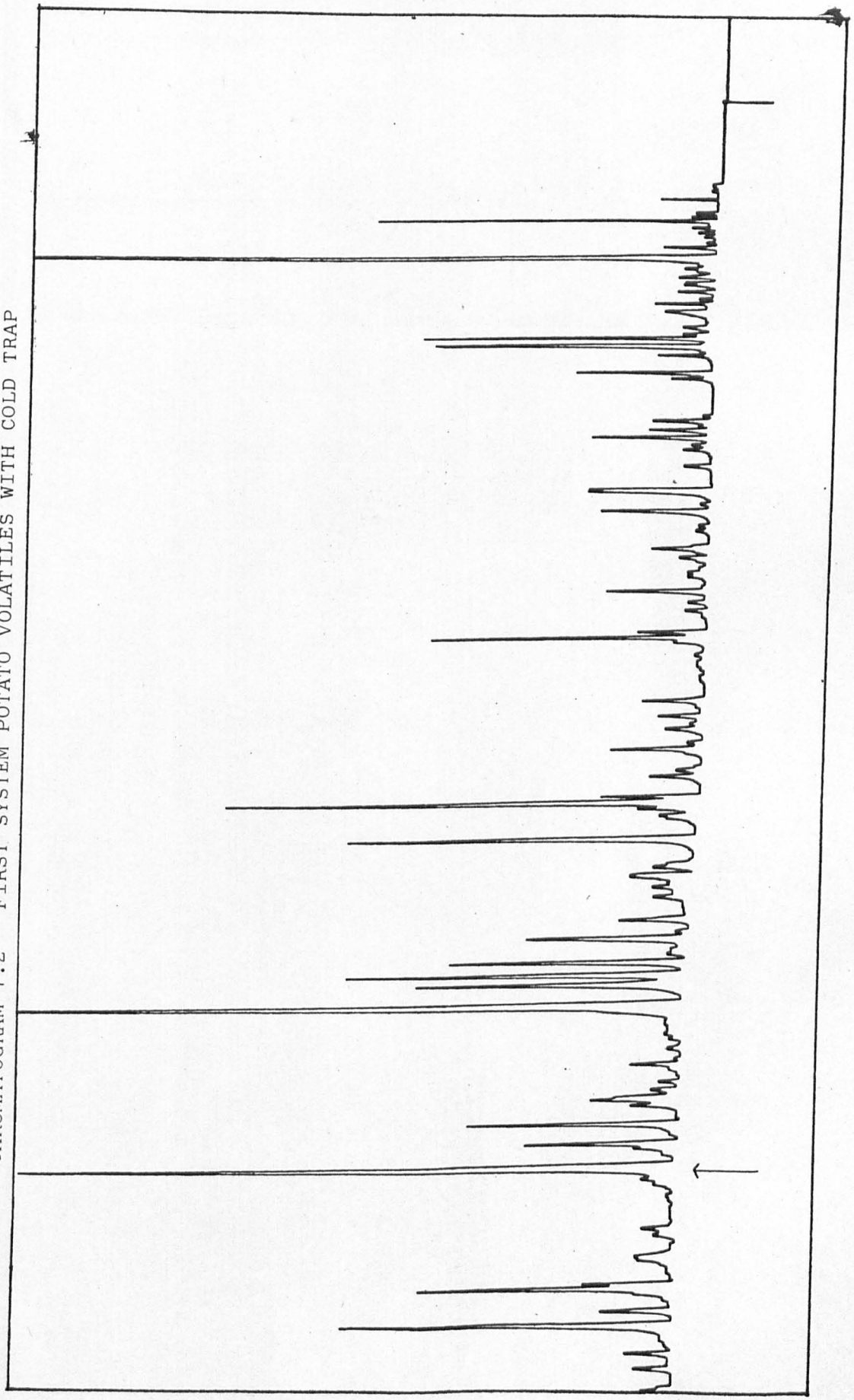
column and temperature programme A started 10 min after the desorption of the precolumn commenced, Chromatogram 7.3. A comparison of Chromatograms 7.2 and 7.3 showed that on-column cold trapping greatly improved the resolution of the volatiles, the peaks in Chromatogram 7.3 were broad and tailing, whereas they were sharp and well resolved in Chromatogram 7.2. This clearly demonstrated the necessity of thermally focussing the volatiles after desorption into a small volume before resolution on a narrow bore capillary column.

On the basis of these results, the analysis system was considered to be acceptable. However, the capillary column was degraded within a short period of time as several of the subsequent samples of potato volatiles contained very large amounts of ethanol, especially the volatiles sampled from potatoes under soft rot disease stress. It is known that potatoes under disease stress from soft rot infections produce large amounts of ethanol (Varns, J.L. & Glynn, M.T., 1979).

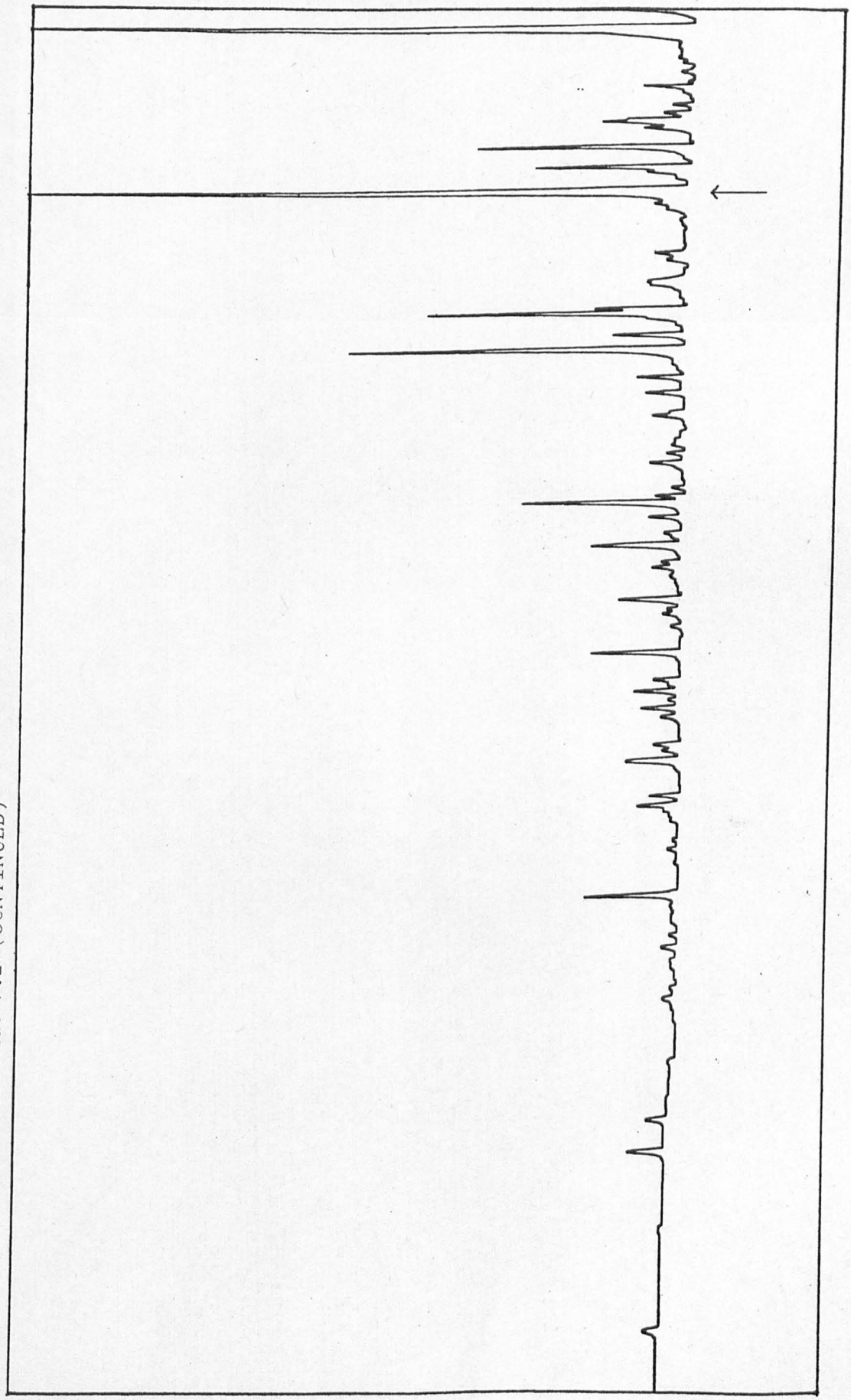
When these samples of potato volatiles containing large amounts of ethanol were desorbed onto the capillary column the C.W.20M phase was stripped off, this after a short period of time ruined the column. Therefore, although the on-column cold trapping technique had proved to be successful, it was considered to be too expensive to risk damaging thin filmed silica capillary columns using this technique. The sample volumes could have been decreased to bring the amount of ethanol desorbed onto the capillary column to within acceptable limits. However, this would have meant that the amounts of trace potato volatiles collected would have been undetectable.



CHROMATOGRAM 7.1 BLANK RUN FIRST SYSTEM

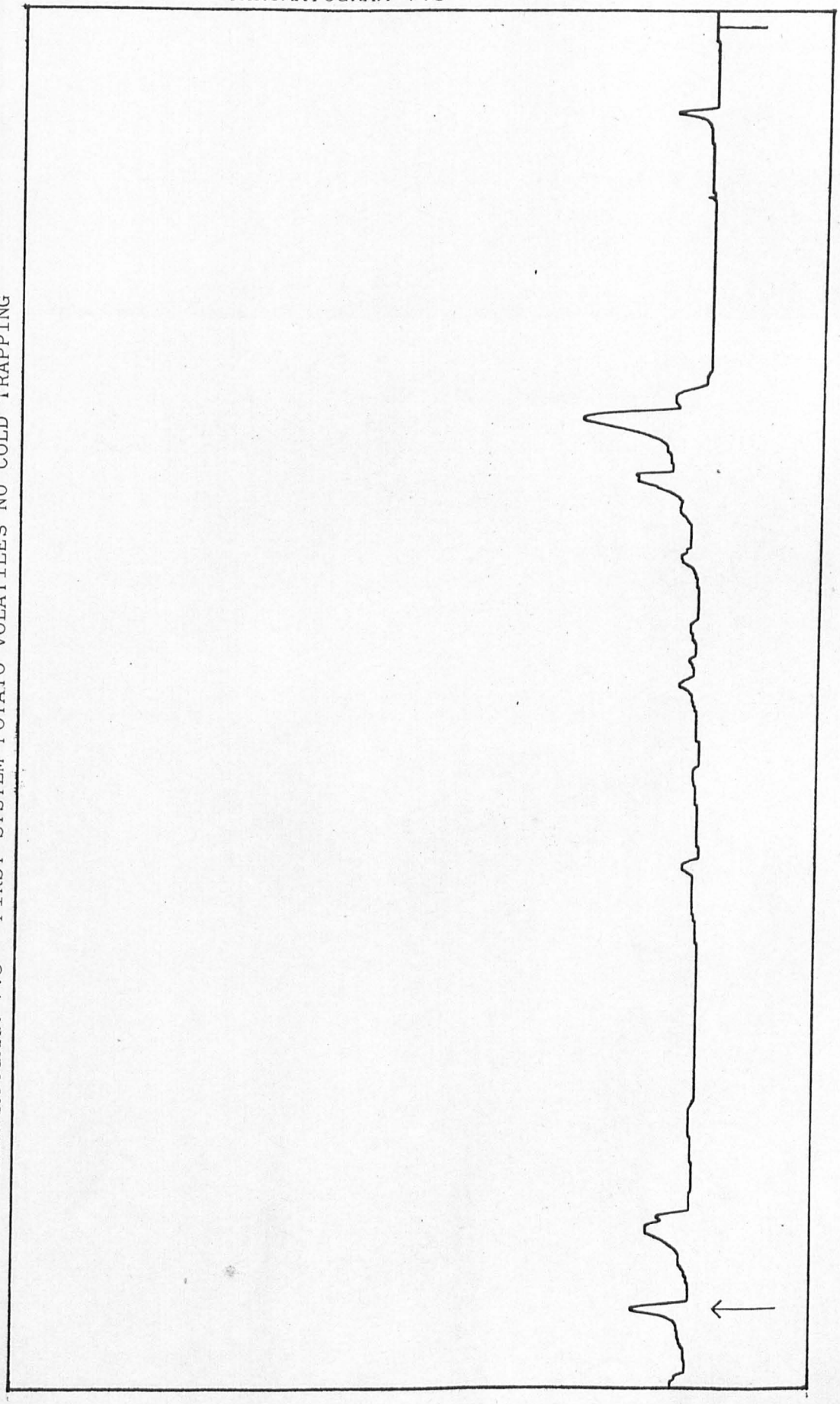


CHROMATOGRAM 7.2 FIRST SYSTEM POTATO VOLATILES WITH COLD TRAP



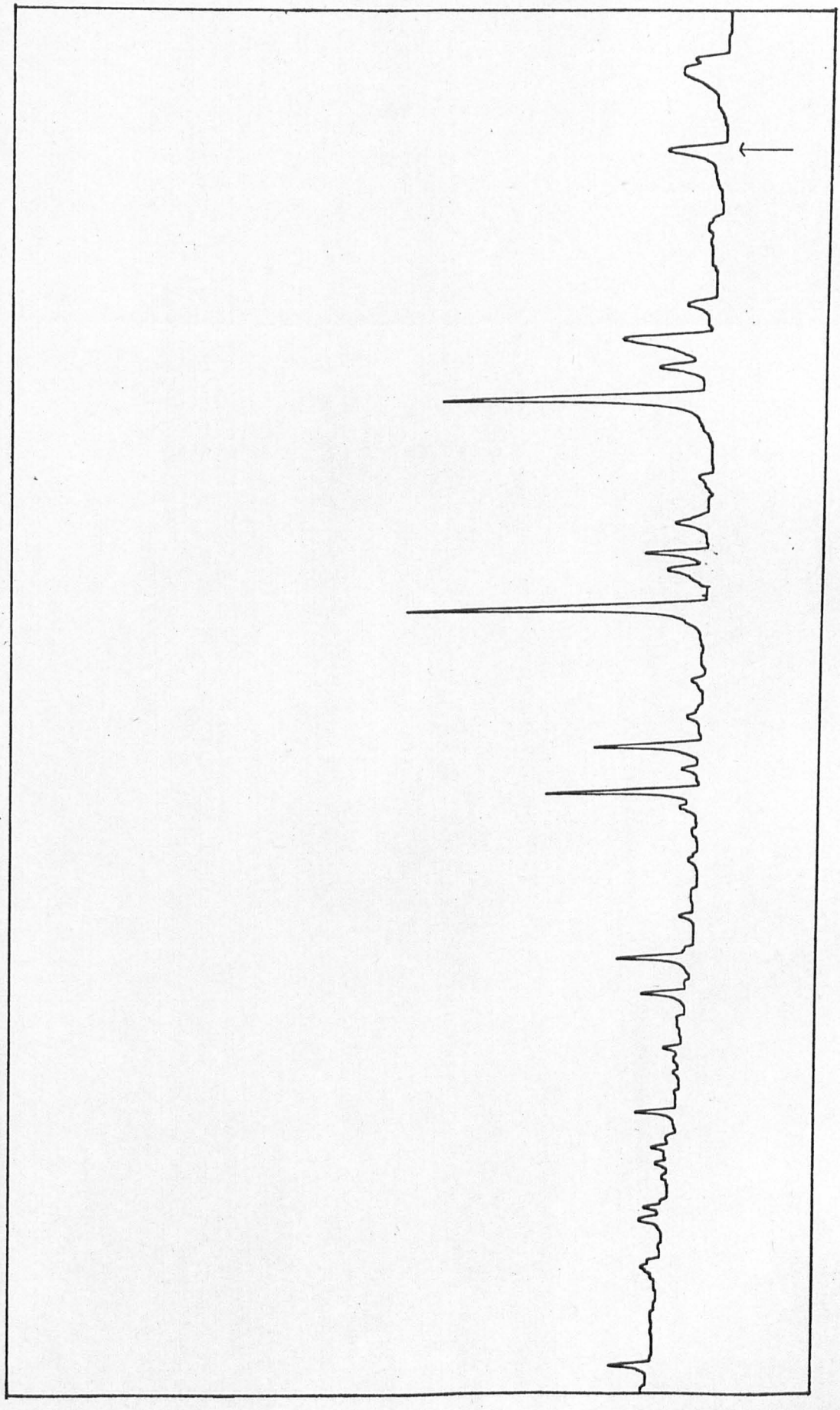
CHROMATOGRAM 7.2 (CONTINUED)

CHROMATOGRAM 7.3



CHROMATOGRAM 7.3 FIRST SYSTEM POTATO VOLATILES NO COLD TRAPPING

CHROMATOGRAM 7.3 (CONTINUED)



CHROMATOGRAM 7.3 (CONTINUED)

Therefore, thin filmed silica capillary columns were not thought to have suitable sample capacities for this form of analysis, and this particular method of on-column cold trapping, System 1, was abandoned.

7.2.2 System 2

The results of analysis System 1 showed that the principle of on-column cold trapping was successful, the major drawback was the low sample capacity of the capillary column used. The second system again employed cold trapping the potato volatiles on the top portion of the capillary column. However, a glass S.C.O.T. capillary column was used as these columns have higher sample capacities than thin filmed silica columns and are less prone to damage by sample overload.

The desorption block and heated switching valve were used unaltered and as described in System 1, the heated switching valve was mounted on top of the G.C. with the outlet inserted through the injection port, the heating block was positioned adjacent to the heated valve enclosure. A glass S.C.O.T. capillary column was connected to the outlet from the switching valve. A copper sleeve T piece 20 mm long was positioned round the top of the capillary column, cold $N_2$, $\nu$-150°C, was passed through the other arm of the T piece, this served as the cold trap. A flow diagram of the gas supplies to the precolumn and switching valve is illustrated in Figure 7.4.

The apparatus was operated as follows:

1. The valve was set to Position 1, and the precolumn was connected to the valve at the side of the valve enclosure with the $^1/4$ inch/$^1/16$ inch reducing union. The injection port head was connected to the other end of the precolumn, the precolumn was then flushed with carrier gas for 5 min.

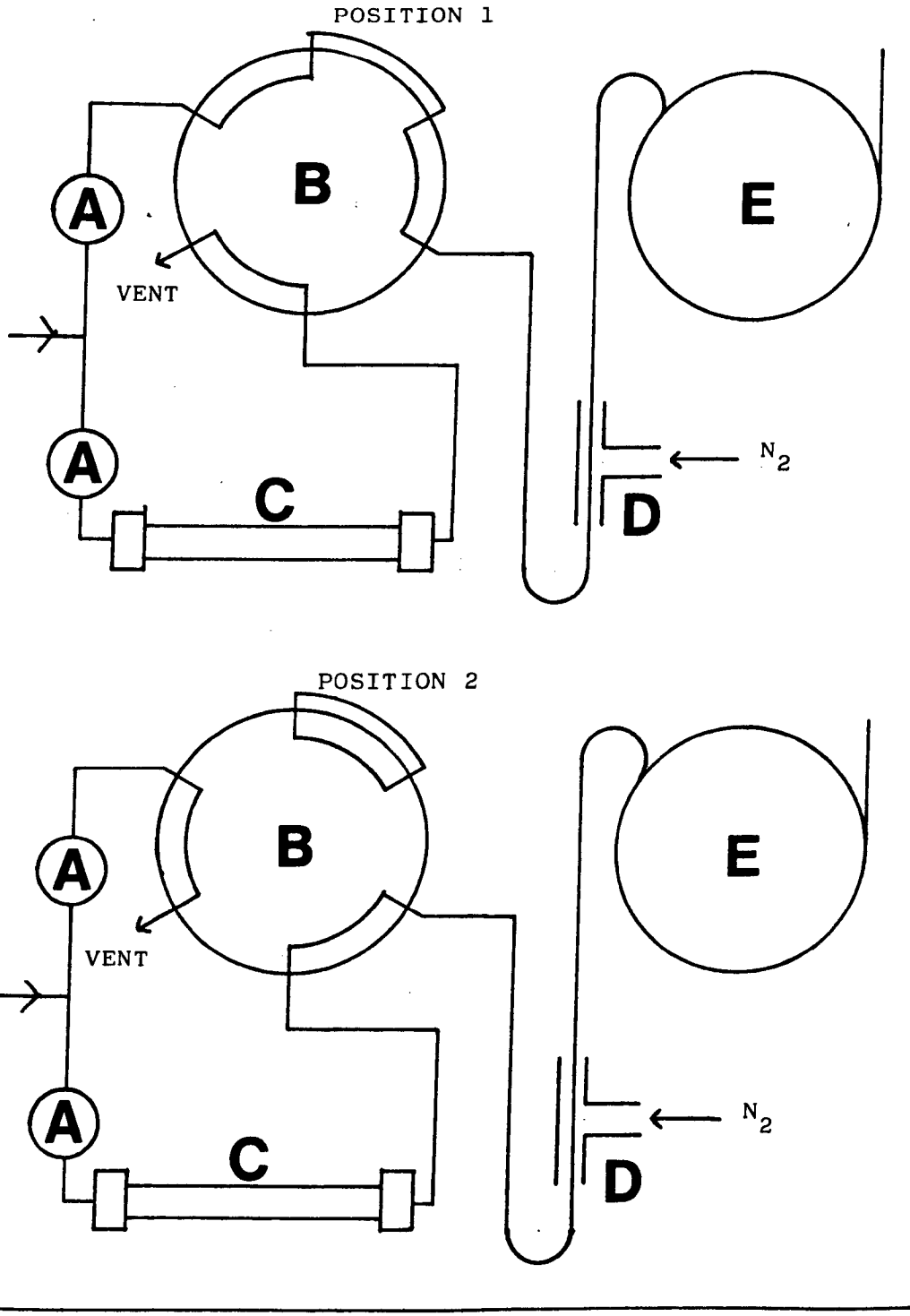
2. The cold trap sleeve was then cooled by passing cold $N_2$, ~-150°C, into the side of the copper sleeve. The $N_2$ was cooled by passing $N_2$ at 50 cc $min^{-1}$ through a 5 m 1/8 inch O.D. copper coil immersed in liquid $N_2$.
3. The valve was then switched to Position 2, the heating block was placed round the precolumn and the precolumn was desorbed for 10 min - 15 min. The volatiles were routed through the switching valve and trapped on the top section of the capillary column cooled by the copper sleeve cold trap. The carrier gas flow rate during desorption was 2 cc $min^{-1}$ - 4 cc $min^{-1}$.
4. After the desorption period, the flow of cooled $N_2$ to the copper sleeve cold trap was stopped and the cold trap allowed to heat to oven temperature. The temperature programme was started at the same time as the flow of cooled $N_2$ to the trap was stopped.

Gas Chromatography

| | | |
|---|---|---|
| G.C.: | | Pye 104 (Pye Unicam Ltd). |
| Column: | | OV17 S.C.O.T. glass capillary column, 73 metre, 1 mm O.D., 0.5 mm I.D. (S.G.E., U.K. Ltd). |
| Carrier Gas: | | $H_2$ u = 20 cm $sec^{-1}$. |
| Temperature: | Initial | 100°C 10 min. |
| | Rise | 2°C $min^{-1}$. |
| | Hold | 200°C. |
| | Detector | 270°C. |
| | Injector | 230°C. |
| Detector: F.I.D. | | $H_2$ 20 cc $min^{-1}$. |
| | | $O_2$ 200 cc $min^{-1}$. |
| | | $N_2$ 15 cc $min^{-1}$. |

FIGURE 7.4
FLOW DIAGRAM SYSTEM 2

A FLOW CONTROLLER
B SWITCHING VALVE
C PRECOLUMN
D T-PIECE
E CAPILLARY COLUMN



Sensitivity: $5 \times 10^{-10}$ A.

Chart Speed: 5 mm $min^{-1}$.

When this system was assessed, there was no potato volatile collection method in operation, and the assessment was based upon a mixture of dimethylnaphthalene isomers. The dimethylnaphthalenes were chosen as test compounds as they have been shown to be physiologically important potato volatiles (Meigh, D.F., et al, 1973), and it was considered to be important that the system was able to resolve these compounds.

A comparison was made of the resolution of the dimethylnaphthalene isomers using three different injection methods, which were as follows:

A stock solution of a mixture of dimethylnaphthalene isomers (Aldrich Chemical Co. Ltd, Gillingham, England), was made up in glass distilled hexane (Rathburn Chemicals Ltd) at a concentration of 1000 ug $cc^{-1}$.

1. A 0.5 $mm^3$ aliquot of the stock solution was injected onto a freshly conditioned precolumn and desorbed as described, Chromatogram 7.4.
2. A 0.5 $mm^3$ aliquot of the stock solution was injected onto a freshly conditioned precolumn and desorbed as described, except the cold trap was not used, the dimethylnaphthalene isomers were desorbed directly onto the capillary column without intermediate cold trapping, Chromatogram 7.5.
3. A 0.5 $mm^3$ aliquot of the stock solution was injected directly onto the capillary column using a splittless

injector ((Model SCl-Bk) S.G.E., U.K. Ltd) as a comparison to the desorption modes of injection, Chromatogram 7.6.

The separation and peak shape obtained with the desorption modes of injection were poor in comparison to a direct injection of the dimethylnaphthalene isomers onto the column, using the splittless injector. The dimethylnaphthalenes were less well resolved and subject to increased tailing, and no benefits were achieved by the use of intermediate on-column cold trapping, the only effect being an increase in the RT of the dimethylnaphthalenes due to cooling of the oven caused by the $N_2$ cold trap coolant. The lack of success of cold trapping step was because of the high thermal mass of the cold trap sleeve. After the cold trap sleeve had been cooled it took 1 min - 3 min for the sleeve and the cooled section of the capillary column to reach the oven temperature of 100°C. This resulted in the sample being introduced onto the capillary column in a large sample volume, the effect was the same as the direct desorption of the volatiles from the precolumn. This problem was not encountered with the flexible silica capillary column as used in System 1, as the length of column was short and of a low thermal mass, and therefore the rate of reheat was not a limiting factor.

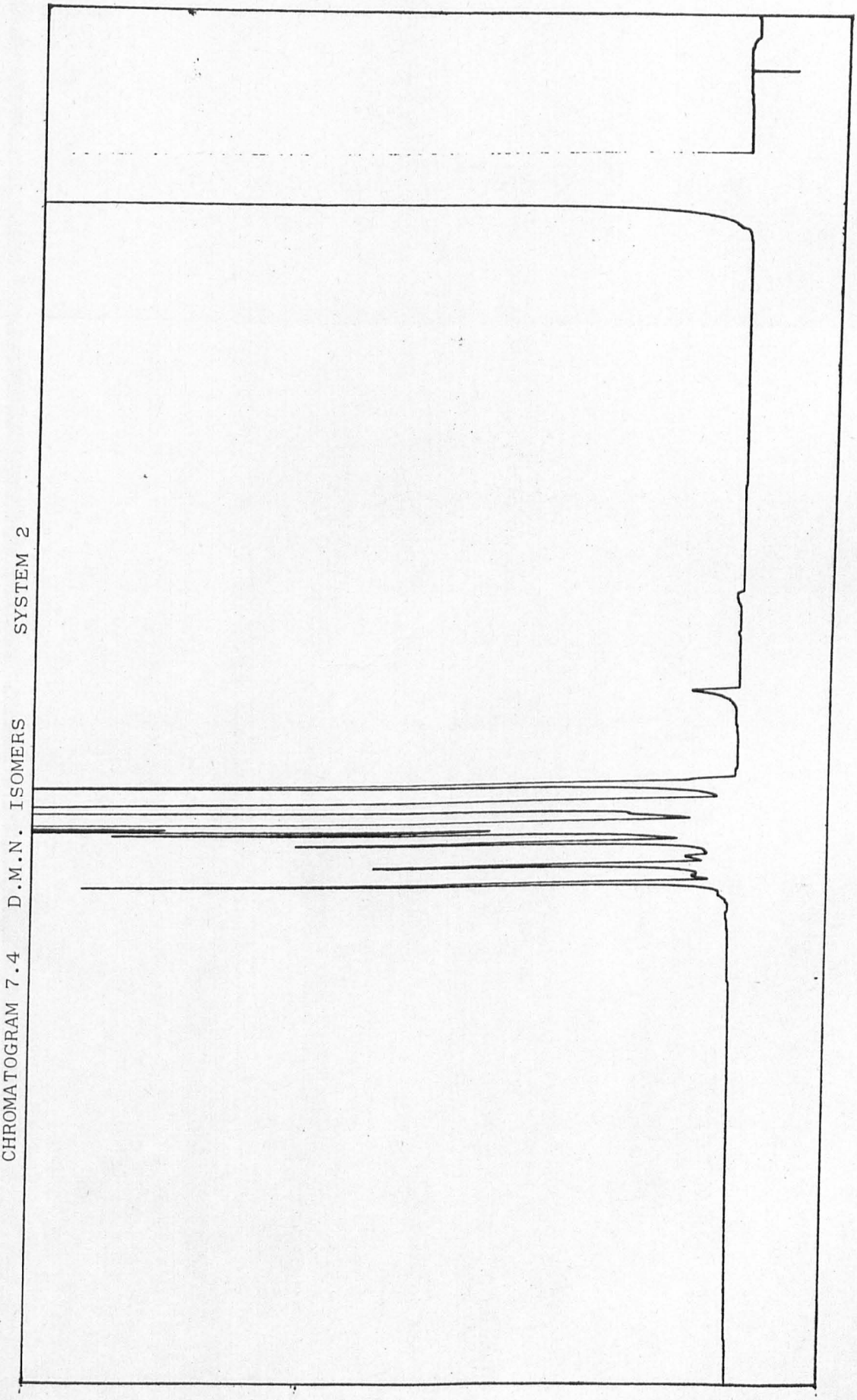
An attempt was made at reheating the cold trap with a heated air flow, by passing air through a heated transfer line (Perkin Elmer Ltd, Beaconsfield, England). However, this proved unreliable, the temperature of the air stream could not be reliably controlled, at times the air stream

was hot enough to damage the column phase, and at times it was ineffective at reheating the cold trap. Further to this, the air stream increased the oven temperature, altering the temperature programme and therefore the retention times of the compounds to be separated.

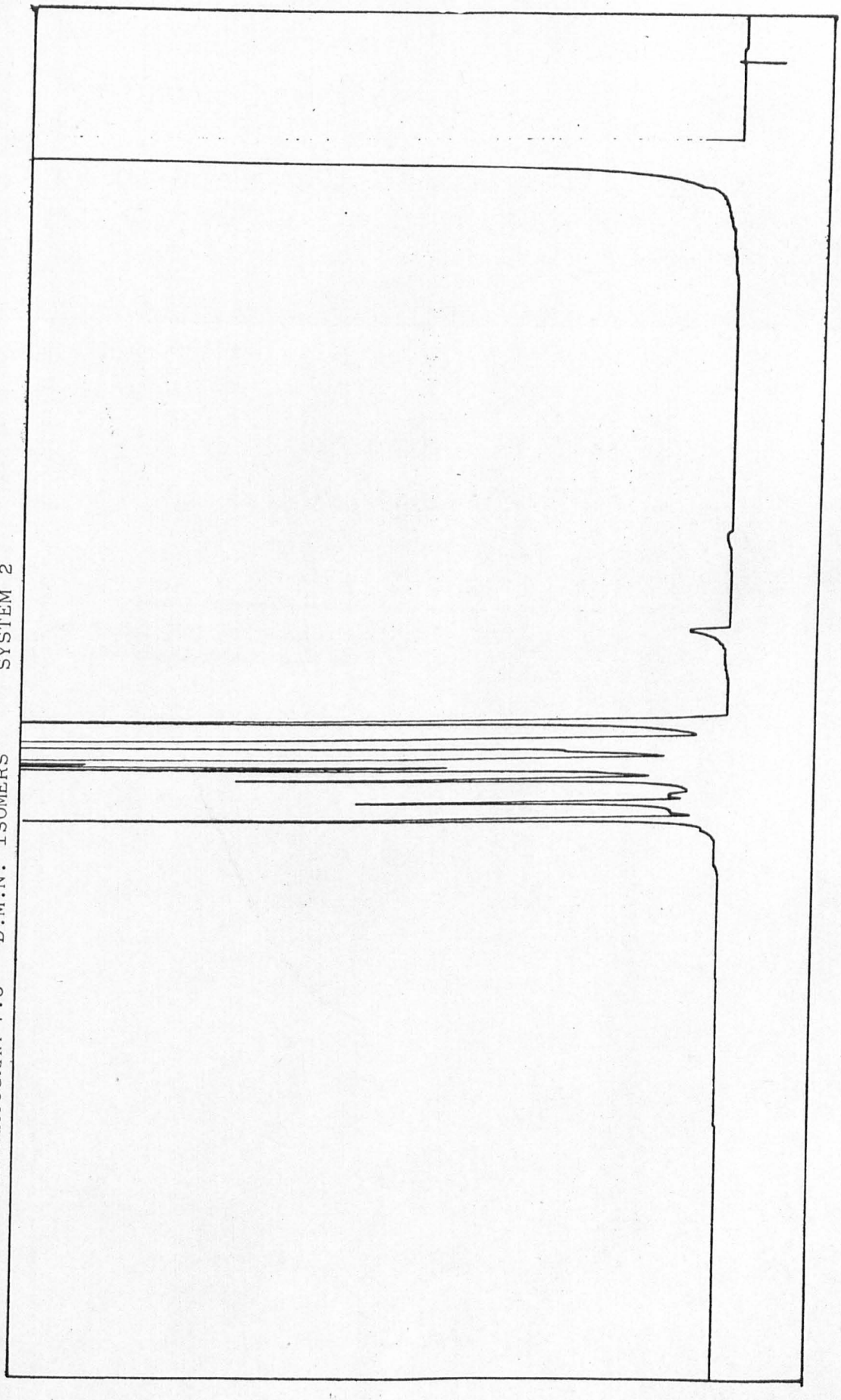
From these results it was obvious that this particular method of on-column cold trapping was unsatisfactory, no benefits were achieved. However, it was concluded that in addition to thermally focussing the volatiles in a cold trap, the trapped volatiles had to be quickly and efficiently vapourised in the cold trap before introduction onto a capillary column, and that these operations should not interfere with their subsequent chromatographic separation.

Therefore this method was abandoned in favour of System 3, where the volatiles were desorbed, cold trapped and reheated external to the G.C. oven.

CHROMATOGRAM 7.4

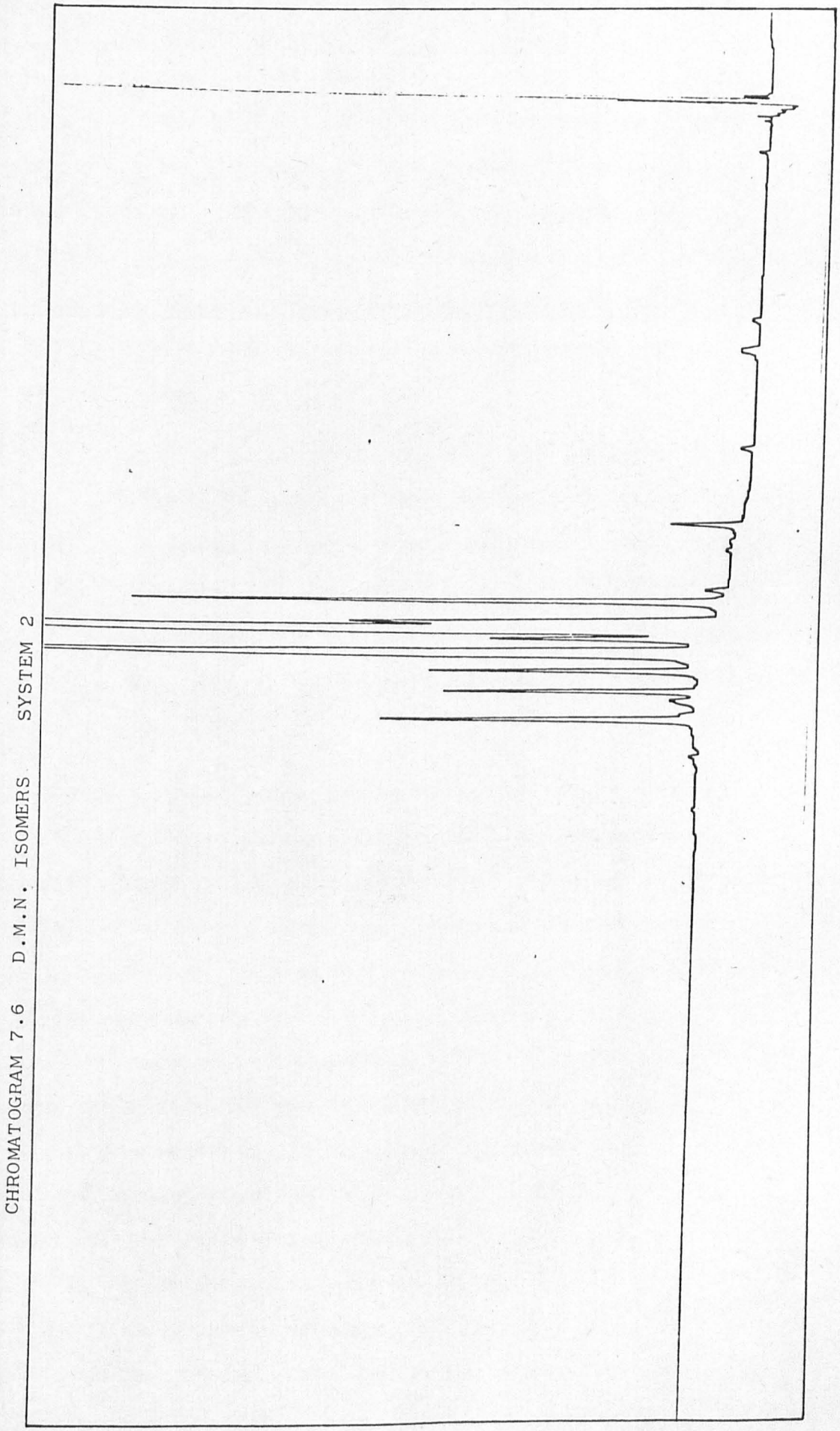


CHROMATOGRAM 7.4 D.M.N. ISOMERS SYSTEM 2



CHROMATOGRAM 7.5 D.M.N. ISOMERS SYSTEM 2

CHROMATOGRAM 7.6



CHROMATOGRAM 7.6 D.M.N. ISOMERS SYSTEM 2

7.2.3 System 3

The results of System 2 showed that although the potato volatiles could be successfully desorbed from the pre-column and concentrated in a small volume cold trap, it was important that the cold trap was quickly and efficiently reheated to ensure the rapid vapourisation of the volatiles before their successful resolution on a capillary column.

The principle of System 3 was to trap the desorbed volatiles in a small volume cold trap external to the G.C. oven, and then to reheat the cold trap ohmically. This method had the advantage that the desorption, cold trapping and reheat were external to the G.C. oven which could be maintained at any desired temperature and would not be affected by these operations.

The desorption block and heated switching valve were as described previously, except that both were mounted on a maranite plate at the side of the G.C. The outlet from the heated switching valve was connected to a 300 mm length of $^{1}/_{16}$ inch O.D., 0.4 mm I.D. glass lined stainless steel tubing with a low volume $^{1}/_{16}$ inch coupling (S.G.E., U.K. ltd), the other end of the glass lined stainless steel tubing was passed through the side of the G.C. into the oven and connected to the capillary column with a $^{1}/_{16}$ inch zero dead volume coupling (S.G.E., U.K. Ltd). The middle section of the glass lined stainless steel tubing was formed into a U 100 mm long, this served as the cold trap. Three electrical connections were made to the cold trap, the first just below the heated valve enclosure, the second at the top

of the cold trap U, the third at the union between the capillary column and the glass lined tubing of the cold trap inside the G.C. oven. This meant that the point from the bottom of the valve enclosure to the top of the cold trap U, and the point from the top of the cold trap U to the capillary column could be heated ohmically by passing a low voltage between these connections, and therefore the volatiles were heated throughout their passage from the precolumn to the capillary column.

A flow diagram of the gas connections between the precolumn, heated switching valve, cold trap and the capillary column is illustrated in Figure 7.5. The complete assembly is shown in Plates 10 and 11.

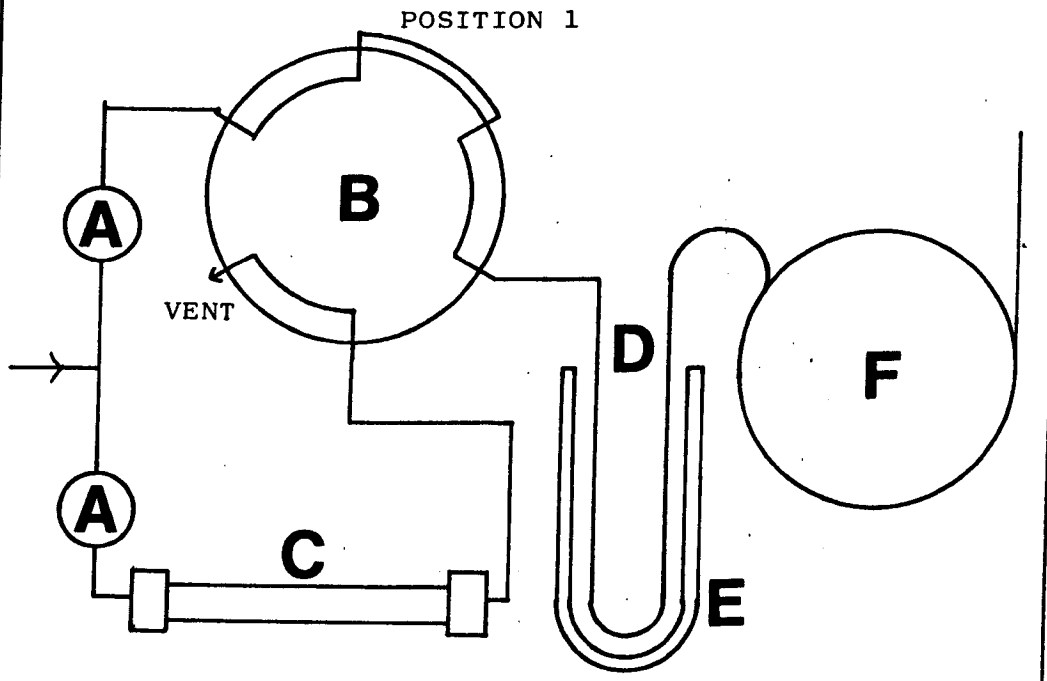
Gas Chromatography

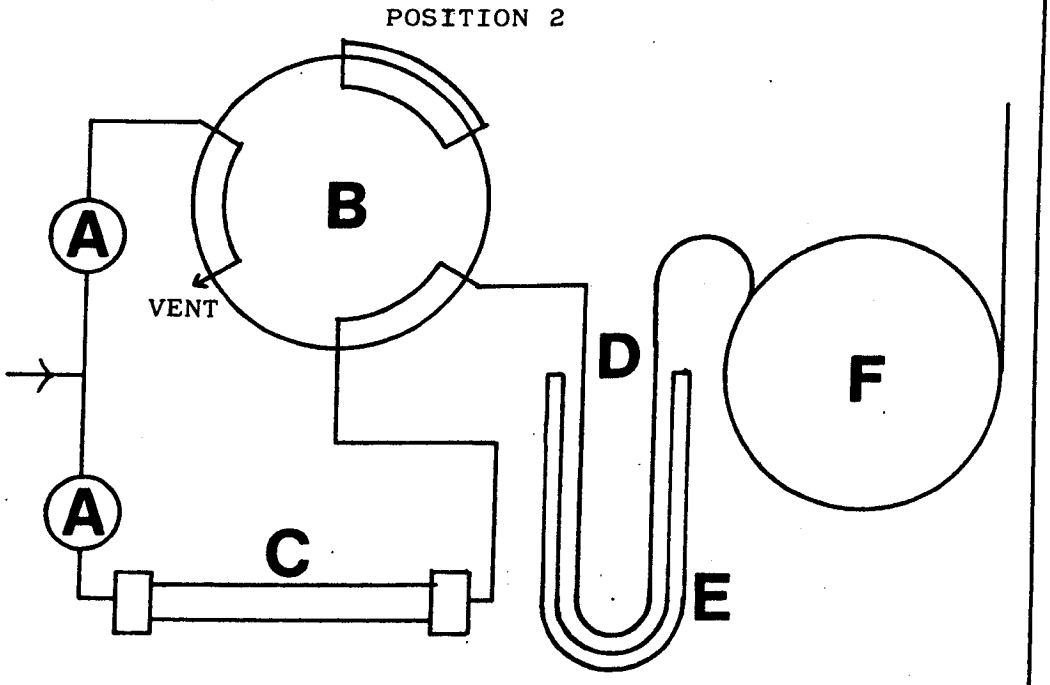
| | | |
|---|---|---|
| G.C.: | | Pye 104 (Pye Unicam Ltd). |
| Column: | | SP2100 S.C.O.T. glass capillary 26 m, 0.5 mm I.D., 1 mm O.D. (S.G.E., U.K. Ltd). |
| Carrier Gas: | | $H_2$ u = 20 cm $sec^{-1}$. |
| Temperature: | | |
| Programme A - | Initial | 50°C 10 min. |
| | Rise | 5°C $min^{-1}$. |
| | Hold | 185°C 20 min. |
| Programme B - | Initial | 100°C 5 min. |
| | Rise | 5°C $min^{-1}$. |
| | Hold | 220°C 20 min. |
| Detector: F.I.D. | | 270°C. |
| Injector: | | Off. |

FIGURE 7.5

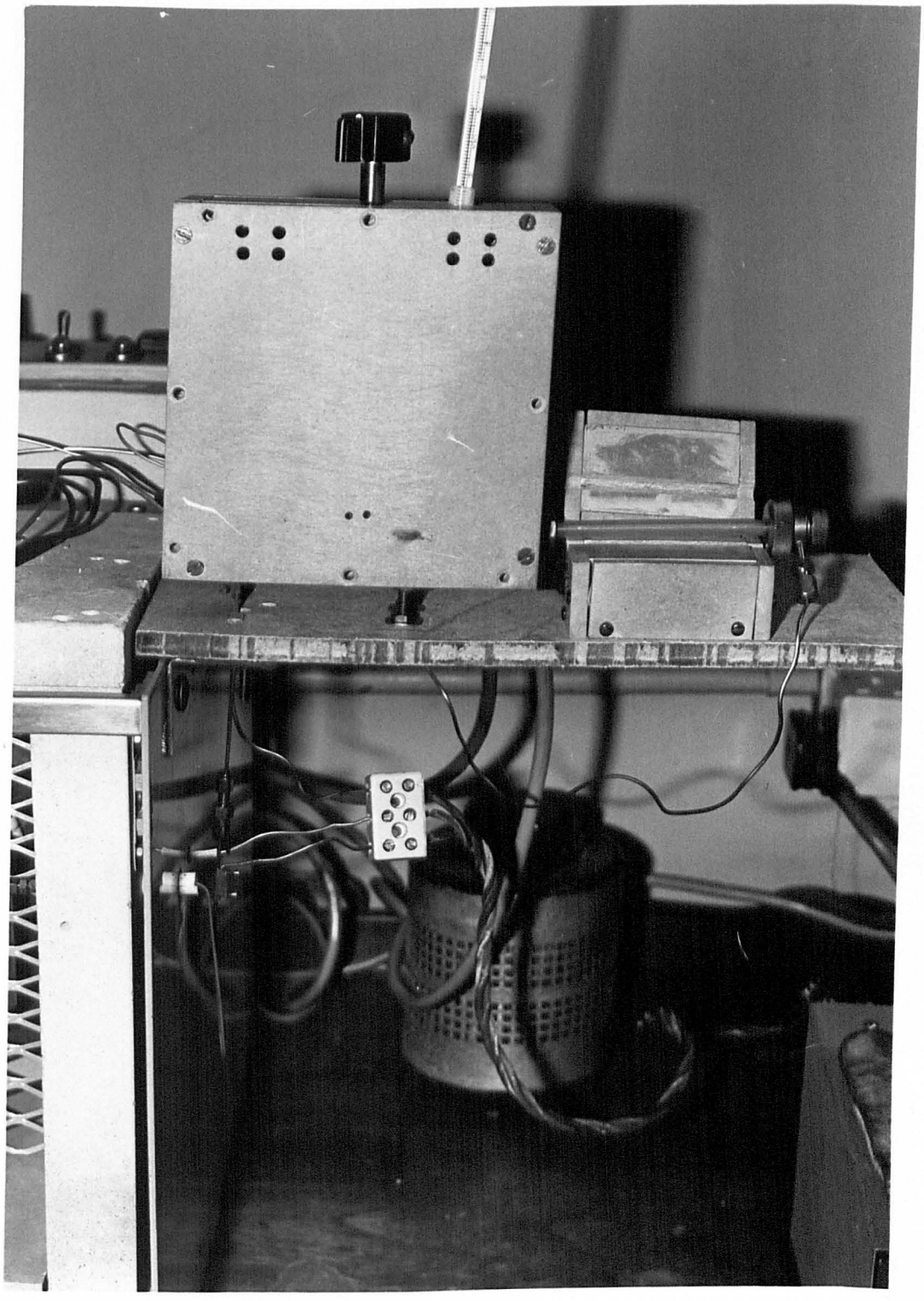
FLOW DIAGRAM SYSTEM 3

| | | | |
|---|---|---|---|
| A | FLOW CONTROLLER | D | GLT COLD TRAP |
| B | SWITCHING VALVE | E | DEWAR FLASK |
| C | PRECOLUMN | F | CAPILLARY COLUMN |

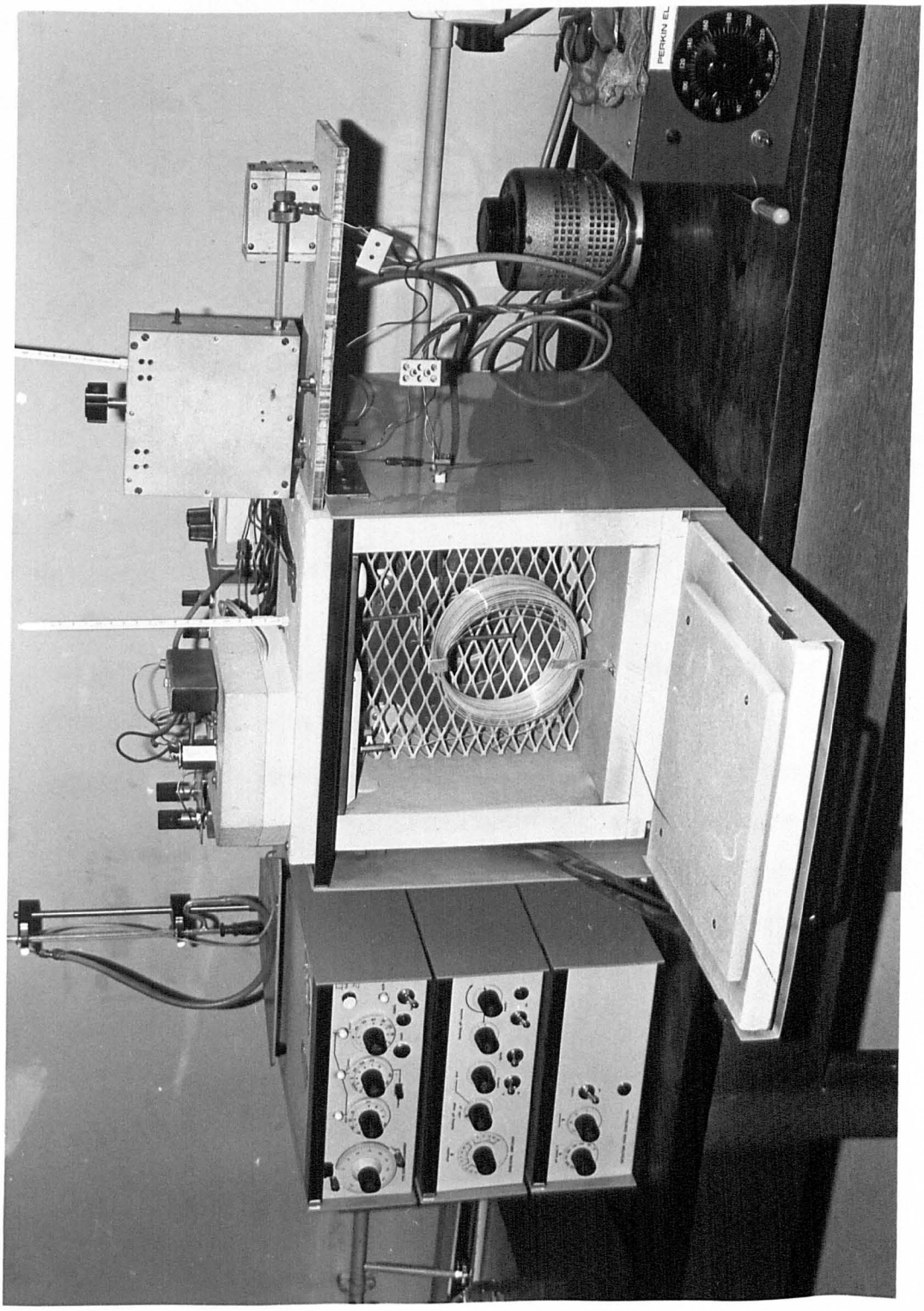


# PLATE 10



# PLATE 11



| | |
|---|---|
| Detector: F.I.D. | $H_2$ 20 cc $min^{-1}$. |
| | $O_2$ 200 cc $min^{-1}$. |
| | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | $5 \times 10^{-10}$ A. |
| Chart Speed: | 5 mm $min^{-1}$. |

The apparatus was operated as follows:

1. The valve was set to Position 1, and the precolumn was connected to the side of the heated valve enclosure with the $^1/_4$ inch/$^1/_{16}$ inch reducing union, the injection port head was connected to the other end of the precolumn. The precolumn was then flushed with carrier gas for 5 min.
2. The U of the glass lined tubing cold trap was immersed in liquid $N_2$ and allowed to cool for 2 min. The point between the bottom of the valve enclosure and the top of the cold trap was heated to 200°C with a low voltage, supplied by a 12V, 15A transformer (Perkin Elmer Ltd, Beaconsfield, England).
3. The valve was then switched to Position 2, the heating block placed round the precolumn and the precolumn was desorbed for 10 min - 15 min. The volatiles were routed through the switching valve into the glass lined tubing cold trap. The carrier gas flow rate during desorption was 2 cc $min^{-1}$ - 4 cc $min^{-1}$.
4. After the desorption period, the point between the top of the cold trap and the inlet to the capillary column was heated with a low voltage at the same time as the liquid $N_2$ coolant was removed from the cold trap. The trapped volatiles were vapourised and flushed into the capillary column, and the temperature programme of the column oven was started.

It was found that ohimic heating of the cold trap was a very effective means of vapourising the volatiles before they entered the capillary column. In operation the cold trap was heated from -190°C to +200°C - +220°C in 3 sec - 4 sec, this ensured almost instantaneous vapourisation of the volatiles. The volume of the cold trap was 38 $mm^3$ so that at the flow rates used, 2 cc $min^{-1}$ - 4 cc $min^{-1}$, the trap was purged with carrier gas 80 times per second.

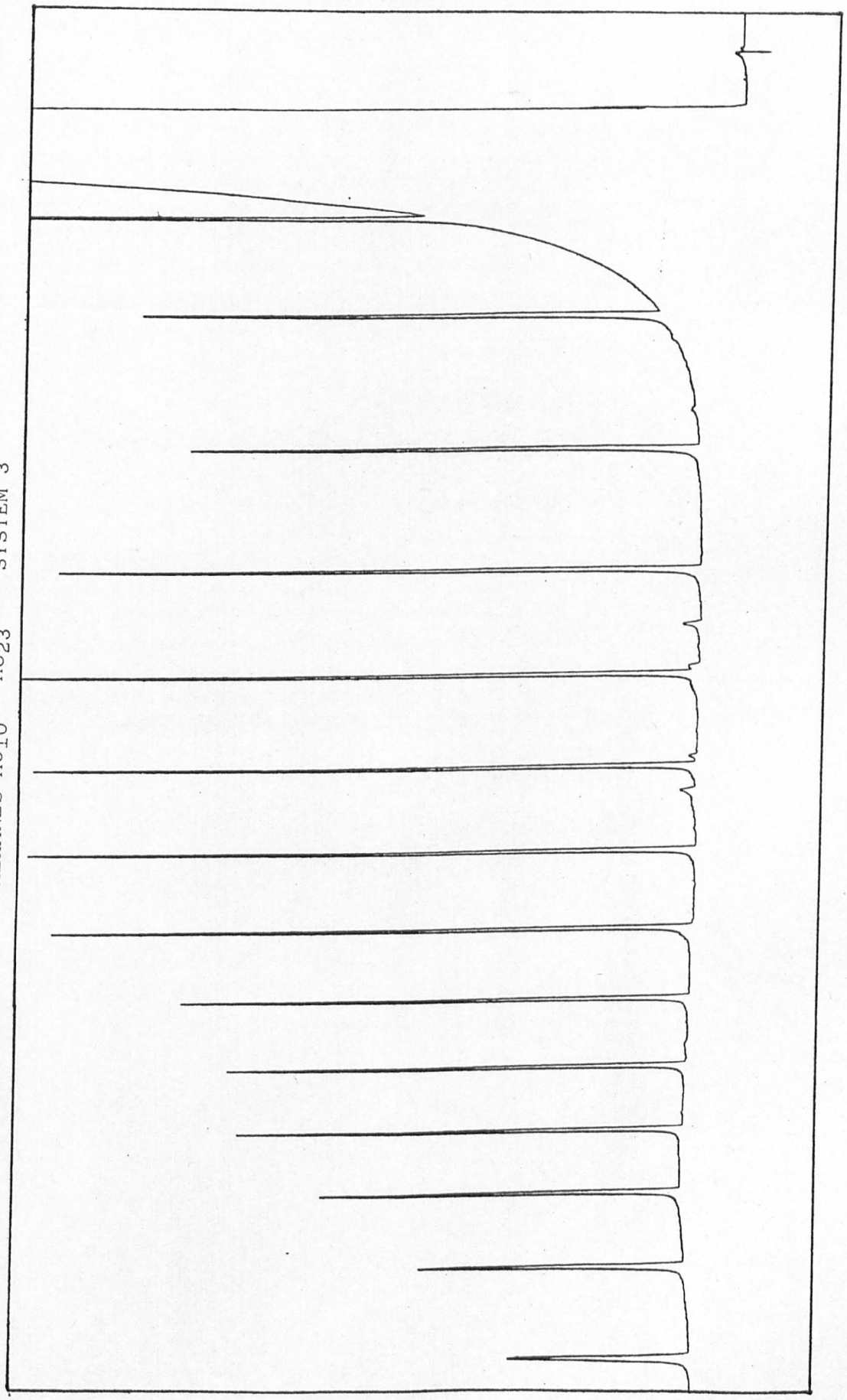
A blank run was performed on the system by desorbing a freshly conditioned precolumn using the procedure as described, and using temperature Programme A, Chromatogram 7.7, the blank run showed that apart from the air peak, there were no background volatiles contributed by the system. The system was also assessed using a series of n-alkanes, a stock solution of n-alkanes $nC_{10}$ - $nC_{24}$ (Phase Separations Ltd) were made up in glass distilled hexane (Rathburn Chemicals Ltd) each at a concentration of 100 ug $cc^{-1}$, a 0.5 $mm^3$ aliquot of this stock solution was injected onto a freshly conditioned precolumn. The precolumn was then desorbed as described, Chromatogram 7.8, this run showed that the n-alkanes were well resolved, with no tailing and that the higher n-alkanes were introduced into the capillary column, this run was made using temperature Programme B. Samples of Cyprus potato headspace volatiles obtained using the All Glass system, Chapter 5, were analysed using programme A, Chromatogram 7.9 shows a typical trace obtained. The potato volatiles were well resolved, for the column used, the peaks were sharp with no

CHROMATOGRAM 7.7 BLANK RUN ON SYSTEM 3

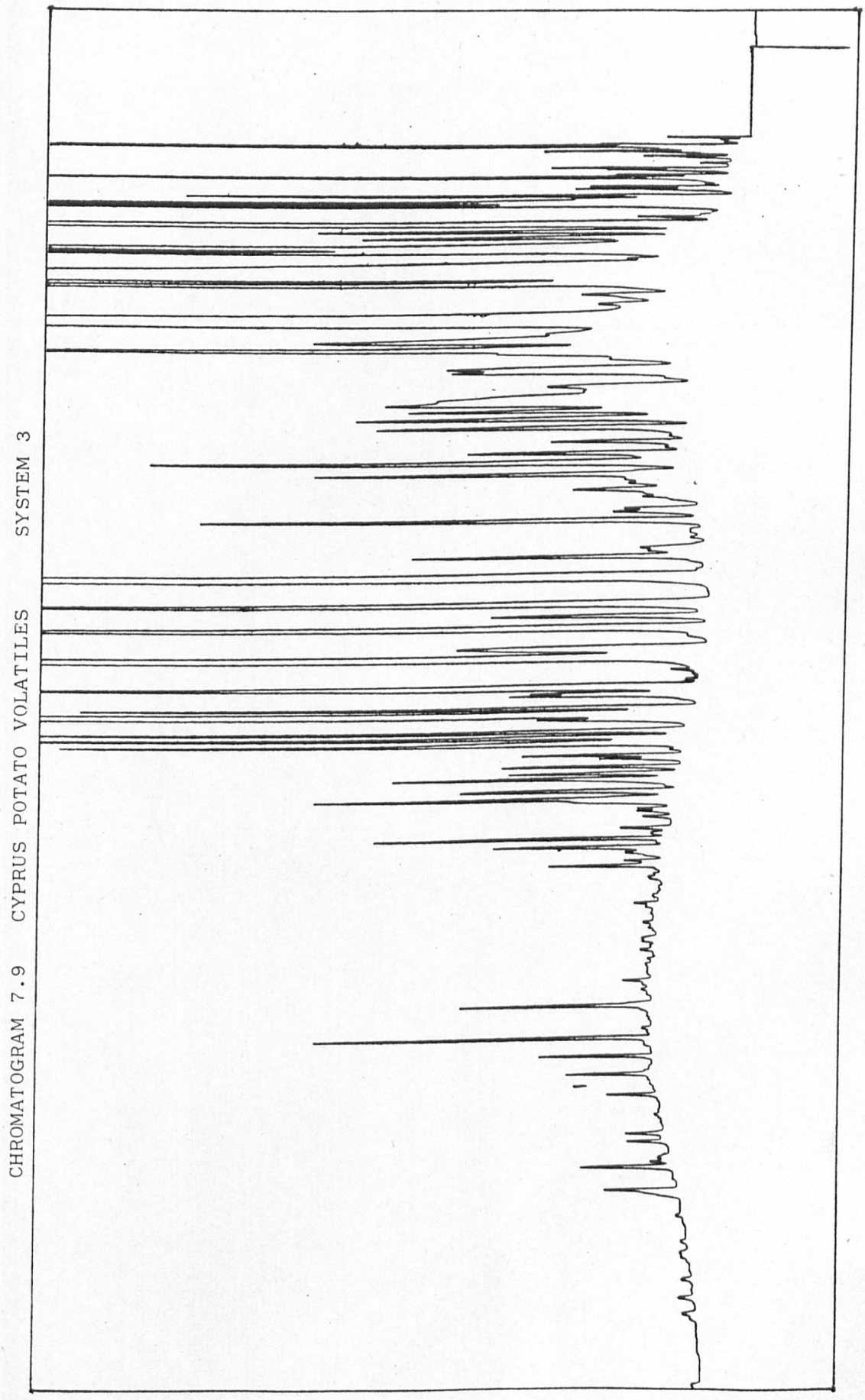


CHROMATOGRAM 7.7

CHROMATOGRAM 7.8



CHROMATOGRAM 7.8 n-ALKANES $nC_{10}$ - $nC_{23}$ SYSTEM 3


CHROMATOGRAM 7.9 CYPRUS POTATO VOLATILES SYSTEM 3

appreciable tailing, indicating that the desorption system and column did not have excessive dead volumes, active sites or cold zones.

It was concluded that this procedure for desorbing potato volatiles into a capillary column was satisfactory, System 3 was the analysis procedure adopted for most of the potato volatile analyses in this project.

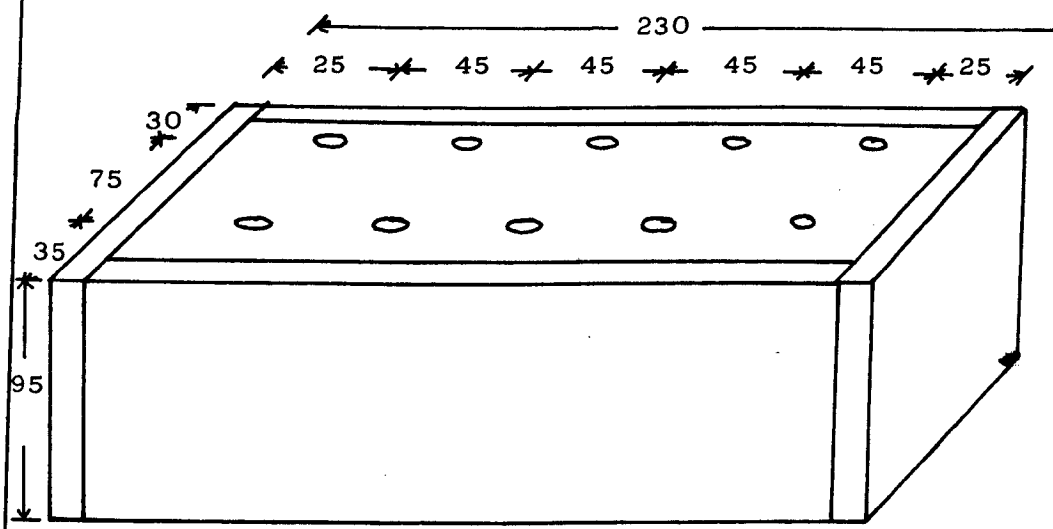
### 7.2.4 Precolumn Conditioning

The precolumns were purified by heating under a $N_2$ flow, this effectively removed any sorbed volatiles from the precolumn, the procedure was described as conditioning.

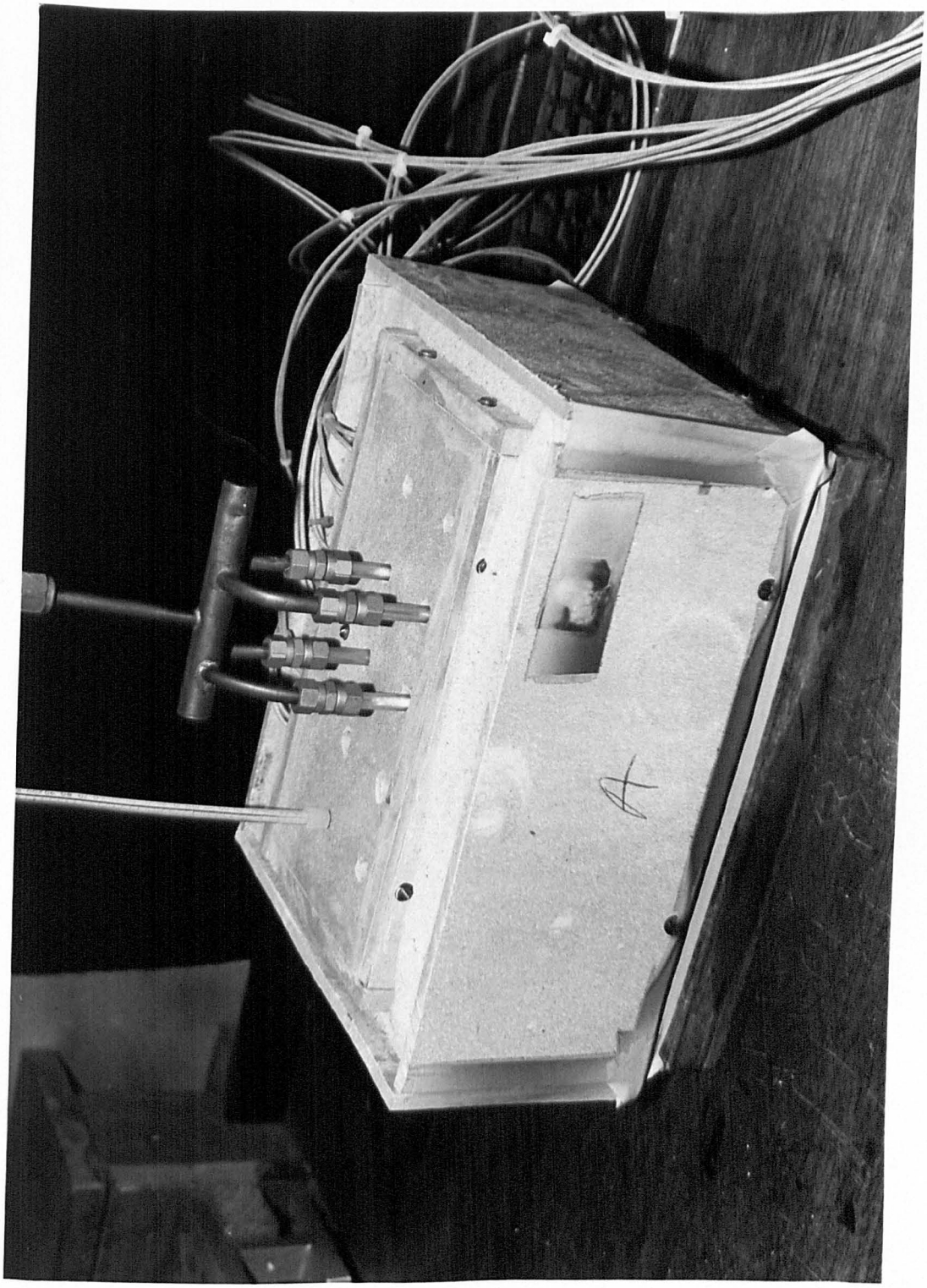
For the reasons outlined in the introduction to Chapter 3, thermal conditioning was chosen as the best means of purifying the precolumns, it proved to be a simple and effective method. The precolumns were conditioned by heating under a flow of $N_2$ at 20 cc $min^{-1}$ - 30 cc $min^{-1}$ at a temperature of 320°C for a minimum of 2 hr. An aluminium heating block was constructed for the purpose of heating the precolumns, the block was designed so that ten precolumns could be conditioned simultaneously.
The heating block was constructed as follows:
A block of aluminium 200 mm long, 65 mm deep, 120 mm wide was drilled to take six 150W cartridge heaters (Hedin Ltd), the block was drilled with ten equally spaced 7 mm diameter holes into which the precolumns were inserted. The whole block was surrounded with $^3/_8$ inch maranite heat insulation. A diagram of the heating block, Figure 7.6, shows the dimensions of the heating block. The heating block was placed in an asbestos lined box and surrounded by sand to provide further thermal insulation. When operational, the heating block was maintained at a temperature of 310°C - 330°C.
A manifold was constructed from ¼ inch O.D. copper tubing (Alltech Associates) to enable four precolumns to be purged with $N_2$ during conditioning, the precolumns were connected to the manifold with ¼ inch couplings ((Dralim, Phase Separations Ltd). The heating block with four precolumns and the manifold is shown in Plate 12.

FIGURE 7.6
CONDITIONING BLOCK

ALL DIMENSIONS IN MILLIMETRES



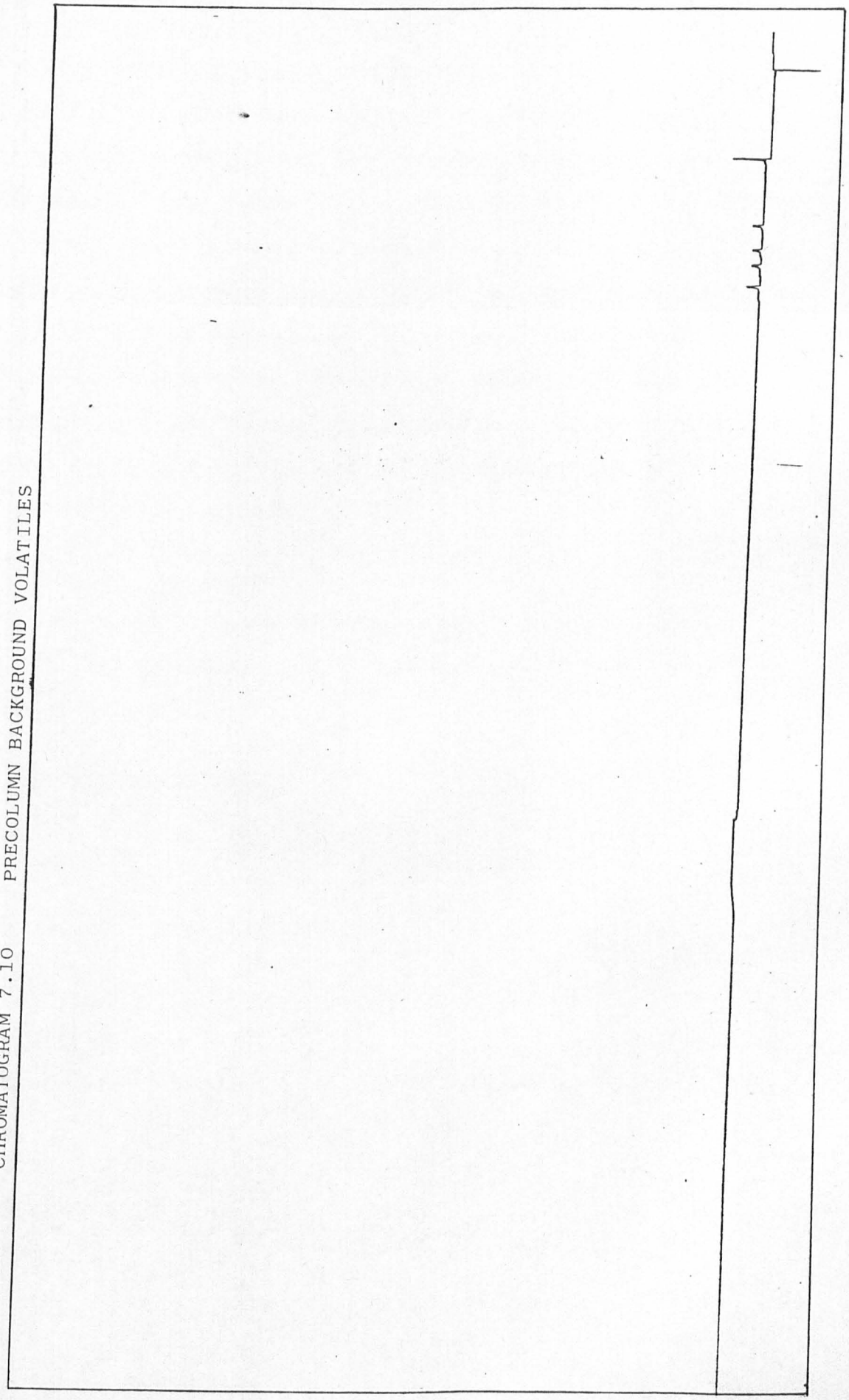
# PLATE 12



The following procedure was adopted for conditioning the precolumns:

1. Four precolumns were coupled to the gas manifold and purged with $N_2$ at 20 cc $min^{-1}$ - 30 cc $min^{-1}$ for 1 min.
2. The precolumns were then inserted into the heating block and heated for a minimum period of 2 hr.
3. The precolumns were then removed from the heating block and allowed to cool to room temperature under the $N_2$ gas flow.
4. The precolumns were disconnected from the manifold and sealed with PTFE caps (Jencons Scientific Ltd) and stored till used. The PTFE caps were made from 30 mm lengths of $^3/_8$ inch O.D. PTFE rod drilled to a depth of 20 mm with a 6 mm drill.

Chromatogram 7.10, shows the trace obtained from a freshly conditioned precolumn analysed using the G.C. conditions described in System 3 of this chapter. There were little background volatiles apart from the air peak. It was found that the precolumns could be kept in the same state of purity as shown in Chromatogram 7.10 for a period of up to seven days when sealed with PTFE caps and stored at 20°C. However, most of the precolumns were used for sampling within 4 hr of conditioning, and the sampled precolumns were analysed within 4 hr. This reduced the possibility of background volatiles being accummulated on the precolumn before and after sampling and the loss of sampled volatiles before analysis.



CHROMATOGRAM 7.10 PRECOLUMN BACKGROUND VOLATILES

### 7.2.5 Retention Time Reproducibility

At this point in the project it was apparent that access to G.C.M.S. facilities for the identification of potato volatiles was limited. It was thought that the identification of potato volatiles would have to be on a tentative basis by the comparison of retention time (RT) or retention index (RI) of the unknown volatiles to the RT or RI of authentic standards. Therefore, before this was undertaken, the gas chromatographic analysis system was assessed for the reproducibility of the retention times of a series of n-alkane standards.

A standard solution of n-alkanes, $nC_{10}$ - $nC_{24}$ (Phase Separations Ltd) was made up in glass distilled hexane, (Rathburn Chemicals Ltd), each n-alkane at a concentration of 100 ug $cc^{-1}$.

Gas Chromatography

| | | |
|---|---|---|
| G.C.: | | Pye 104 (Pye Unicam Ltd). |
| Column: | | SP2100 glass S.C.O.T. 26 m, 0.5 mm I.D., 1 mm O.D. (S.G.E., U.K. Ltd). |
| Carrier Gas: | | $H_2$ u = 20 cm $sec^{-1}$. |
| Temperature: | | |
| Column: | Initial | 100°C 10 min. |
| | Rise | 4°C $min^{-1}$. |
| | Final | 220°C 30 min. |
| Detector: | F.I.D. | 270°C. |
| Detector: | F.I.D. | $H_2$ 20 cc $min^{-1}$. |
| | | $O_2$ 200 cc $min^{-1}$. |
| | | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | | 5 x $10^{-10}$ A. |

The n-alkane standard solution was chromatographed as follows:

Aliquots, 0.5 $mm^3$, of the standard solution were injected onto a freshly conditioned precolumn with a 1 $mm^3$ syringe ((700 series, Hamilton) Phase Separations Ltd). After injection, the precolumn was sealed with PTFE caps and allowed to equilibrate for 30 min. The precolumns were then desorbed according to the method described in this chapter, System 3. The chart recorder was set at a high chart speed, 5 cm $min^{-1}$, to enable the accurate measurement of the n-alkanes retention times. The n-alkane standard solution was run six times, between each run the G.C. oven was allowed to equilibrate at the starting temperature of 100°C for at least 30 min. The results of the six runs are shown in Table 7.1.

The conditions under which the gas chromatographic runs were made, were as far as possible optimum for reproducible retention times. The oven was given sufficient time for equilibration before the start of the run, the temperature rise of 4°C $min^{-1}$ was at a rate which the oven was capable of maintaining and the sample did not overload the column. The coefficient of variation decreased with increasing retention time, the short retention time volatiles were subject to greater variability. The range of retention times for each n-alkane was large, the smallest range was $nC_{10}$ - 24 seconds, the largest $nC_{23}$ - 55 seconds. It was thought that the range of the retention times was too large to justify the identification of an unknown potato volatile solely by the comparison of retention time with an authentic

TABLE 7.1 Retention Times of $nC_{10}$ - $nC_{24}$ n-alkanes

| n-alkane | RETENTION TIME (seconds) 1 | 2 | 3 | 4 | 5 | 6 | $\bar{x}$ | S.D. | C.V. | Range |
|---|---|---|---|---|---|---|---|---|---|---|
| $nC_{10}$ | 343 | 340 | 362 | 364 | 355 | 364 | 355 | 11 | 3.1 | 24 |
| $nC_{11}$ | 532 | 532 | 566 | 578 | 556 | 578 | 557 | 21 | 3.7 | 46 |
| $nC_{12}$ | 832 | 837 | 873 | 880 | 859 | 880 | 860 | 21 | 2.4 | 48 |
| $nC_{13}$ | 1118 | 1125 | 1161 | 1159 | 1140 | 1159 | 1143 | 20 | 1.7 | 43 |
| $nC_{14}$ | 1368 | 1372 | 1404 | 1397 | 1380 | 1399 | 1386 | 15 | 1.1 | 36 |
| $nC_{15}$ | 1579 | 1588 | 1615 | 1605 | 1591 | 1608 | 1598 | 14 | 0.8 | 36 |
| $nC_{16}$ | 1771 | 1778 | 1804 | 1788 | 1778 | 1795 | 1785 | 12 | 0.7 | 33 |
| $nC_{17}$ | 1946 | 1953 | 1977 | 1961 | 1951 | 1968 | 1959 | 12 | 0.6 | 31 |
| $nC_{18}$ | 2109 | 2116 | 2136 | 2124 | 2112 | 2126 | 2120 | 10 | 0.5 | 27 |
| $nC_{19}$ | 2258 | 2268 | 2289 | 2275 | 2265 | 2280 | 2272 | 11 | 0.5 | 31 |
| $nC_{20}$ | 2407 | 2414 | 2436 | 2419 | 2412 | 2424 | 2418 | 10 | 0.4 | 29 |
| $nC_{21}$ | 2548 | 2556 | 2580 | 2561 | 2556 | 2565 | 2561 | 11 | 0.4 | 32 |
| $nC_{22}$ | 2707 | 2721 | 2745 | 2729 | 2726 | 2736 | 2727 | 13 | 0.5 | 38 |
| $nC_{23}$ | 2902 | 2928 | 2957 | 2940 | 2942 | 2947 | 2936 | 19 | 0.6 | 55 |
| $nC_{24}$ | 3185 | 3194 | 3228 | 3209 | 3216 | 3216 | 3208 | 16 | 0.5 | 43 |

standard. The main factor contributing to the poor retention time reproducibility was the oven temperature control and the basic oven design. The Pye 104 oven is of a high thermal mass and therefore not particularly suited to the precise temperature control which is necessary for accurate capillary gas chromatographic analyses. Therefore, the most reliable means of identification of the unknown potato volatiles was by mass spectral identification.

# CHAPTER 8

# COMPARISON OF COLLECTION METHODS

## 8.1 INTRODUCTION

The project at this point had reached the stage where two methods for the collection of potato volatiles had been developed, the All Glass system, Chapter 5, and the Vacuum Distillation system, Chapter 6. A satisfactory system had also been developed for the introduction of the volatiles from a Tenax G.C. precolumn onto a capillary column, System 3, Chapter 7. It was now thought that it would be worthwhile at this stage to compare the profiles of potato volatiles obtained using the All Glass system, the Vacuum Distillation system and from a steam distillation using the Likens/Nickerson solvent extraction head (Likens, S.T., Nickerson, G.B., 1964). The Likens/Nickerson extraction method has been frequently used for the collection of potato volatiles and can be regarded as a routine method of obtaining potato volatiles, especially in the field of potato flavour research.

Potatoes from the same source were used for each isolation technique. The isolation and analysis of the potato volatiles obtained by all three methods were carried out within twelve days, to ensure the potatoes were of the same physiological age.

## 8.2 EXPERIMENTAL

A sample of 40 kg of potatoes, c.v. Record, were obtained from commercial sources, the potatoes had been grown using normal commercial practice and were not treated with any chemicals post-harvest. The potatoes were graded to remove diseased or damaged tubers, the tubers were then washed and allowed to dry before they were stored in the dark at 10°C before use. After 12 wks storage, the potatoes were regraded to obtain 16 kg of healthy tubers of uniform size. The regraded tubers were then stored at 15°C for 1 wk before use, by the time the tubers were used for experimentation they had broken dormancy and had sprouts 20 mm - 50 mm long, these tubers were used for all the experiments in this chapter.

The volatiles collected using the All Glass system and the Vacuum Distillation system were analysed using System 3 described in Chapter 7. The volatiles obtained using the Likens/Nickerson method were in the form of an ether extract, this extract was reduced in volume for G.C. analysis and was analysed by injecting an aliquot of the extract onto a freshly conditioned precolumn and desorbing the precolumn as described in System 3, Chapter 7.

### Gas Chromatography

| | |
|---|---|
| G.C.: | Pye 104 (Pye Unicam Ltd). |
| Column: | C.W.20M S.C.O.T. glass, 43 m, 1 mm O.D., 0.5 mm I.D. (S.G.E., U.K. Ltd). |
| Carrier Gas: | $H_2$ u = 25 cm $sec^{-1}$. |

| Temperature: | |
|---|---|
| Initial | 50°C 10 min. |
| Rise | 5°C $min^{-1}$. |
| Hold | 185°C 40 min. |
| Detector: F.I.D. | 270°C. |
| Detector: F.I.D. | $H_2$ 20 cc $min^{-1}$. |
| | $O_2$ 200 cc $min^{-1}$. |
| | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | 5 x $10^{-10}$ A. |
| Chart Speed: | 5 mm $min^{-1}$. |

8.2.1 All Glass System

The All Glass system was cleaned with hot water and detergent, rinsed with deionised water followed by acetone and then dried at 220°C for 24 hr before use. The apparatus was then assembled, the $N_2$ was set at a flow rate of 250 cc $min^{-1}$ and the $O_2$ set at a flow rate of 50 cc $min^{-1}$. The apparatus was then flushed with the air mix for 2 hr to remove any residual laboratory air. All the headspace samples from the apparatus were 10 $dm^3$ volume and taken at a flow rate of 300 cc $min^{-1}$. The sampled precolumns were sealed with PTFE caps and analysed within 3 hr of being sampled. The apparatus was kept at 15°C and in the dark throughout the experiment.

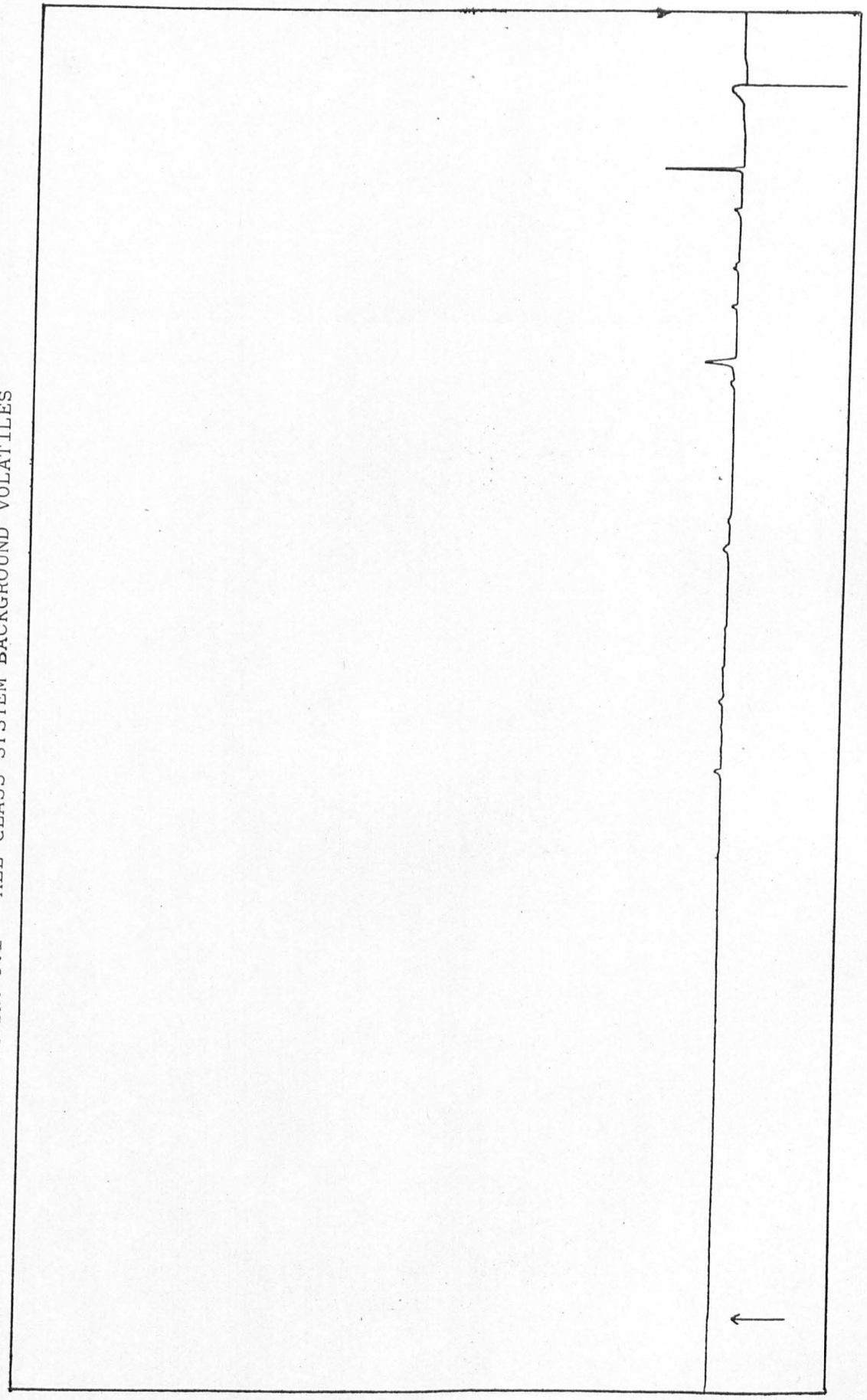
After the 2 hr flushing period, a sample was taken of the air from the containment vessel, Chromatogram 8.1, which shows that the levels of background volatiles in the system were low, only a few short RT compounds, at low levels were

present. After this sample was taken, the containment vessel was sealed with PTFE caps and left for 16 hrs to allow any volatiles to desorb off the containment vessel and accummulate in the headspace. After the 16 hr period, the air supply was reconnected to the containment vessel and a sample was taken from the containment vessel, Chromatogram 8.2, the background volatiles that accumulated over the 16 hr period were low, comparable to those in Chromatogram 8.1. It was concluded that the background levels of the volatiles contributed by the system were acceptable.

The apparatus was then opened and the containment vessel filled with 12.5 kg of potatoes, the apparatus was resealed and flushed with air for 2 hr. After the 2 hr flush, a sample was taken of the potato volatiles, Chromatogram 8.3, it was noted that there were substantial amounts of potato volatiles that had retention times between 40 min - 50 min. After this sample was taken the apparatus was sealed with PTFE caps for 16 hr to allow the potato volatiles to accumulate in the headspace. After the 16 hr period, the air supply was reconnected and a sample of the potato volatiles was taken, Chromatogram 8.4. The levels of numbered peak potato volatiles obtained were substantially lower than those in Chromatogram 8.3, which suggested that the production of volatiles was inhibited if the potatoes were enclosed in a sealed, static headspace. The lower levels of volatiles was not due to respiratory stress as the $O_2$ level within the sealed vessels was more than sufficient for potato respiration during the 16 hr period.

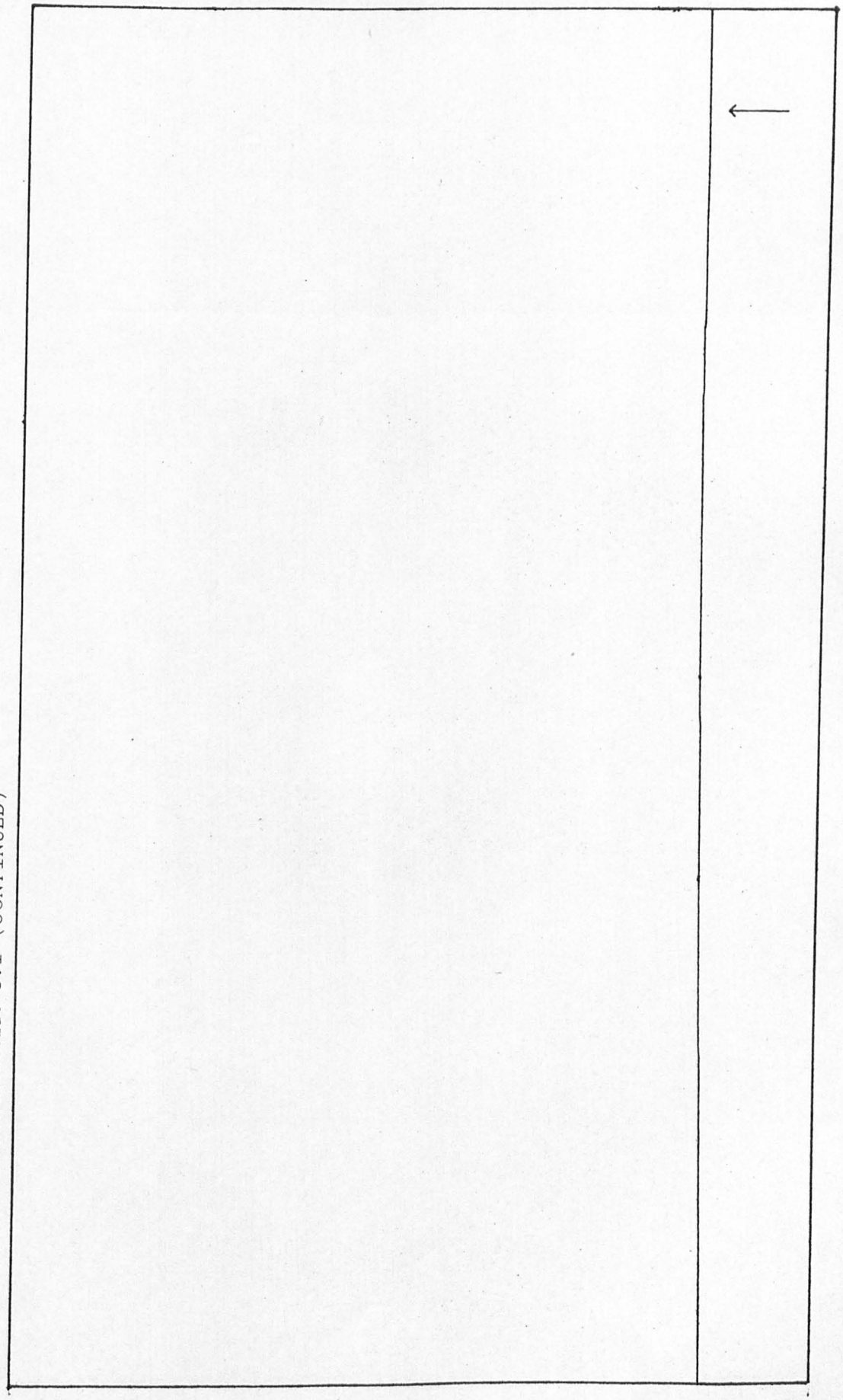
After the sample for Chromatogram 8.4 was taken the vessels were flushed with 50 $dm^3$ of air and then sampled, Chromatogram 8.5, the levels of potato volatiles in Chromatogram 8.5 were similar to those in Chromatogram 8.3, which was taken under similar conditions. The apparatus was then flushed with air for a further 24 hr and then two consecutive samples were taken of the potato volatiles, Chromatogram 8.6, Chromatogram 8.7, the levels of potato volatiles were similar in both chromatograms. The major differences between the volatiles in Chromatograms 8.3, 8.5, 8.6 and 8.7, all obtained under the same conditions, were in the levels of the early eluting volatiles.

This difference was due to breakthrough of these compounds on the Tenax G.C. precolumns which have low sample capacities for very volatile polar compounds at the high sample volumes and flow rates used. However, it was thought that these compounds with short retention times were of minor interest, whereas the compounds that eluted between 40 min - 50 min were of more interest as they had retention times similar to authentic dimethylnaphthalene isomers. The breakthrough of these early eluting potato volatiles was assessed, a second precolumn was connected in series using a PTFE coupling to the precolumn used to collect the potato volatiles for Chromatogram 8.4, so that any volatiles that broke through the first precolumn were trapped on the second precolumn. The analysis of the second precolumn, Chromatogram 8.8, showed that there was breakthrough of the short retention time volatiles, but not of the volatiles that had retention times greater than 10 mins.



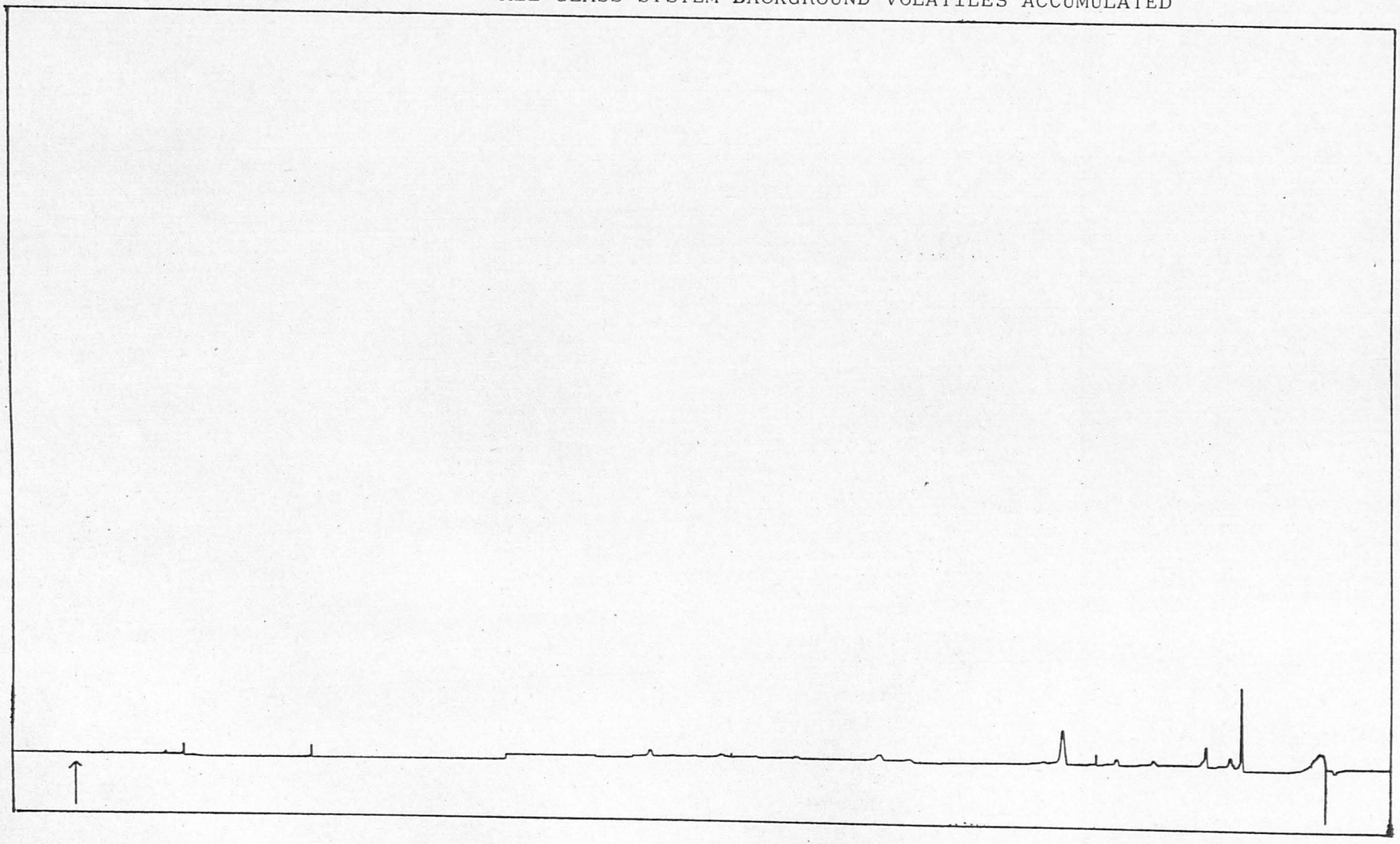
CHROMATOGRAM 8.1 ALL GLASS SYSTEM BACKGROUND VOLATILES

CHROMATOGRAM 8.1 (CONTINUED)

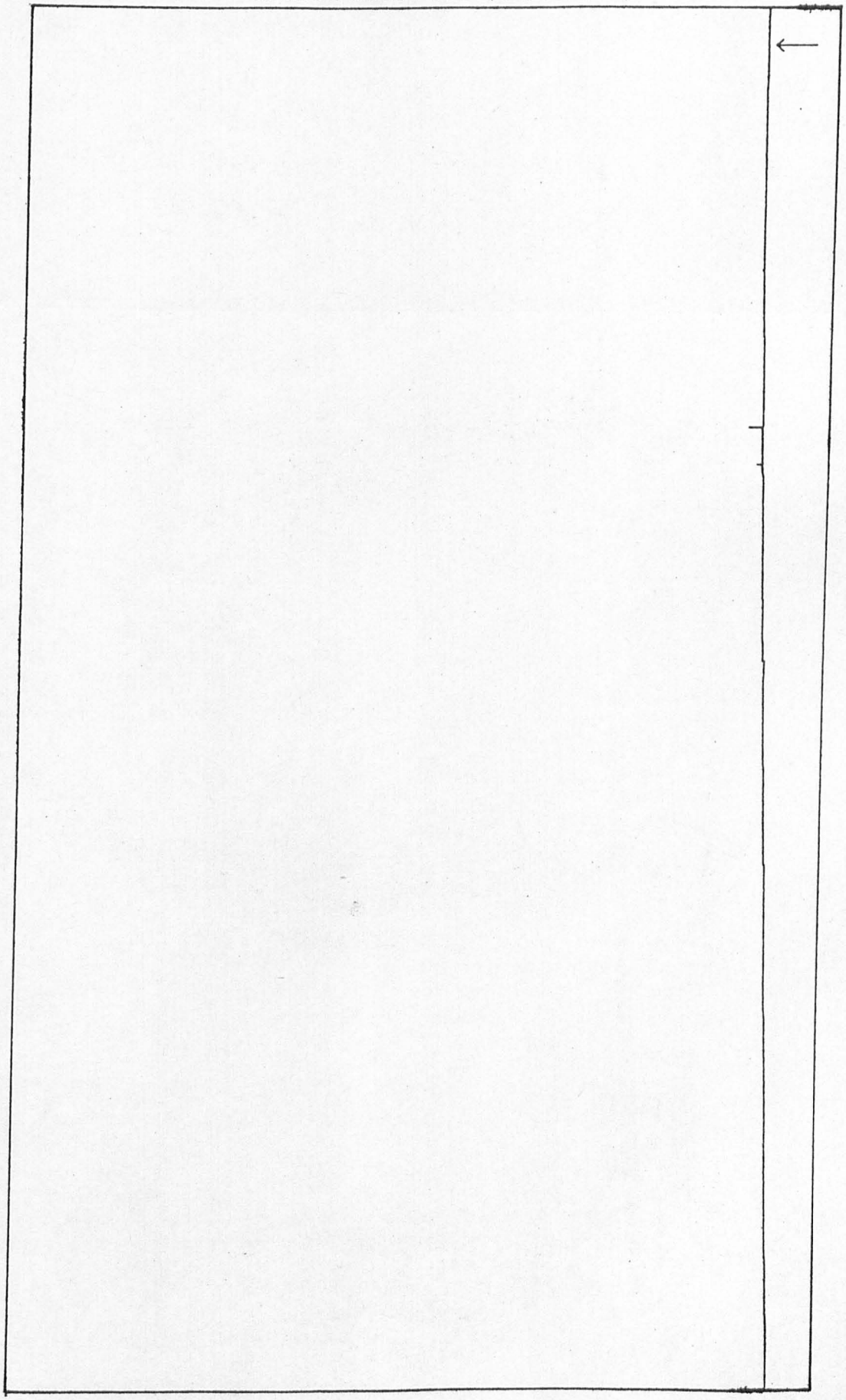


CHROMATOGRAM 8.1 (CONTINUED)

CHROMATOGRAM 8.2 ALL GLASS SYSTEM BACKGROUND VOLATILES ACCUMULATED

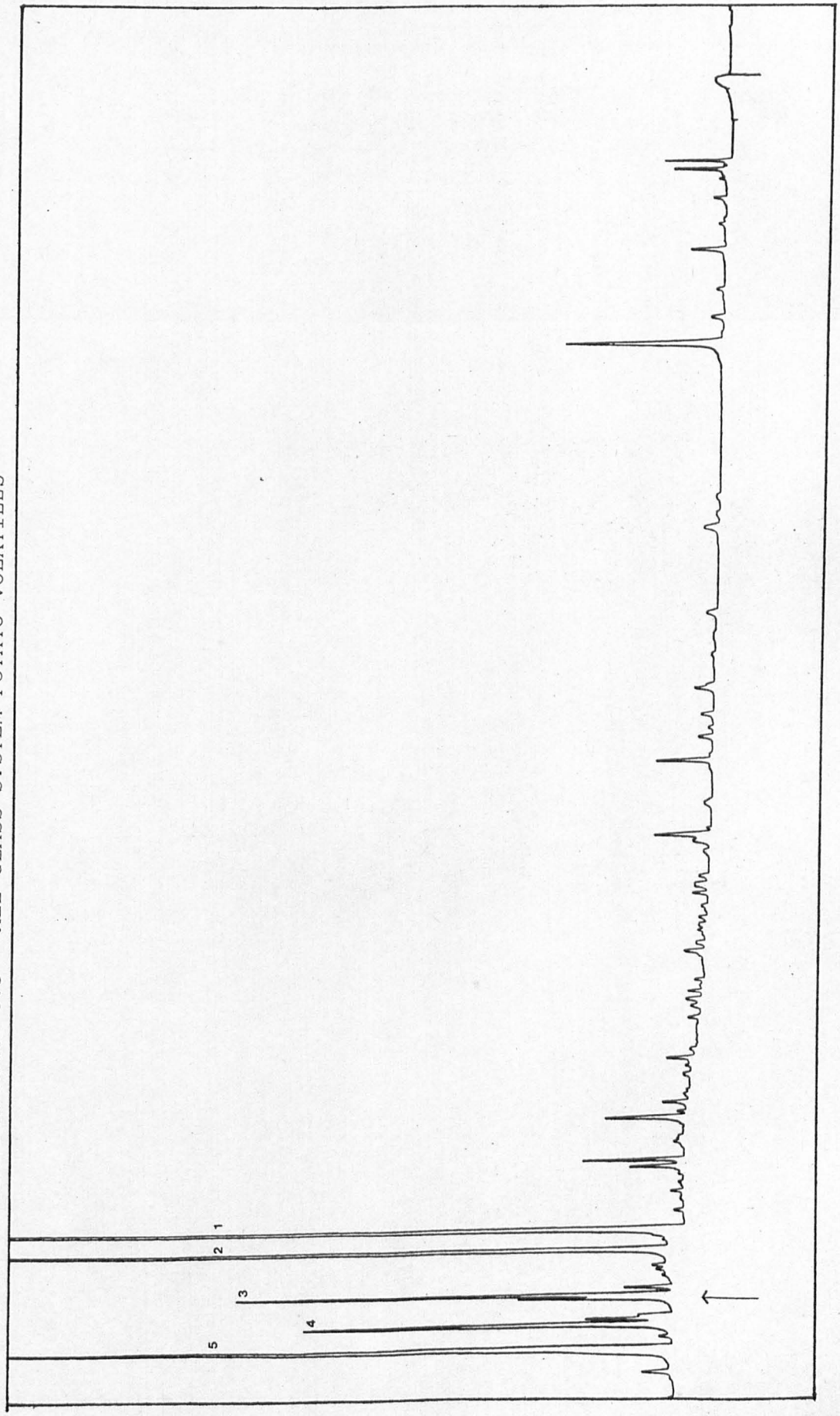


CHROMATOGRAM 8.2 (CONTINUED)



CHROMATOGRAM 8.2 (CONTINUED)

CHROMATOGRAM 8.3 ALL GLASS SYSTEM POTATO VOLATILES



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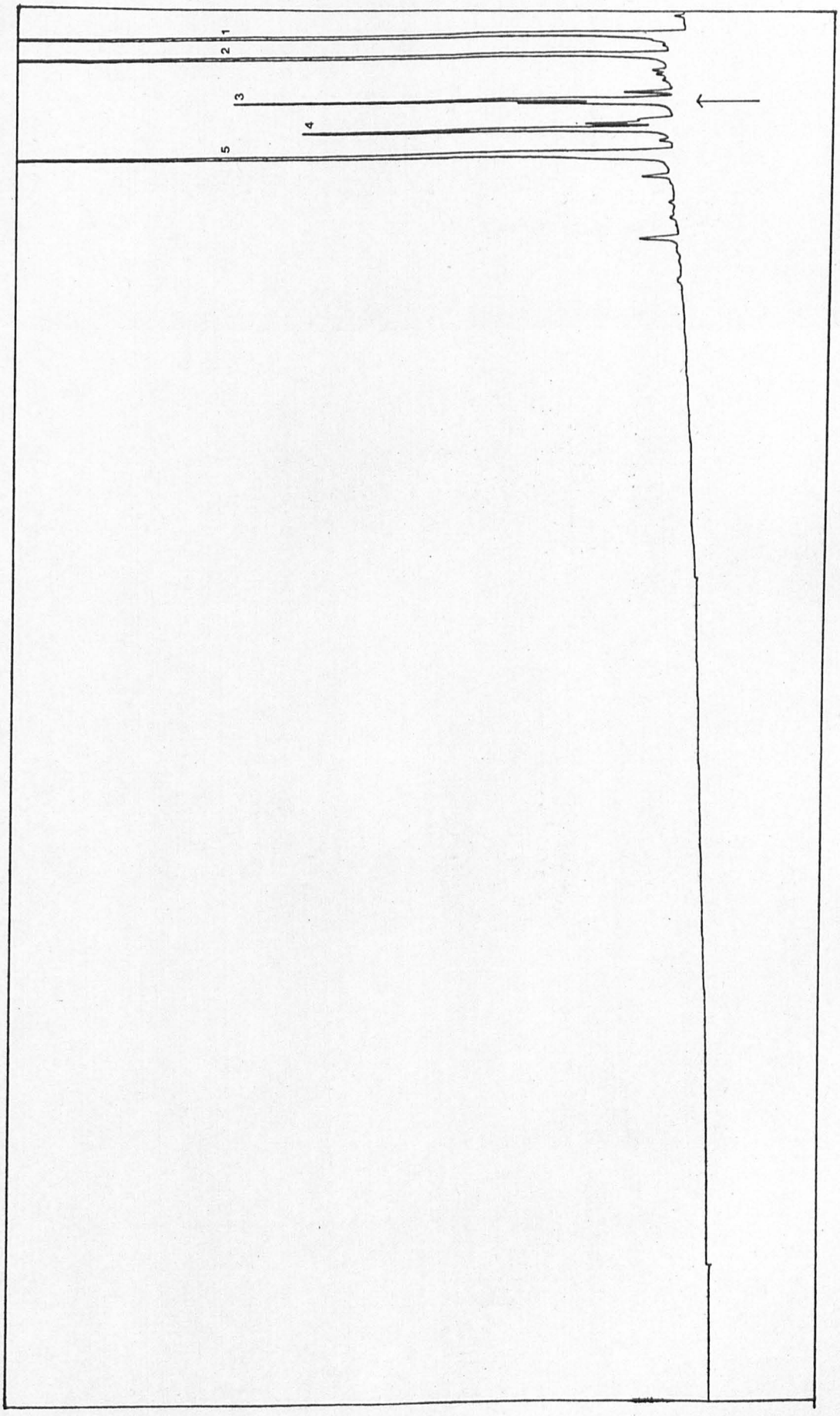
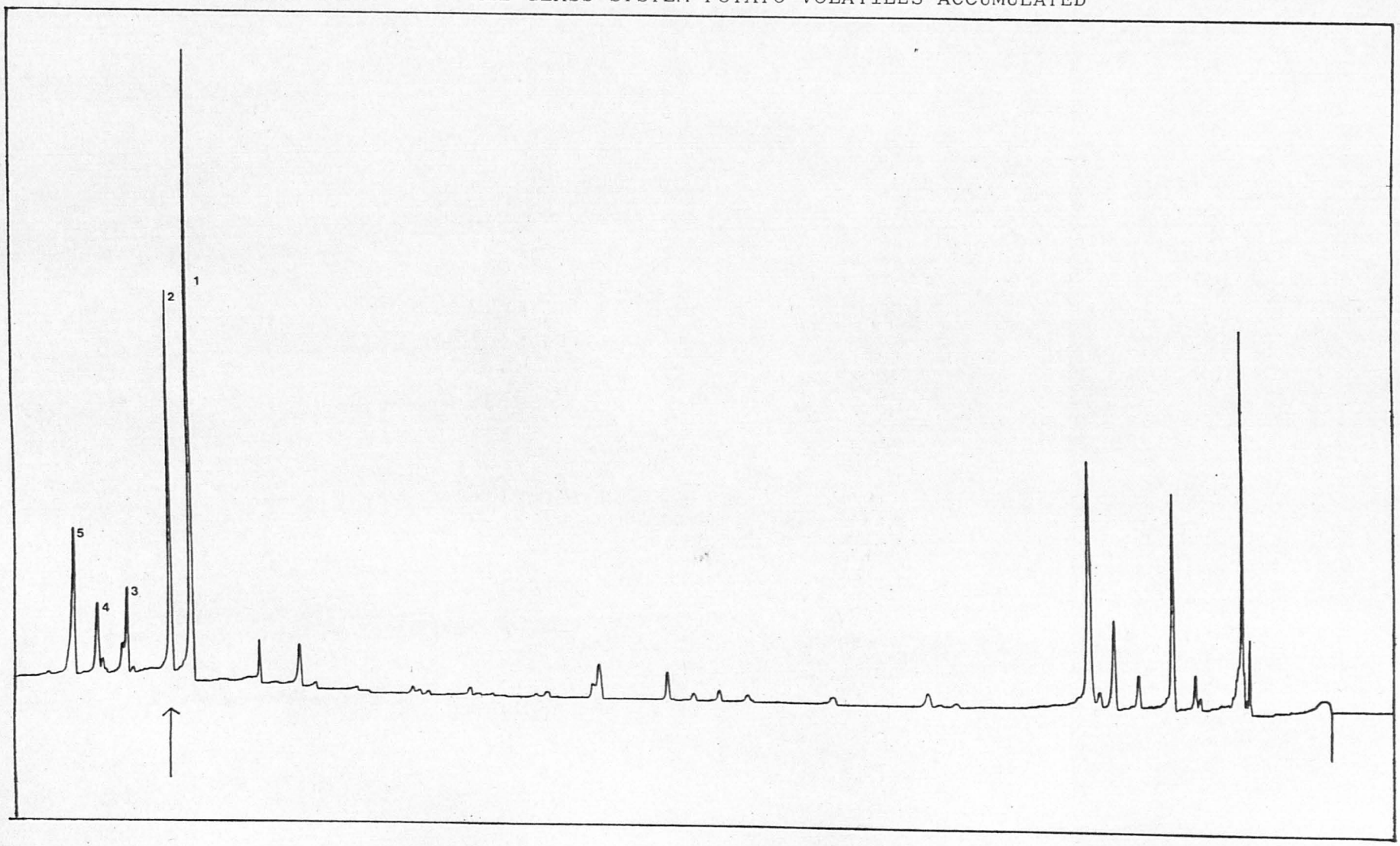


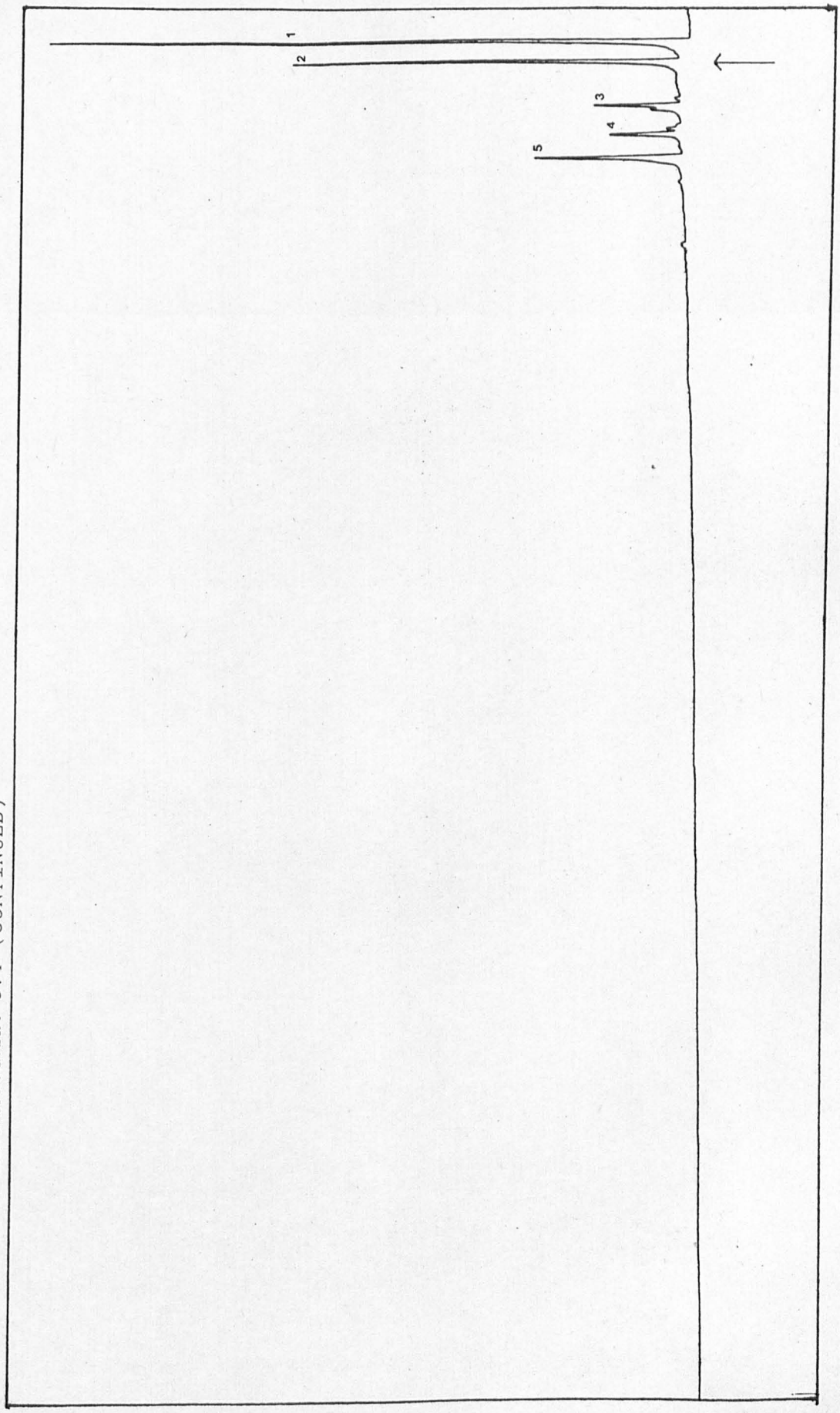

CHROMATOGRAM 8.3 (CONTINUED)

CHROMATOGRAM 8.4 ALL GLASS SYSTEM POTATO VOLATILES ACCUMULATED




CHROMATOGRAM 8.4

CHROMATOGRAM 8.4 (CONTINUED)

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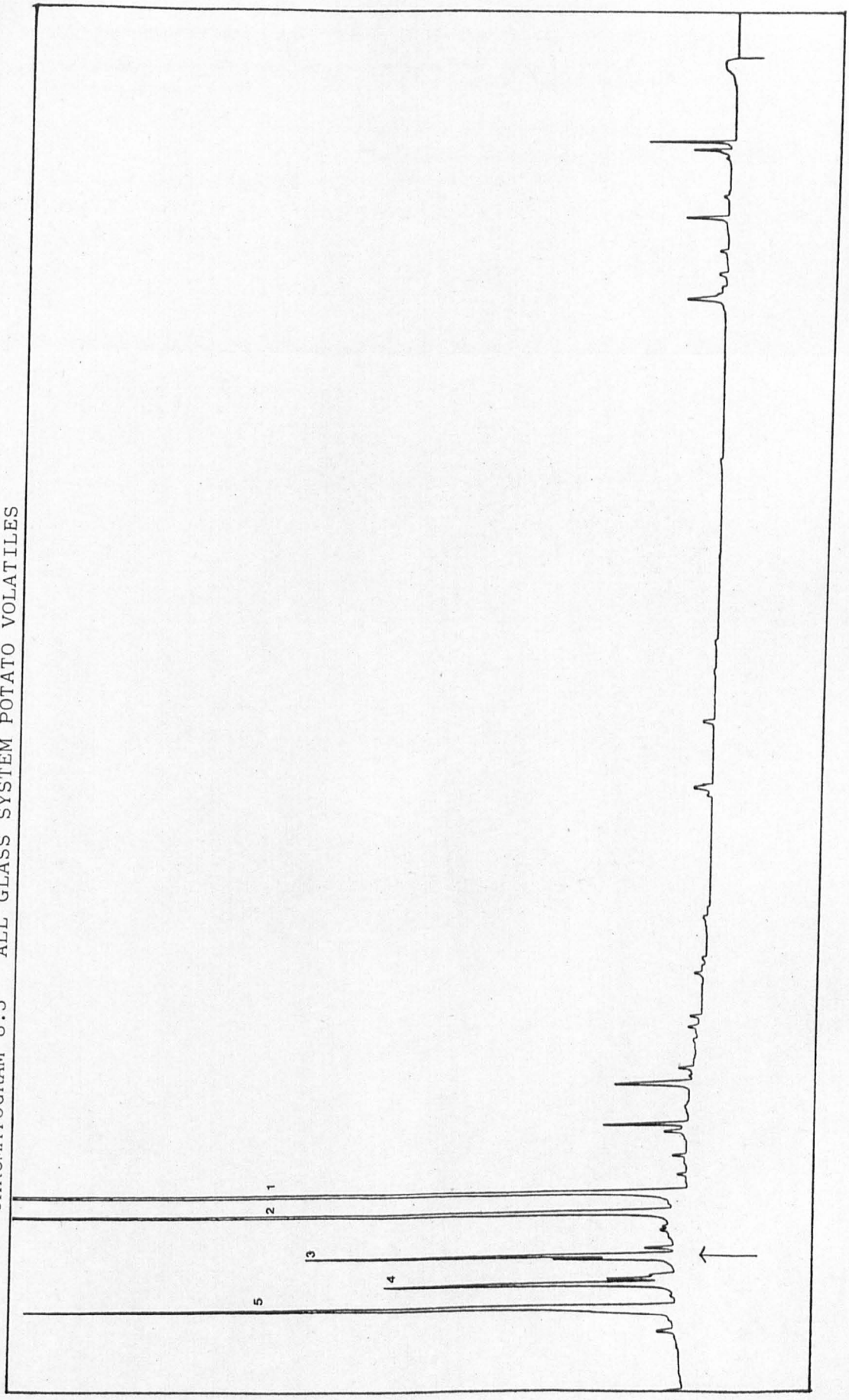
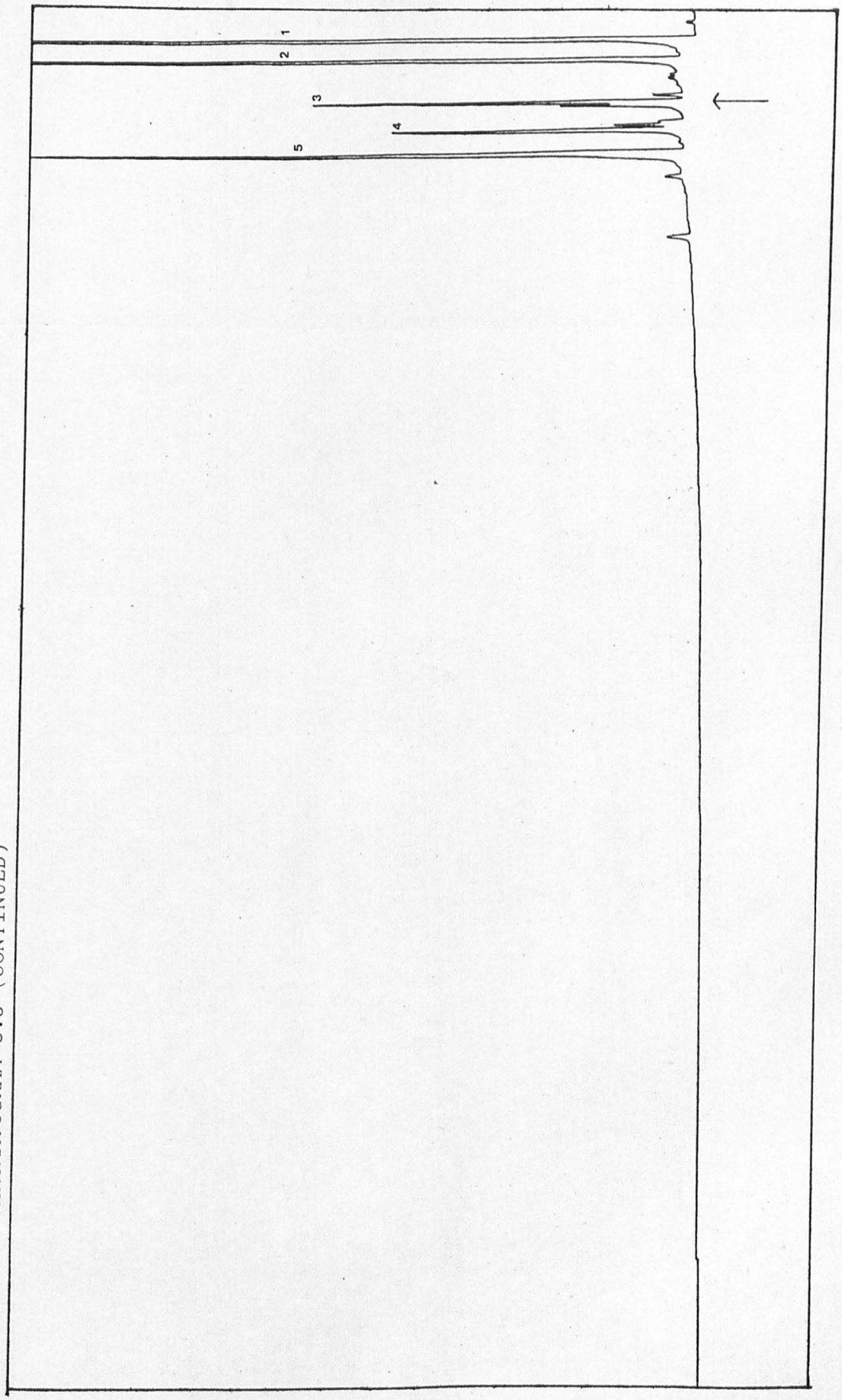
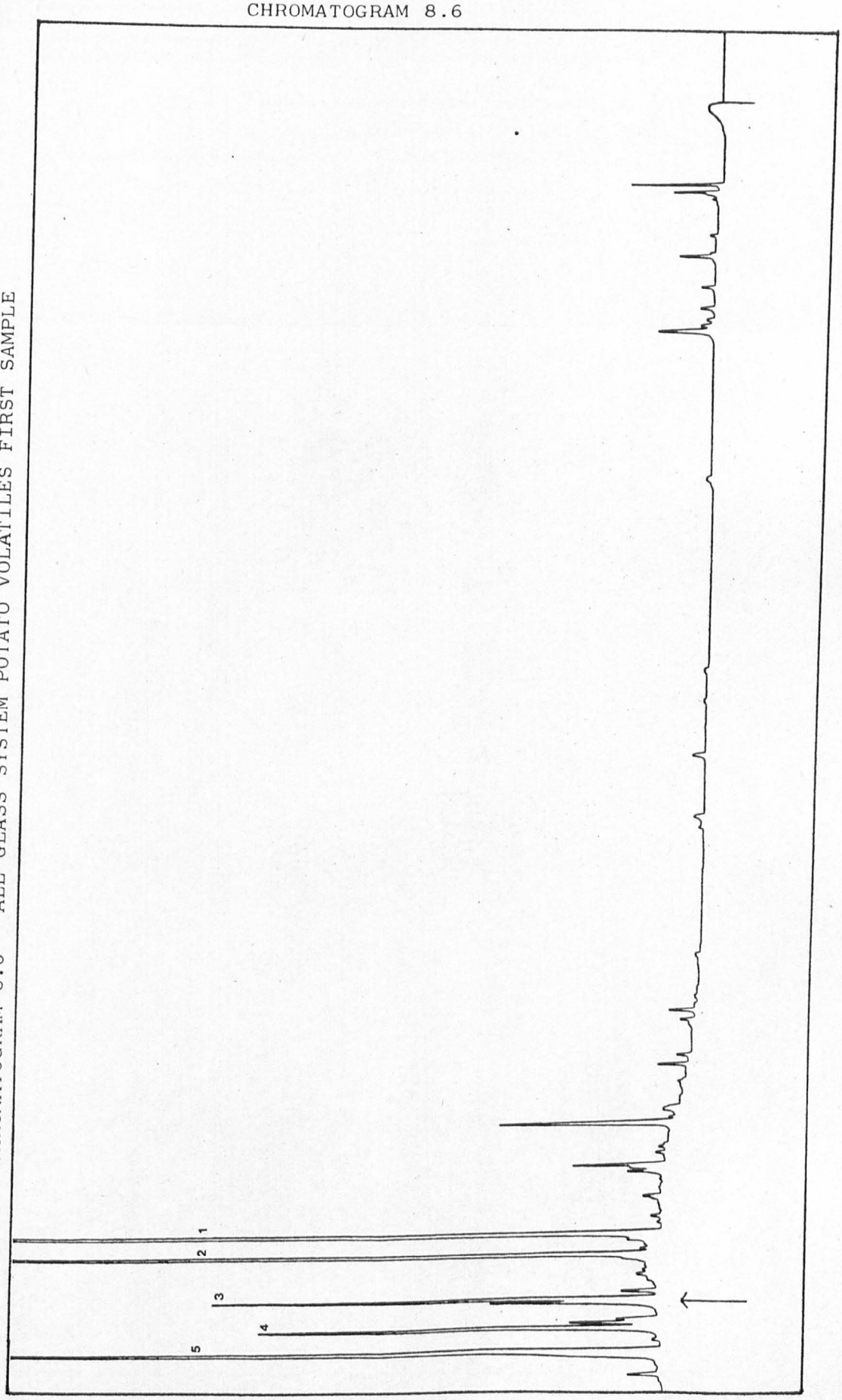

CHROMATOGRAM 8.5 ALL GLASS SYSTEM POTATO VOLATILES
1
2
3
4
5

CHROMATOGRAM 8.5 (CONTINUED)



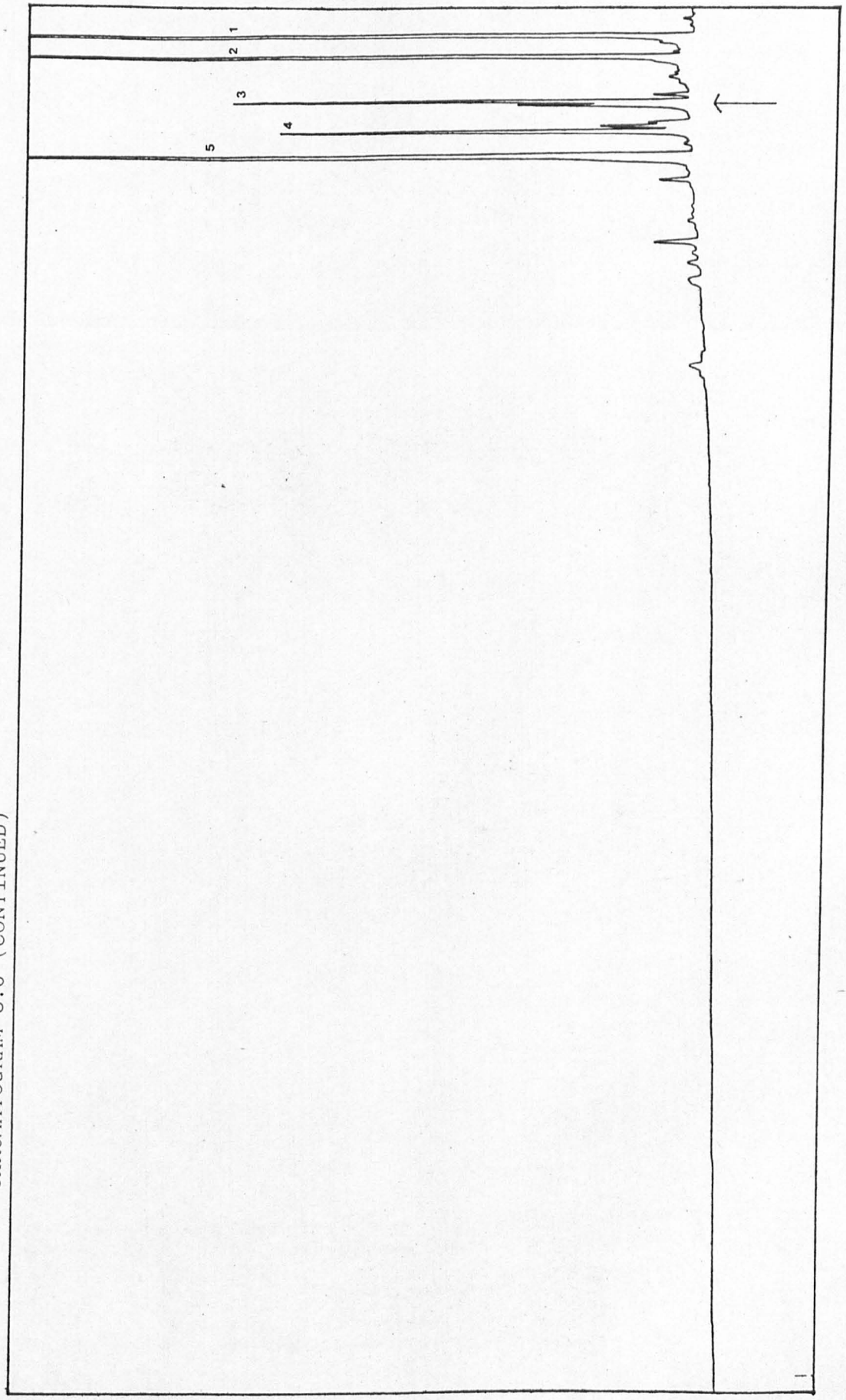

CHROMATOGRAM 8.5 (CONTINUED)

CHROMATOGRAM 8.6

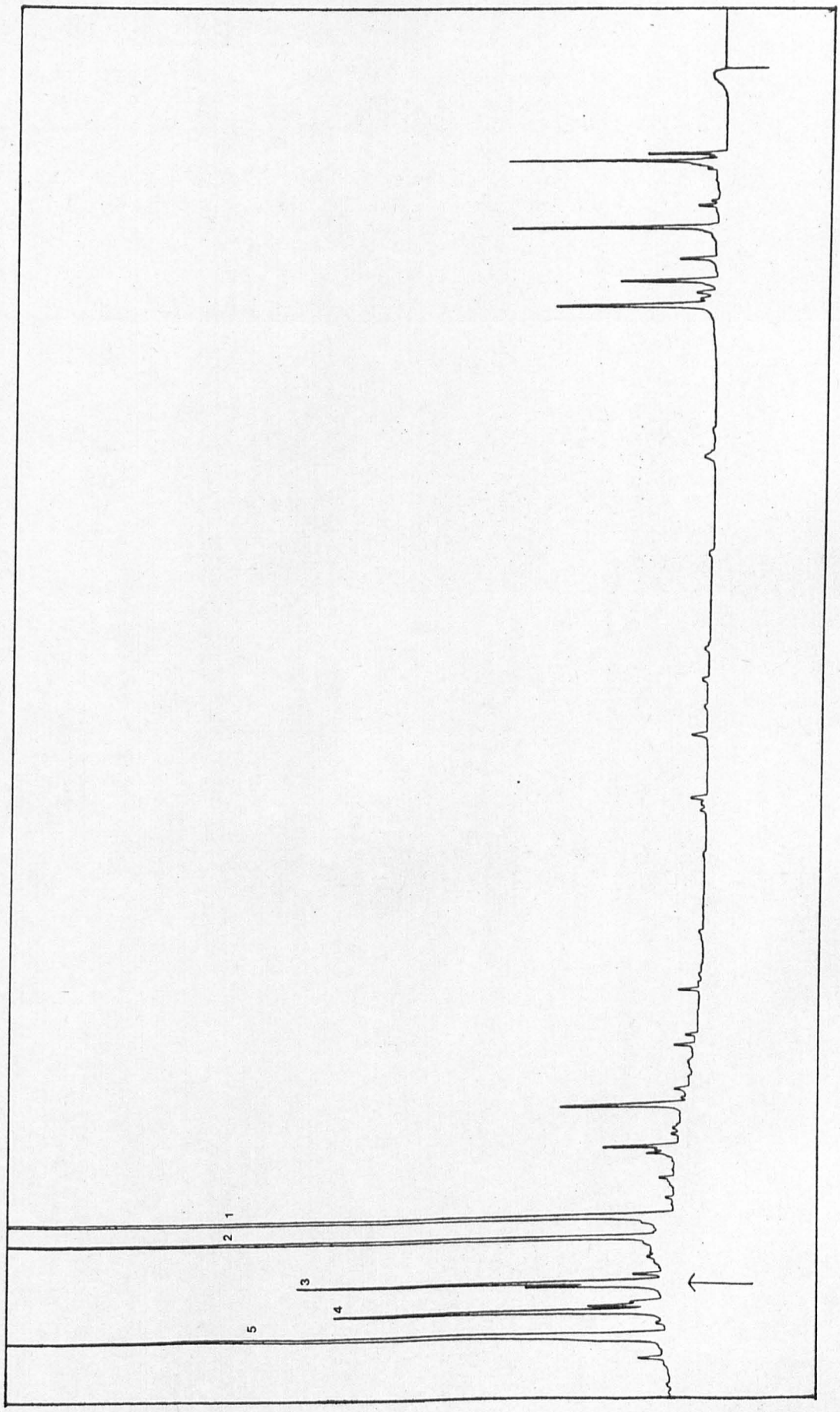



CHROMATOGRAM 8.6 ALL GLASS SYSTEM POTATO VOLATILES FIRST SAMPLE

CHROMATOGRAM 8.6 (CONTINUED)

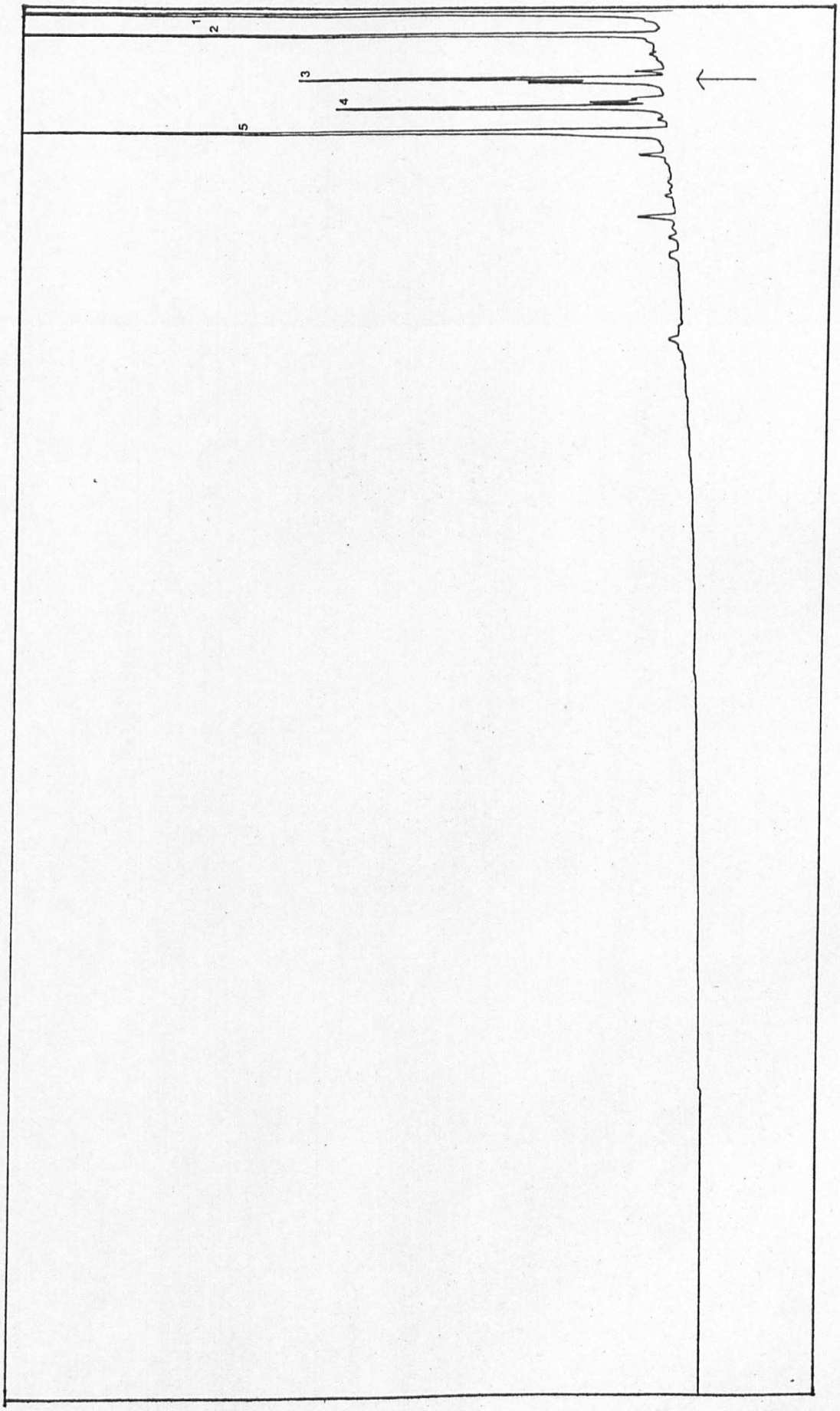



CHROMATOGRAM 8.6 (CONTINUED)



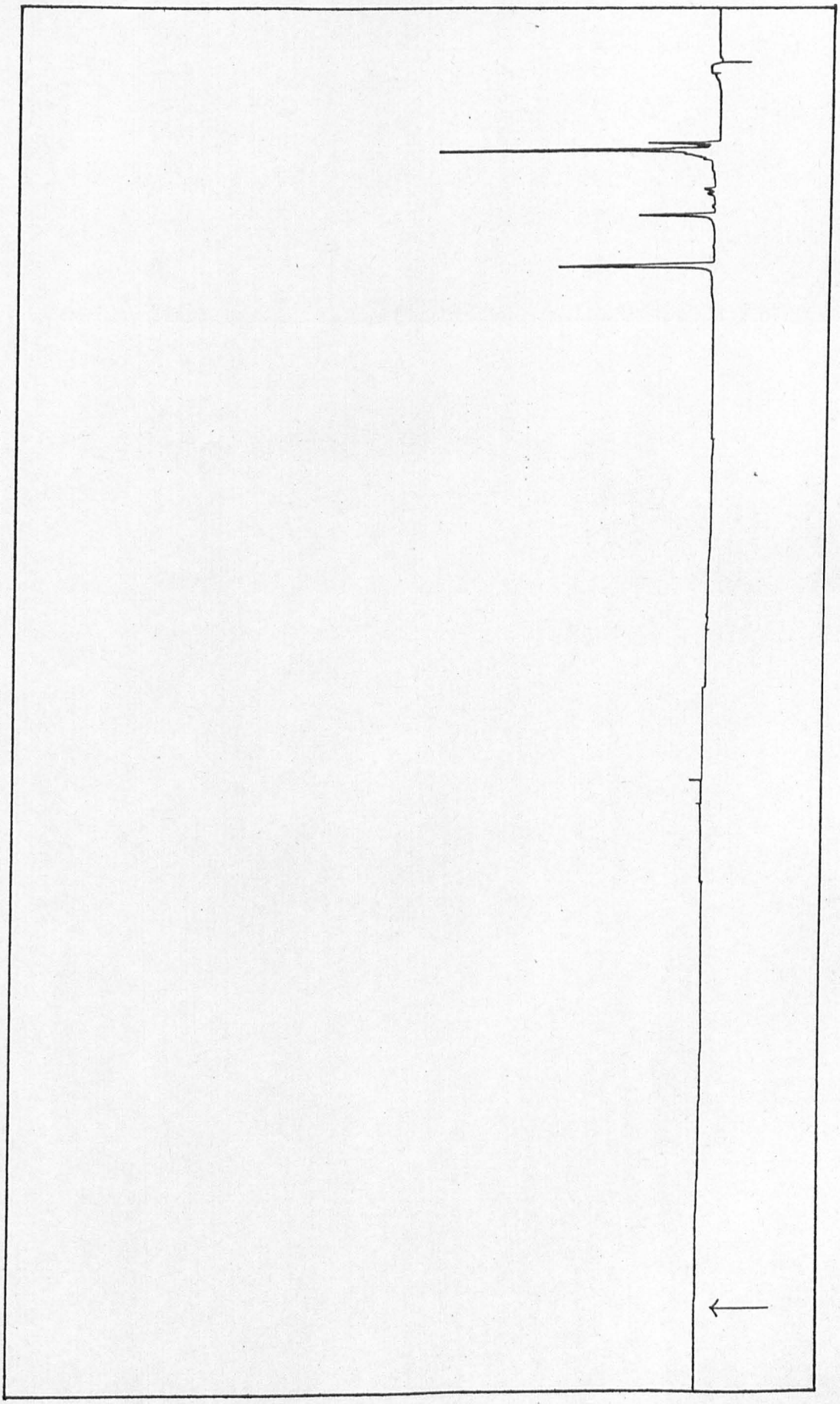

CHROMATOGRAM 8.7 ALL GLASS SYSTEM POTATO VOLATILES SECOND SAMPLE

CHROMATOGRAM 8.7 (CONTINUED)



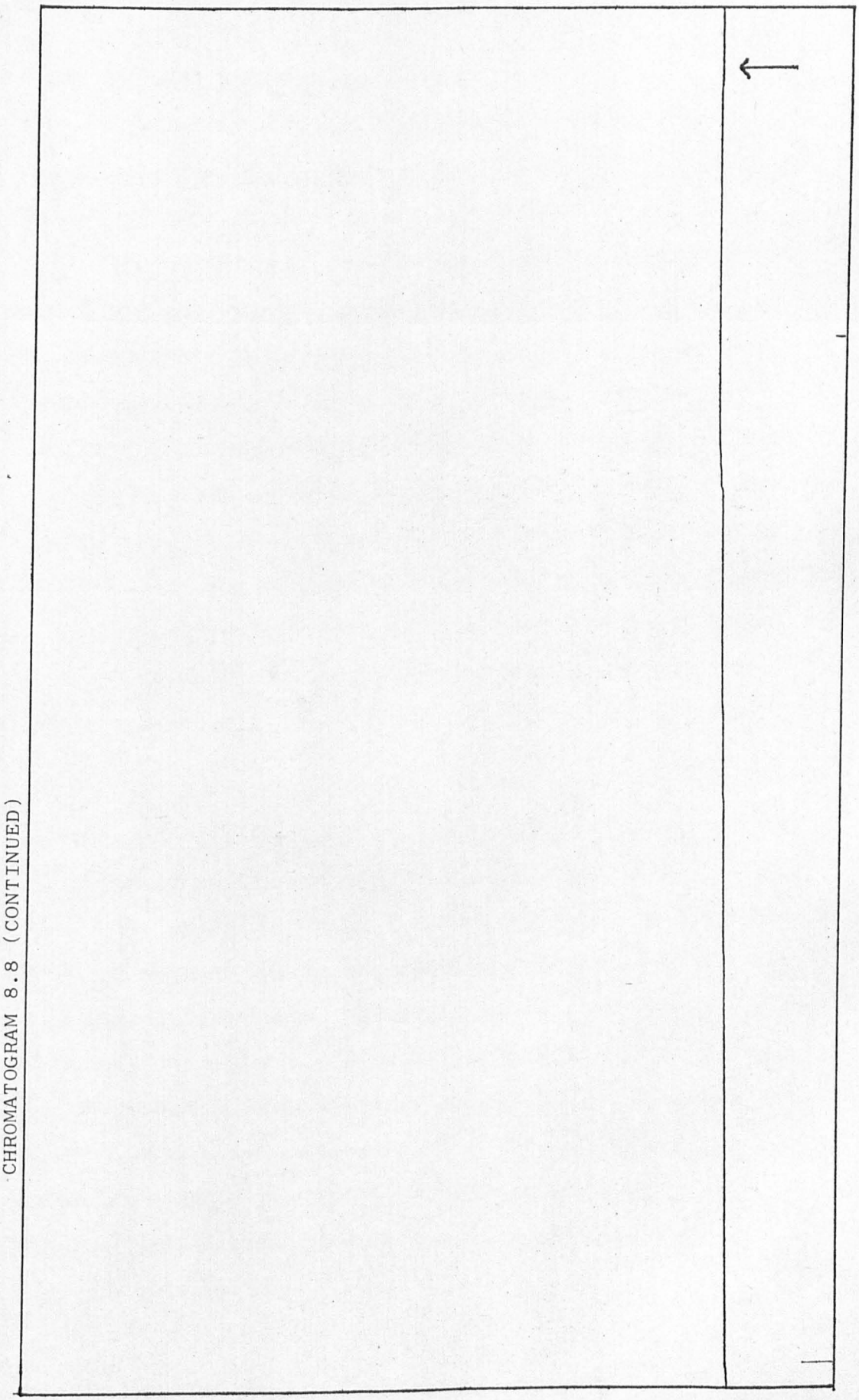

CHROMATOGRAM 8.7 (CONTINUED)

CHROMATOGRAM 8.8



CHROMATOGRAM 8.8 ALL GLASS SYSTEM BREAKTHROUGH VOLATILES

CHROMATOGRAM 8.8 (CONTINUED)



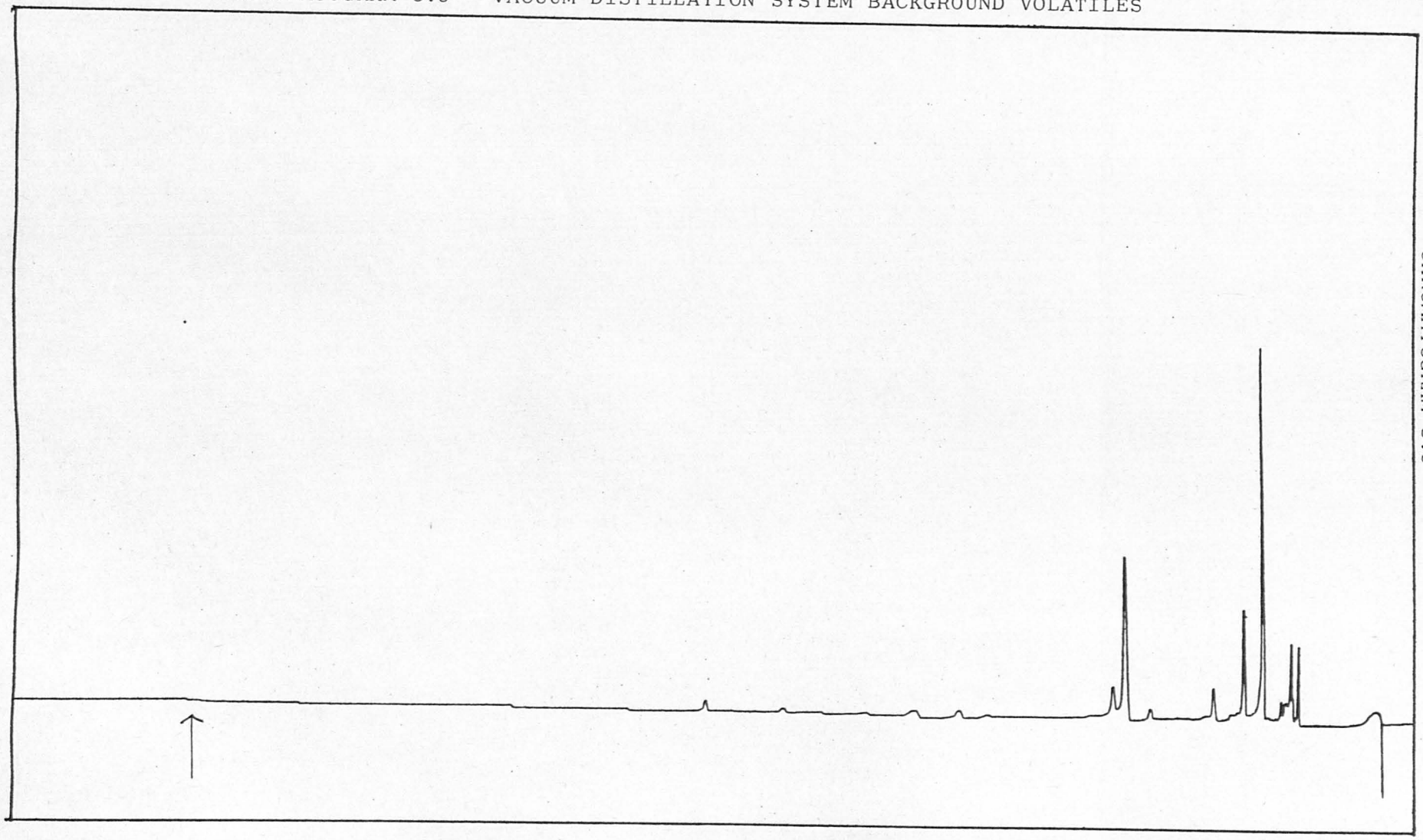
CHROMATOGRAM 8.8 (CONTINUED)

8.2.2 Vacuum Distillation System

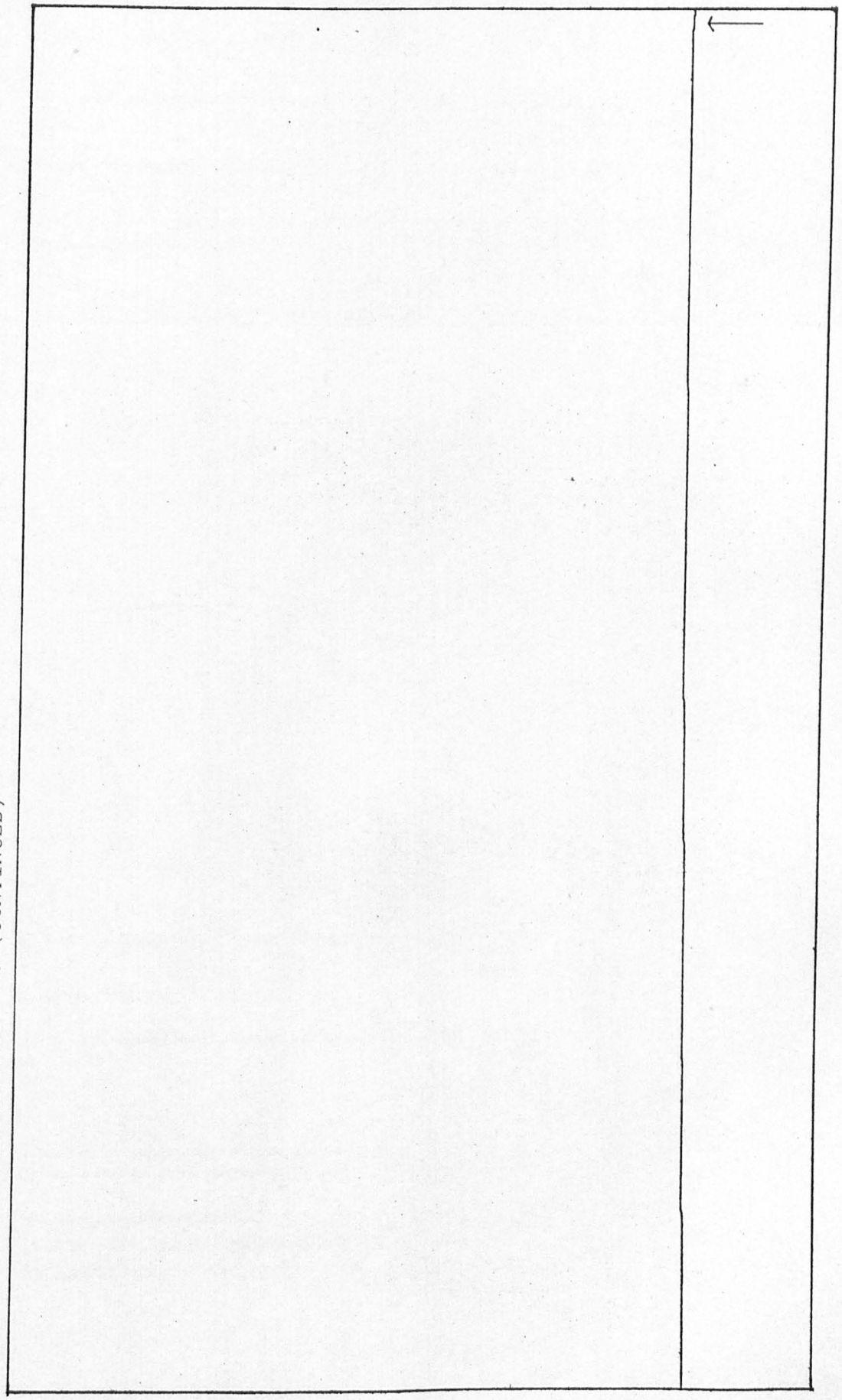
The apparatus as described in Chapter 6 was thoroughly cleaned in hot water and detergent, rinsed with deionised water followed by acetone and then heated at 220°C for 24 hr. The apparatus was assembled and a vacuum distillation of the empty apparatus was carried out according to the method described in Chapter 6. The analysis of the background volatiles from the cold trap, Chromatogram 8.9, showed that there were a few compounds that eluted within the first 10 mins with no major peaks thereafter. It was concluded that the level of background volatiles contributed by the system was acceptable, the peaks that were present in Chromatogram 8.9 were probably due to leaks in the seals of the system that allowed the laboratory air into the cold trap.

The apparatus was then filled with 2.7 kg of the potatoes and a vacuum distillation of the potatoes was carried out. The analysis of the volatiles from the cold trap, Chromatogram, 8.10, shows that higher levels of potato volatiles were obtained by this system than by the All Glass system. It is interesting to note that peaks 1 - 5 in Chromatogram 8.10 correspond to peaks 1 - 5 in Chromatograms 8.3, 8.4, 8.5, 8.6 and 8.7 obtained by the All Glass system. Although it is not possible to confirm peaks 1 - 5 are due to the same compounds without the aid of mass spectral identification. Their retention times and relative proportions suggest that they are the same.

CHROMATOGRAM 8.9 VACUUM DISTILLATION SYSTEM BACKGROUND VOLATILES

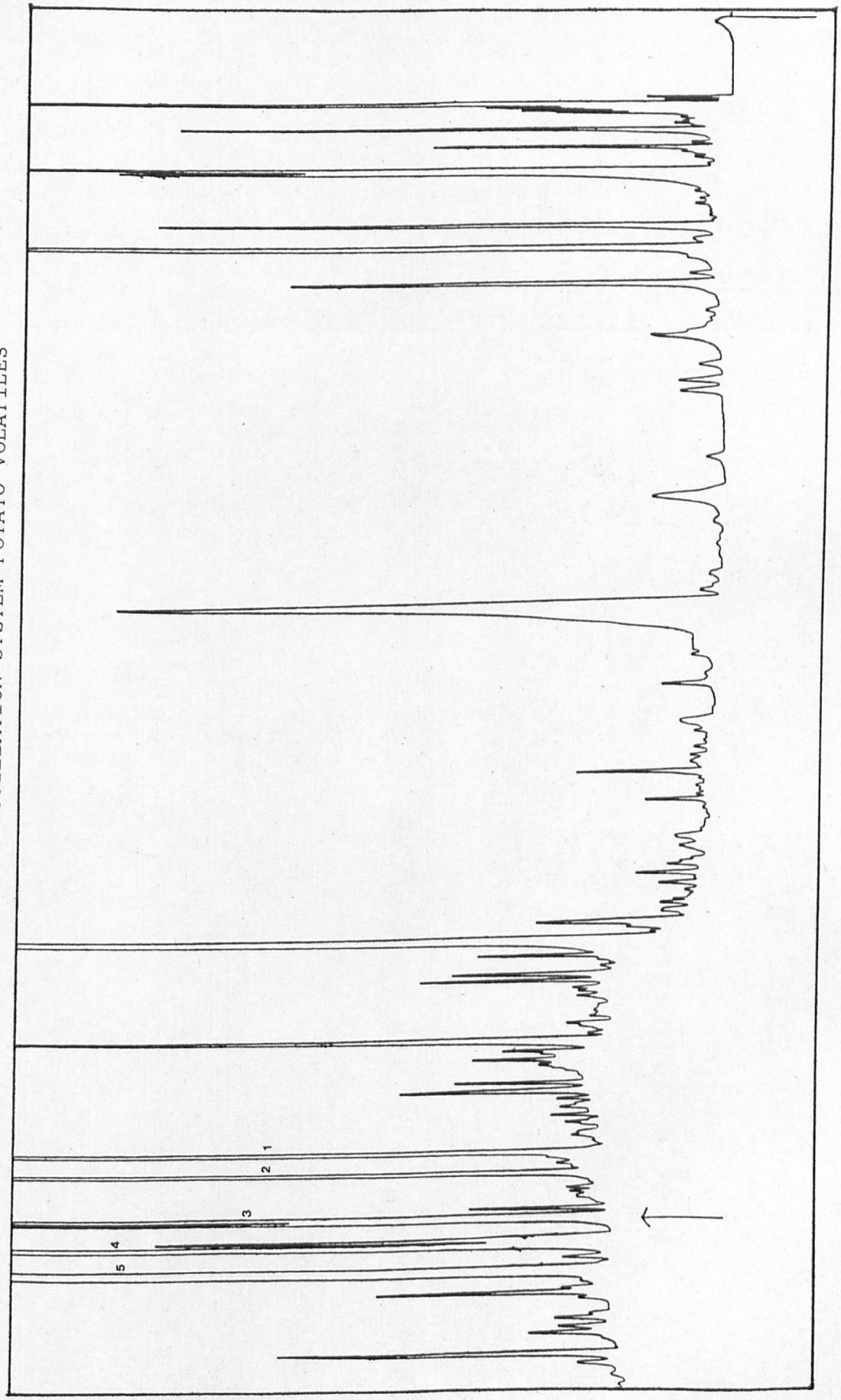


CHROMATOGRAM 8.9



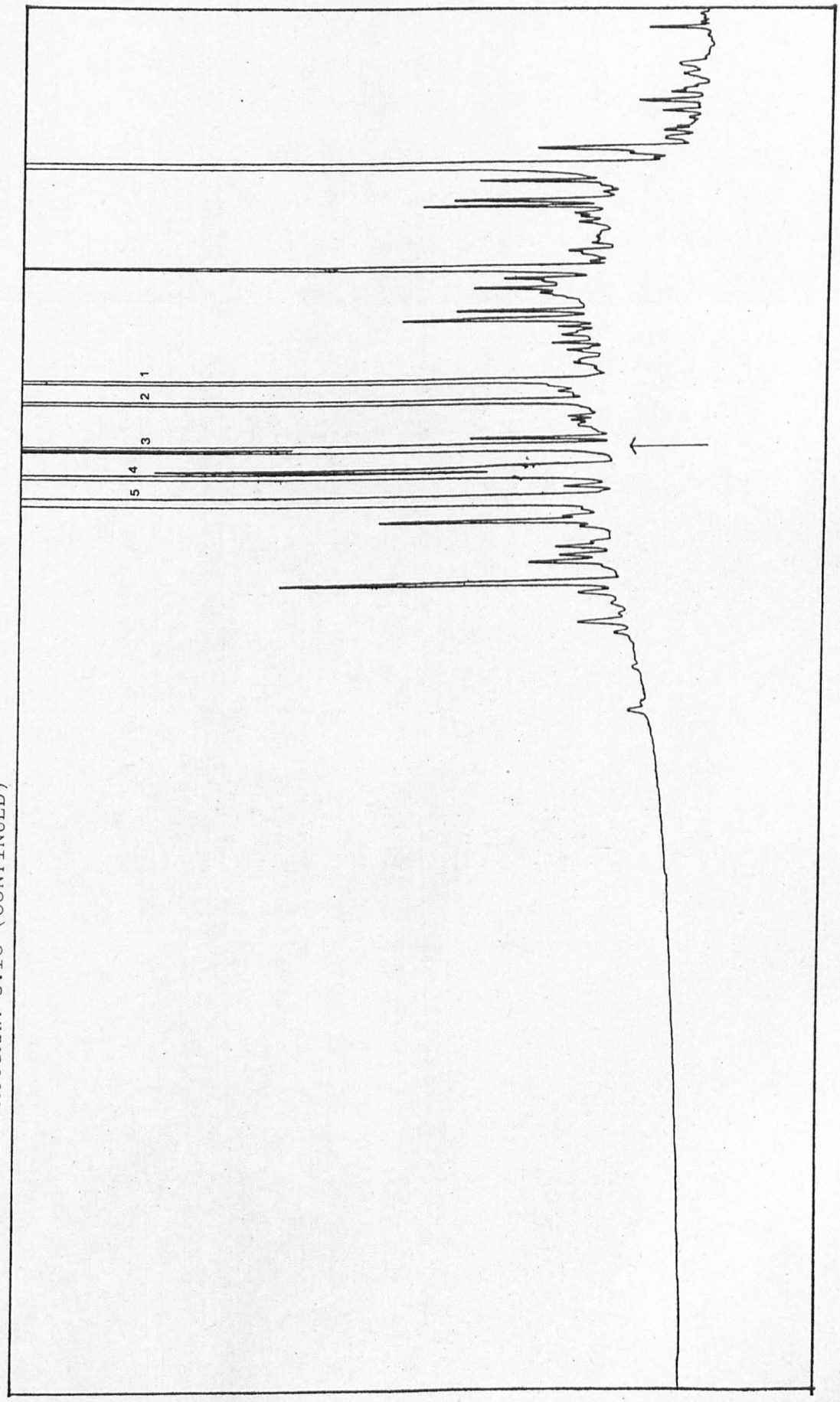
CHROMATOGRAM 8.9 (CONTINUED)

CHROMATOGRAM 8.10




CHROMATOGRAM 8.10 VACUUM DISTILLATION SYSTEM POTATO VOLATILES

CHROMATOGRAM 8.10 (CONTINUED)




CHROMATOGRAM 8.10 (CONTINUED)

8.2.3 Likens/Nickerson Extraction

The apparatus used to extract the potato volatiles was assembled as shown in Plate 13. Before use it was cleaned in hot water, rinsed with acetone and dried at 220°C for 48 hr. The potatoes were contained in a 5 $dm^3$ reaction flask with a 100 mm flange lid (Corning Ltd), the Likens/Nickerson (L/N), extraction head was connected to the reaction flask with a 100 mm flange head (Corning Ltd) the extraction solvent was contained in a 25 cc R.B. flask.

Before an extract was made of the potatoes, a blank run was performed on the system to determine the level of background volatiles contributed by the system, reagent impurities and laboratory atmosphere. The reaction vessel was filled with 2.5 $dm^3$ of freshly distilled water. The 25 cc R.B. flask was filled with 20 cc of freshly distilled diethyl ether (A.R., B.D.H., Poole, England). The distilled water was then set to distill at 10 cc $min^{-1}$ - 20 cc $min^{-1}$, the diethyl ether was set to distill at 3 cc $min^{-1}$ - 4 cc $min^{-1}$, the water and ether condensed on the top 50 mm of the condenser. The distillation/extraction was carried out for 6 hr, during that time the ether was topped up occasionally to maintain the original volume of 20 cc. After the 6 hr period, the distillation/extraction was stopped and the diethyl ether was removed and placed in a 100 cc separating funnel and extracted with 50 cc of 5% $Na_2CO_3$ aqueous solution (A.R., B.D.H., Poole, England). The ether layer was drawn off and dried with anhydrous $MgSO_4$ (A.R., B.D.H., Poole, England) which was then removed by filtration and the dried diethyl ether extract reduced in volume to 20 $mm^3$ - 50 $mm^3$

initially with a rotary evaporator at 20°C - 25°C and then in the final stages under a stream of $N_2$ gas. The extract was then analysed immediately. An aliquot of the extract, 0.1 $mm^3$, was injected onto a freshly conditioned precolumn with a 1 $mm^3$ syringe ((700 Series, Hamilton) Phase Separations Ltd), the precolumn was then analysed according to the method described in Chapter 7, System 3.

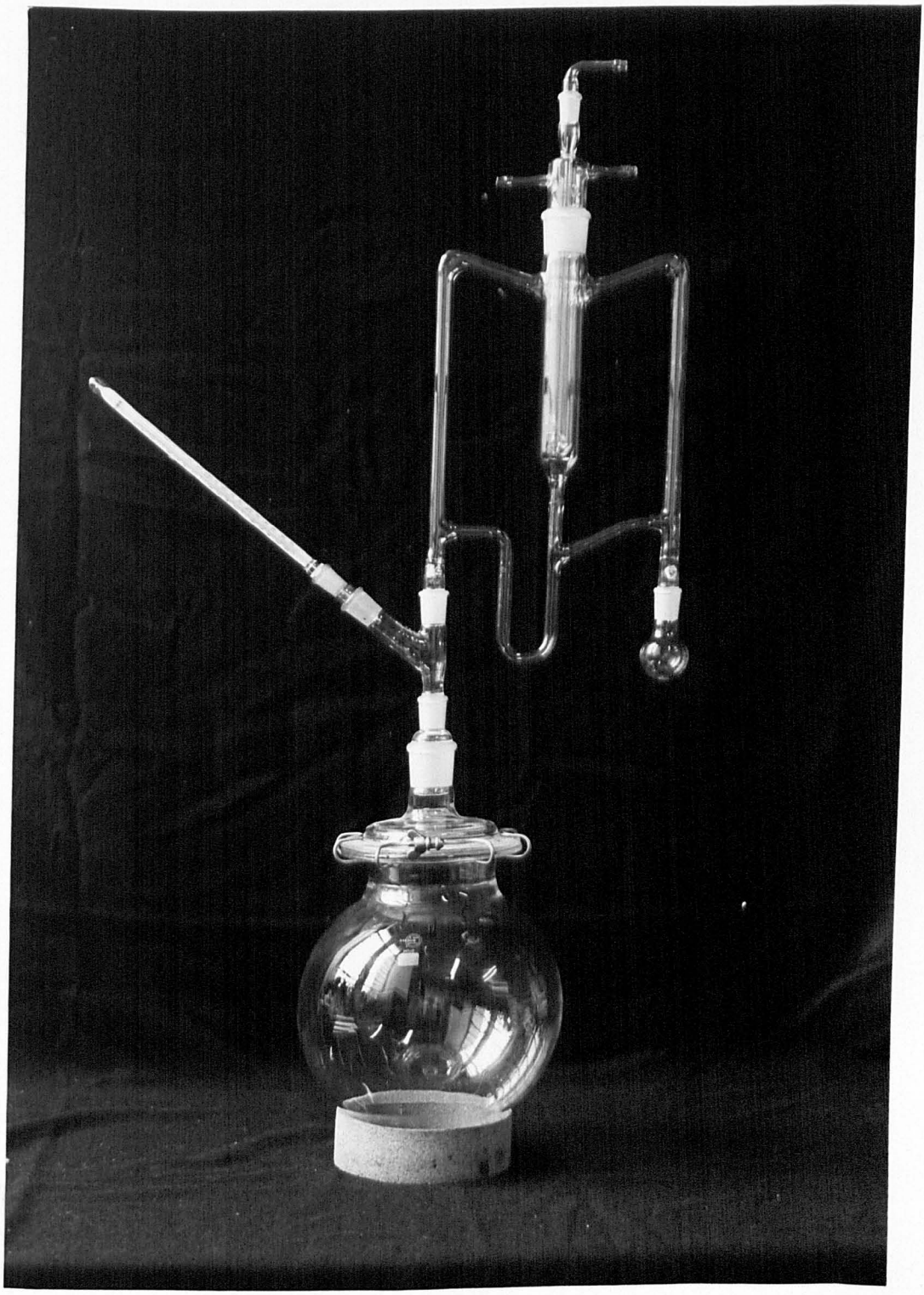
The chromatogram of the distilled water extract, Chromatogram 8.11, had eight peaks that were attributable to background volatiles.

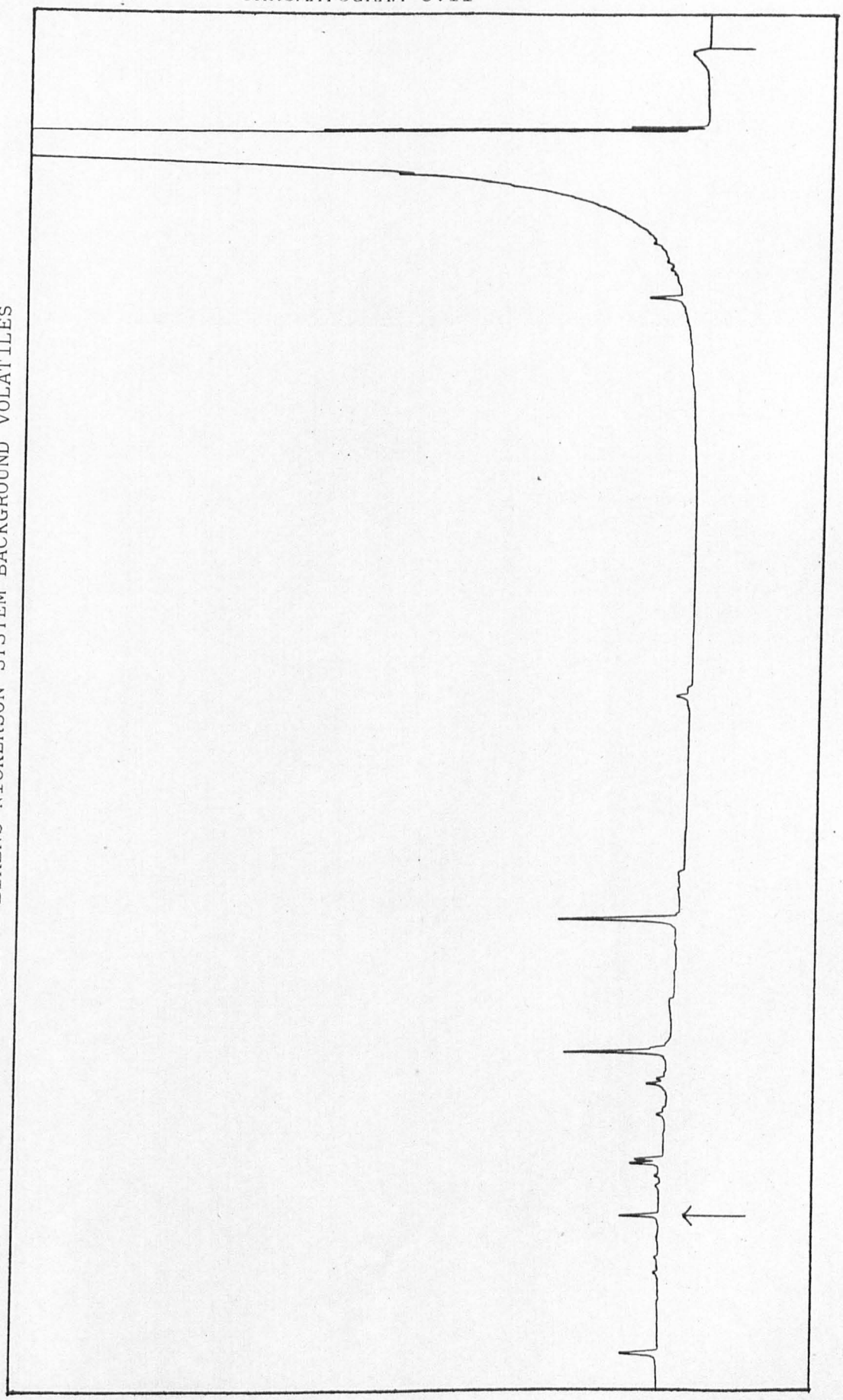
The apparatus was then cleaned and dried as before, the reaction vessel was filled with 2.5 kg of potatoes that had been cut into 20 mm cubes and mixed with 1 $dm^3$ of freshly distilled water, the 25 cc R.B. flask was filled with 20 cc of freshly distilled diethyl ether. The potatoes and water were set to distill at 20 cc $min^{-1}$ - 25 cc $min^{-1}$, the diethyl ether at 3 cc $min^{-1}$ - 4 cc $min^{-1}$. The distillation/extraction was carried out for 3 hr. After the 3 hr period, the ether extract in the R.B. flask was removed. The contents of the vessel were discarded and replaced with 2.5 kg of fresh potatoes and 1 $dm^3$ of freshly distilled water, the R.B. flask was filled with 20 cc of freshly distilled diethyl ether. The potatoes were extracted as before for a a period of 3 hr. After the 3 hr extraction, the diethyl ether extract was bulked with previous extract. The extracts were placed in a 250 cc extraction funnel and extracted with 100 cc of

5% $Na_2CO_3$ (aqueous), to remove the free fatty acids. The diethyl ether layer was drawn off and dried with $MgSO_4$ anhydrous, this was then removed by filtration and the dried diethyl ether extract was reduced in volume to 20 $mm^3$ - 50 $mm^3$.

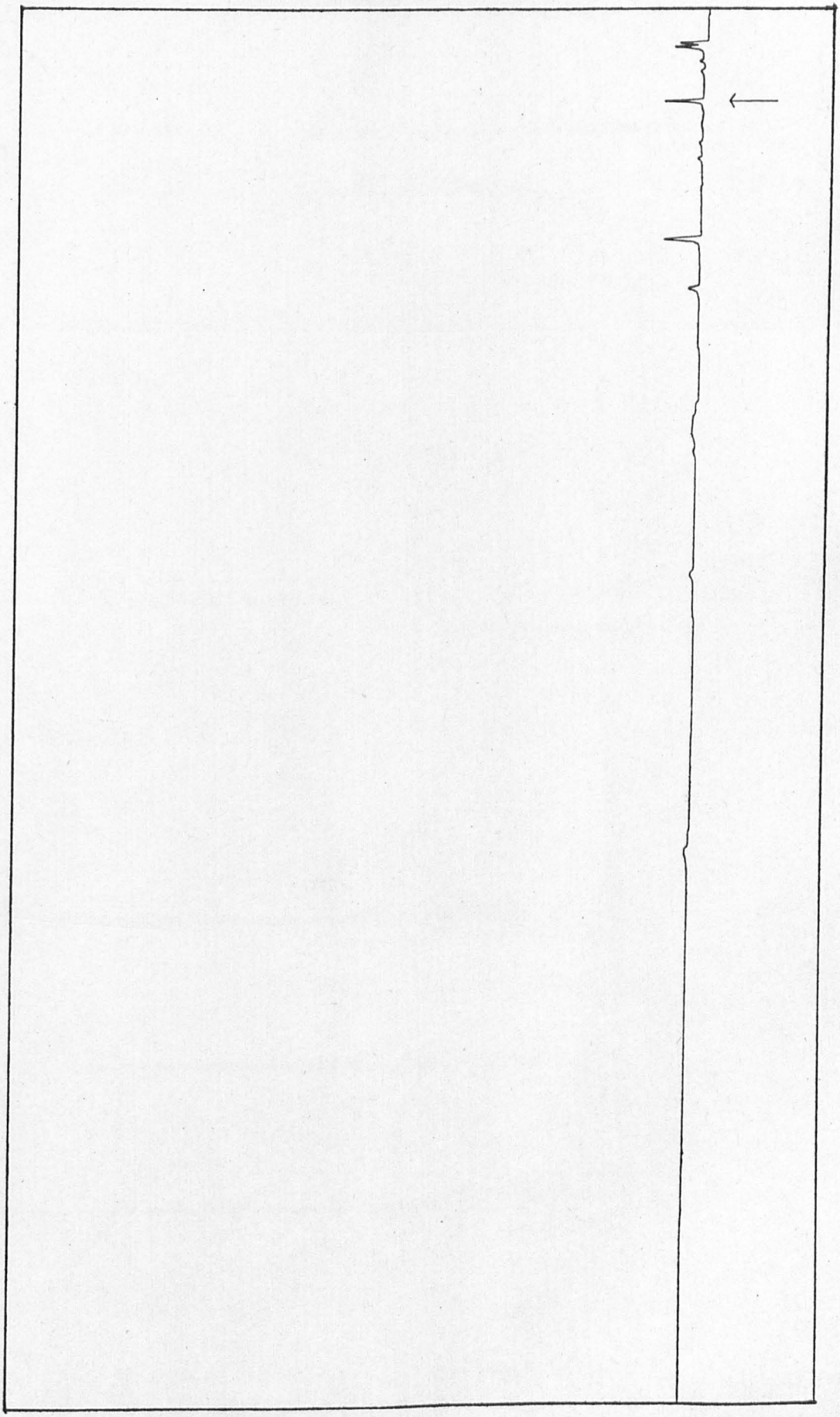
The extract had a strong odour of boiled potatoes, a 0.1 $mm^3$ aliquot of the extract was analysed using the method described for the distilled water extract. The analysis of the diethyl ether extract, Chromatogram 8.12, shows that there were a wide range of compounds extracted by the L/N extraction method, and in comparison to the other two methods, a large amount of volatiles with very long retention times were obtained.

# PLATE 13



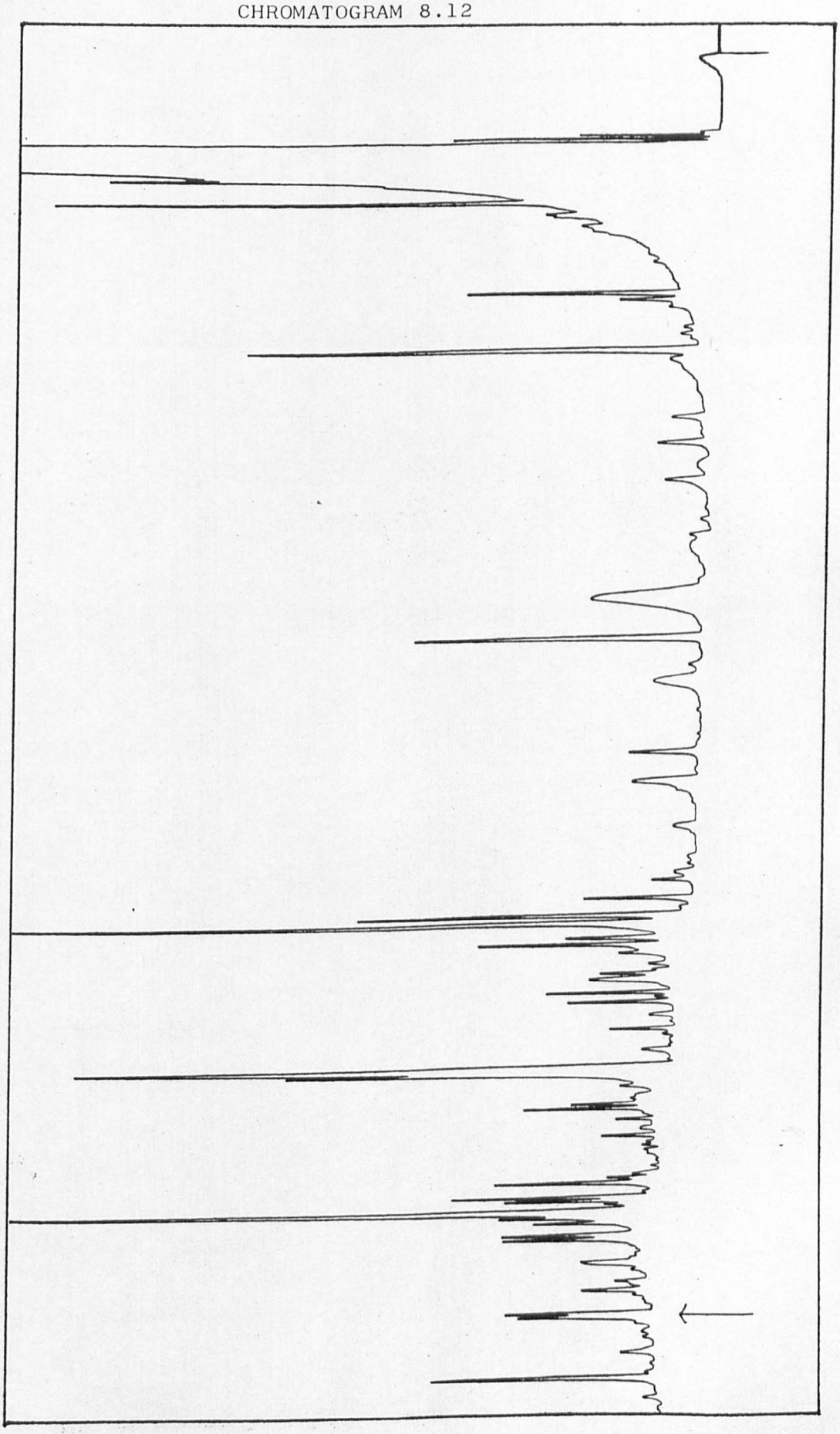


CHROMATOGRAM 8.11 LIKENS-NICKERSON SYSTEM BACKGROUND VOLATILES



CHROMATOGRAM 8.11 (CONTINUED)

CHROMATOGRAM 8.12



CHROMATOGRAM 8.12 LIKENS-NICKERSON SYSTEM POTATO VOLATILES

CHROMATOGRAM 8.12 (CONTINUED)

CHROMATOGRAM 8.12 (CONTINUED)

## 8.3 DISCUSSION

The most noticeable difference between the three systems under discussion was in the range of volatiles in the profiles obtained. It was concluded that each system differed in terms of the levels of volatiles that were collected. As the samples were not subjected to analysis by G.C.M.S., it is not possible to state whether each system isolates the same potato volatiles except in different amounts, or whether completely different volatiles are obtained. However, from their retention times and relative proportions, it was assumed that peaks 1 - 5 in Chromatograms 8.3, 8.4, 8.5, 8.6, 8.7 (All Glass system) and 8.10 (Vacuum Distillation system) were due to the same compounds. The retention times for peaks 1 - 5 in Chromatograms 8.3, 8.4, 8.5, 8.6, 8.7 and 8.10 are given in Table 8.1. Without such a clear pattern as that obtained for peaks 1 - 5, no further similarities were drawn between Chromatograms 8.1 - 8.12.

TABLE 8.1 Retention Times of Peaks 1 - 5

| | PEAK RT (sec) | | | | |
|---|---|---|---|---|---|
| Chromatogram | 1 | 2 | 3 | 4 | 5 |
| 8.3 | 2556 | 2604 | 2700 | 2766 | 2814 |
| 8.4 | 2550 | 2594 | 2700 | 2760 | 2808 |
| 8.5 | 2544 | 2586 | 2688 | 2754 | 2784 |
| 8.6 | 2568 | 2616 | 2712 | 2772 | 2832 |
| 8.7 | 2532 | 2580 | 2676 | 2736 | 2796 |
| 8.10 | 2530 | 2577 | 2676 | 2748 | 2796 |
| $\bar{x}$ | 2547 | 2593 | 2692 | 2756 | 2805 |
| S.D. | 14 | 15 | 14 | 13 | 17 |

The most noticeable feature of the volatile profiles by the All Glass system was in the absence of the short RT volatiles, attributable in part to their breakthrough on the Tenax G.C. precolumns. The majority of the headspace volatiles were grouped in the region at the end of the chromatograms, a mixture of dimethylnaphthalene isomers, under the same chromatographic conditions, eluted within this region. As the dimethylnaphthalenes have been shown to be present in potato volatiles (Meigh, D.F., et al, 1973) it is possible that some of these unknown peaks may be due to dimethylnaphthalenes.

The Vacuum Distillation system in terms of amount of volatiles $kg^{-1}$ of potatoes, isolated more volatiles than the All Glass system, an order of 5 - 10 times more, higher levels of earliery eluting compounds were isolated. However, a similar grouping of volatiles at the end of the chromatogram of the Vacuum Distillation system volatiles was found as that observed in the chromatograms of the All Glass system volatiles. It was thought that the Vacuum Distillation system altered the distribution of the headspace volatiles and the volatiles present within the tuber, the trend being in favour of the headspace, the volatiles were then drawn into the systems cold trap. The volatiles that were isolated by the Vacuum Distllation system were probably due to: volatiles that are normally present in the potato headspace at atmospheric pressure, and therefore isolated by the All Glass system, volatiles not normally present in the headspace at atmospheric pressure and volatiles produced by the cell damage due to the vacuum

applied to the tuber tissue. The differences between the volatiles obtained by the Vacuum Distillation and the All Glass systems were likely to be due to the last two factors stated above.

The L/N steam distillation technique isolated a wide range of potato volatiles, the most noticeable feature being the presence of volatiles with retention times greater than 60 mins, which were not present in the volatiles isolated by the other two techniques. Further to this, no comparisons could be drawn between the L/N volatile profile and the profiles obtained by the All Glass and the Vacuum Distillation systems. The ether extract of the steam distilled tubers had a distinct boiled potato odour, which was attributed to the fact that the tubers were boiled during the extraction procedure. It was noted that the volatiles flushed from the All Glass system and the cold trap of the Vacuum Distillation system had a raw, earthy potato odour, reflecting at least in organoleptic terms a major difference between the steam distillation (L/N) method and the All Glass, Vacuum Distillation systems.

In terms of ease of use and time taken to obtain a sample, the All Glass system was far superior to the other two systems, a headspace sample was obtained within 35 min and no sample work up was necessary thereafter. The other two systems required 5 hr - 6 hr to obtain a sample, the Vacuum Distillation requiring less sample preparation of the two.

The L/N steam distillation method had the disadvantage of the use of a solvent, when the extract was chromatographed

the solvent front obscured or would make identification difficult for the early eluting volatiles. The All Glass and Vacuum Distillation systems as they employed the solventless Tenax G.C. adsorbent followed by thermal desorption method had no such disadvantage, the early eluting components were well resolved. The main disadvatage of the All Glass and the Vacuum Distillation systems was the breakthrough of the very volatile low molecular weight components. This could have been remedied by increasing the amount of adsorbent used or by decreasing the sample volumes, at the sacrifice of trapping less of the later eluting volatiles. It was not considered to be desirable to decrease the sample volumes at the expense of the more interestiing later eluting volatiles. Increasing the amount of adsorbent would have meant a major alteration to the desorption block, due to the increased precolumn size, this was not thought to be justified.

The main aim of the work was the identification of potato tuber volatiles, and even though, especially with the All Glass system, the volatiles were not trapped in a quantitative manner, a portion of all the volatiles were trapped which it was hoped would be sufficient for their subsequent identification by G.C.M.S.

# CHAPTER 9

# VOLATILE PROFILES RELATED TO DORMANCY

## 9.1 INTRODUCTION

It is known that certain varieties of potatoes have a more pronounced dormancy than others, the main-crop varieties have been classified as to their dormancy on a scale from A, long dormancy, to E, short dormancy (Anon., 1981). In this chapter an investigation was made of the volatiles produced by a variety with a pronounced dormancy, and a variety with a short dormancy. The potato varieties chosen were Golden Wonder, which has been assigned a dormancy rating of B and Record which has a dormancy rating E.

It was thought that the difference in each varieties dormancy would be reflected by the volatiles that they produced. In particular, it was hoped that volatiles produced by dormant tubers would contain volatiles unique to the variety with a long dormancy and that these volatiles were involved with that varieties more pronounced dormancy.

Two parallel experiments were set up in which the volatiles from Golden Wonder and Record potatoes were sampled over a period of time from the dormant tubers. The tubers were stored till they had sprouted and then a further series of samples were taken. A comparison of the volatile profiles would show it there were any differences that were attributable to variety and/or dormancy.

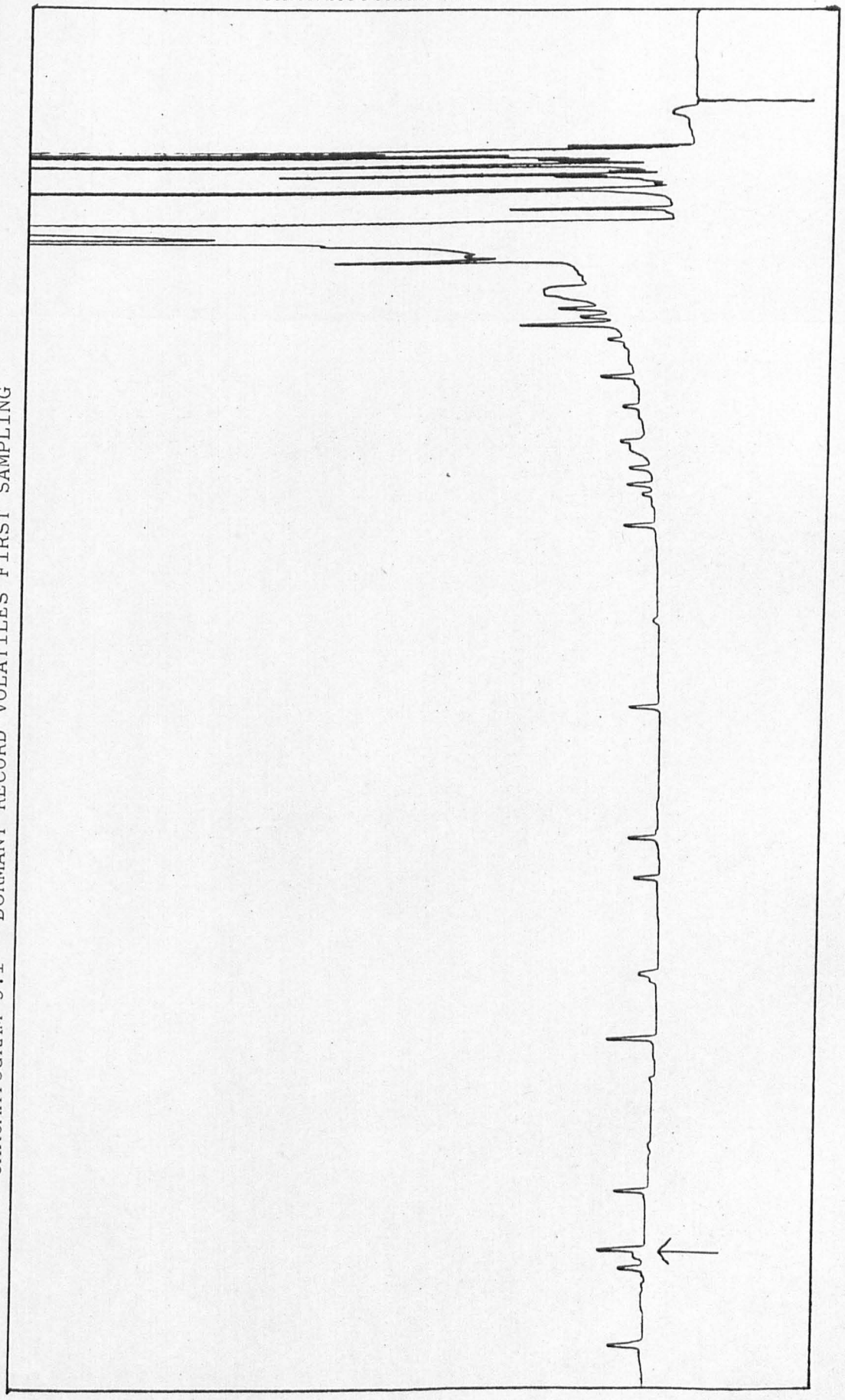
## 9.2 EXPERIMENTAL

The Vacuum Distillation system, Chapter 6, was used to collect the potato volatiles. The potatoes c.v. Golden Wonder and c.v. Record were obtained from commercial sources, they had not been treated with any chemicals post-harvest. The tubers were washed to remove any adhering soil, and then allowed to dry, the tubers were graded to obtain 50 kg of healthy undamaged tubers. They were then put in boxes and stored in the dark at 10°C for 4 wk before use. After 4 wk storage, the potatoes were examined and were thought to be dormant, the eyes were closed with no evidence of any of the tubers having sprouted. A sample of the potato volatiles was then taken from each variety of tubers, Chromatogram 9.1 - Record volatiles, Chromatogram 9.2 - Golden Wonder volatiles. One week after the first set of samples was taken, a second set of samples was taken, Chromatogram 9.3 - Record volatiles, Chromatogram 9.4 - Golden Wonder Volatiles. One week later, a third set of samples was taken, Chromatogram 9.5 - Record volatiles, Chromatogram 9.6 - Golden Wonder volatiles. The remaining potatoes were stored at 10°C for 6 wk till they had sprouted, both varieties sprouted evenly and at the same time. A further three sets of samples were then taken at weekly intervals from both potato varieties; first set: Chromatogram 9.7 - Record volatiles, Chromatogram 9.8 - Golden Wonder volatiles, second set: Chromatogram 9.9 - Record volatiles, Chromatogram 9.10 - Golden Wonder volatiles, third set: Chromatogram 9.11 - Record volatiles, Chromatogram 9.12 - Golden Wonder volatiles. By the time the third set of samples were taken,

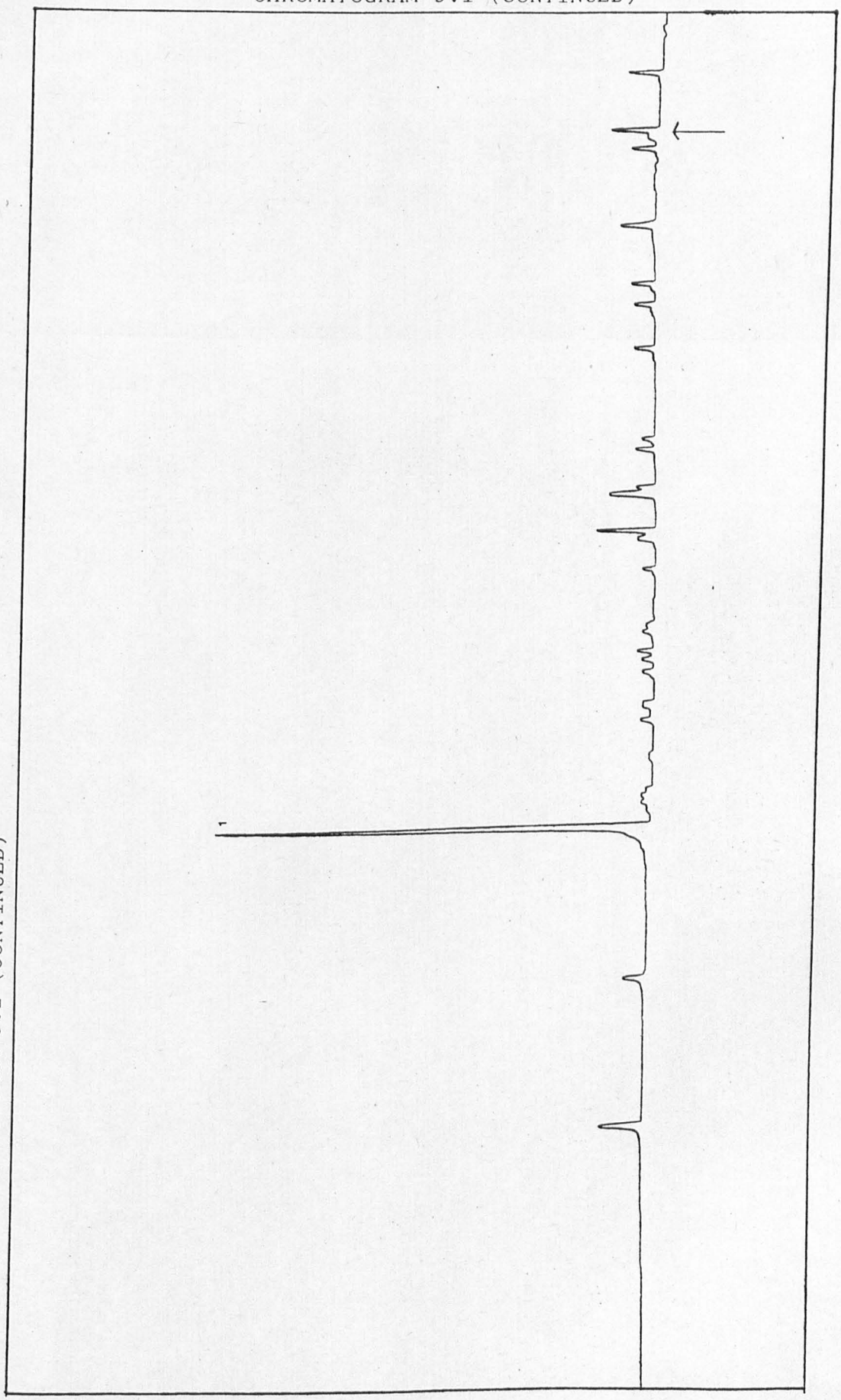
both varieties had sprouts 5 mm - 10 mm long. All the potato volatile samples from the cold trap of the Vacuum Distillation system were 1.0 $dm^3$ in volume, at a flow rate of 20 cc $min^{-1}$ - 25 cc $min^{-1}$. The sampled precolumns were analysed according to the method described by System 3, Chapter 7.

Gas Chromatography

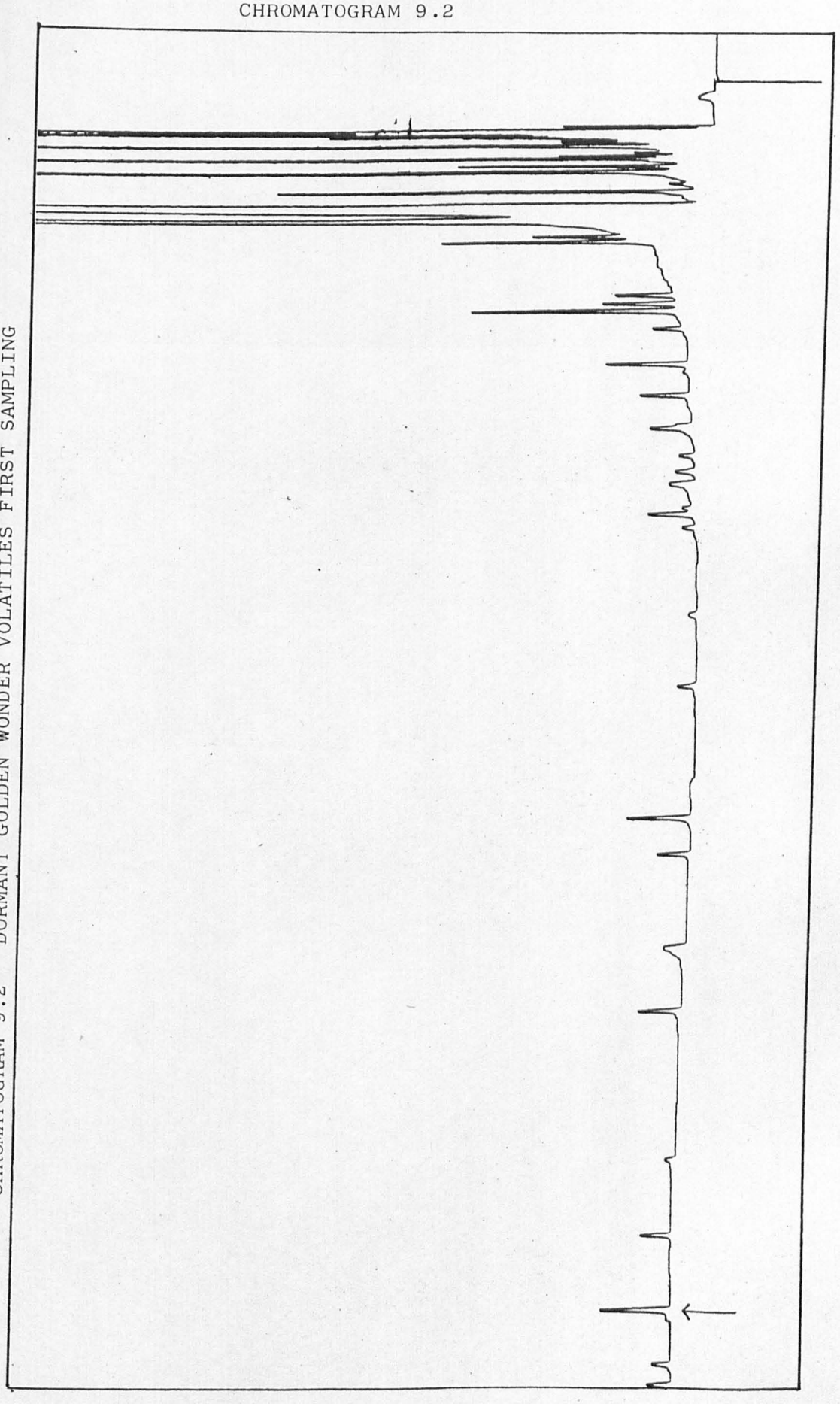
| | | |
|---|---|---|
| G.C.: | | Pye 104 (Pye Unicam Ltd). |
| Column: | | C.W.20M S.C.O.T. glass 43 m, 1 mm O.D., 0.5 mm I.D., (S.G.E., U.K. Ltd). |
| Carrier Gas: | | $H_2$ u = 25 cm $sec^{-1}$. |
| Temperature: | Initial | 50°C 10 min. |
| | Rise | 1°C $min^{-1}$. |
| | Final | 185°C 60 min. |
| Detector: F.I.D. | | 270°C. |
| Detector Gasses: | | $H_2$ 15 cc $min^{-1}$. |
| | | $O_2$ 200 cc $min^{-1}$. |
| | | $N_2$ 15 cc $min^{-1}$. |
| Sensitivity: | | $5 \times 10^{-10}$ A. |
| Chart Speed: | | 5 mm $min^{-1}$. |



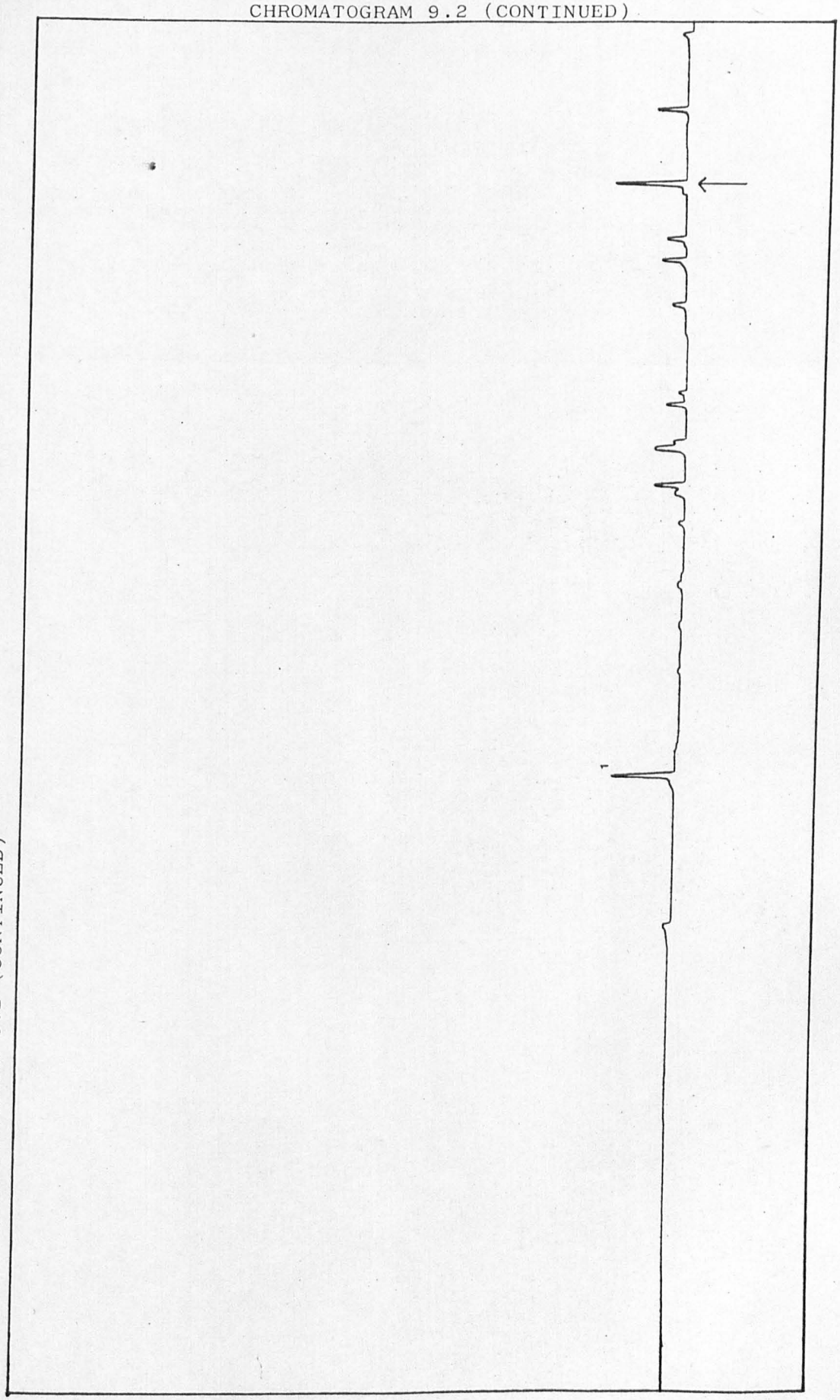
CHROMATOGRAM 9.1 DORMANT RECORD VOLATILES FIRST SAMPLING



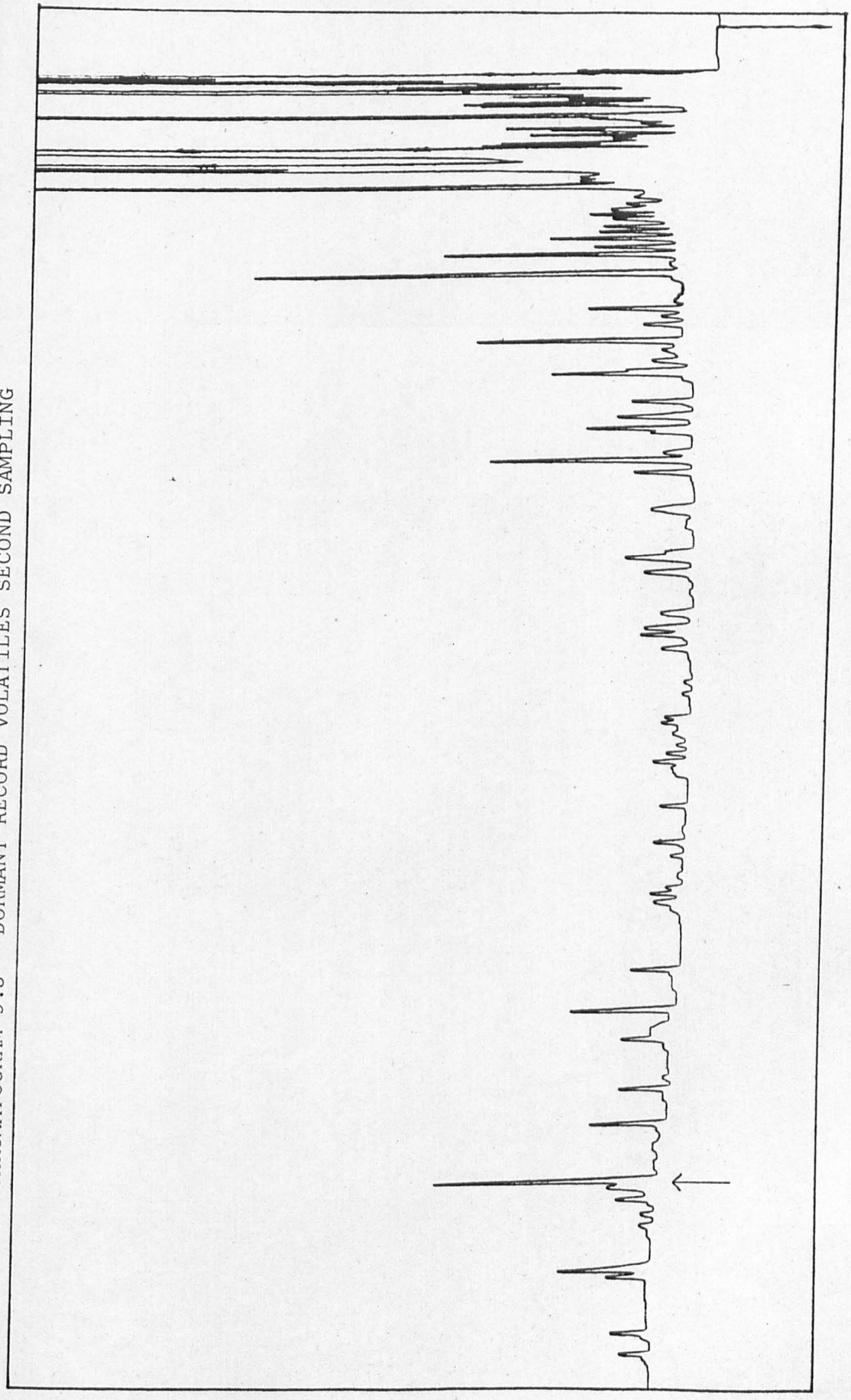

CHROMATOGRAM 9.1 (CONTINUED)



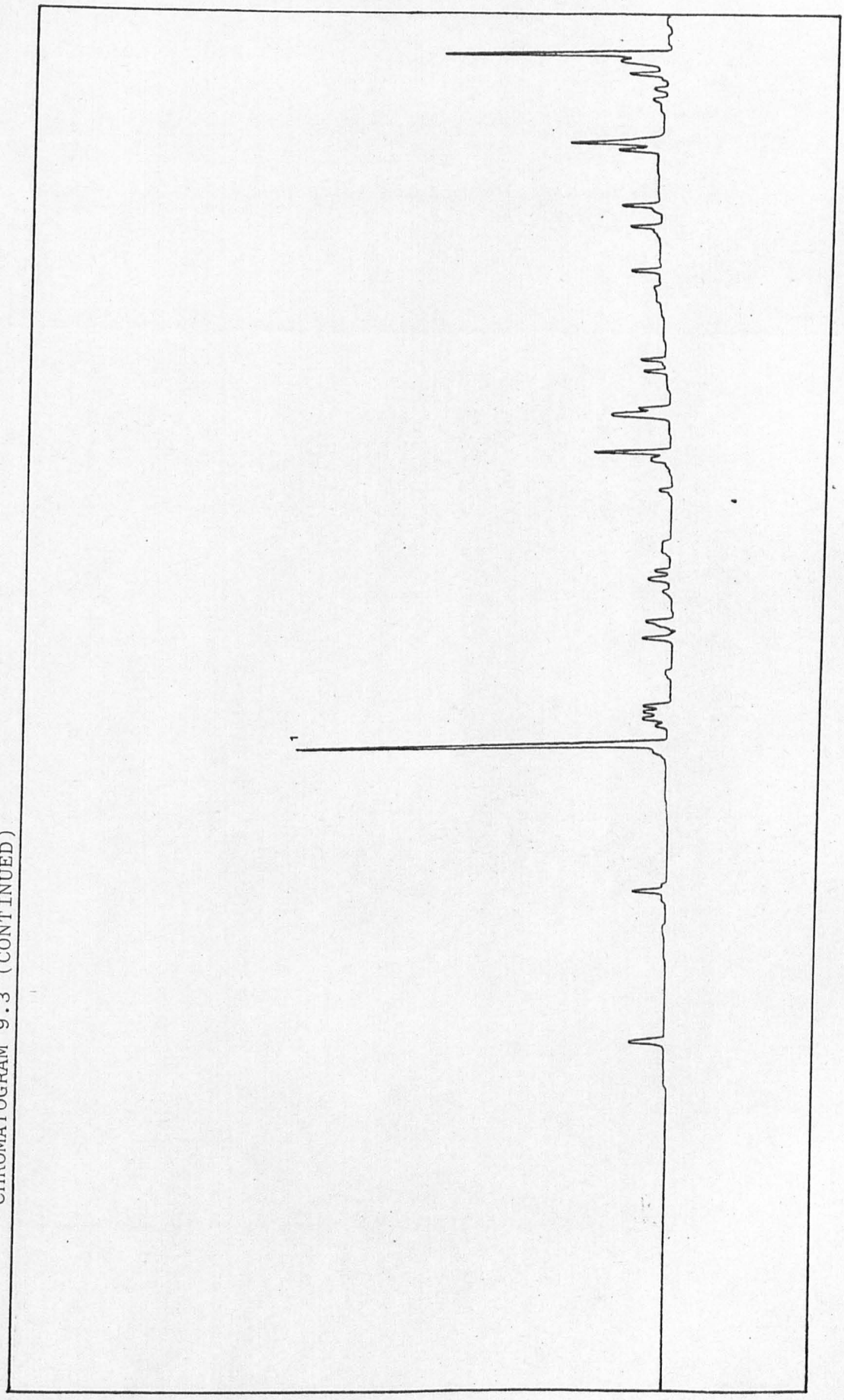
CHROMATOGRAM 9.2 DORMANT GOLDEN WONDER VOLATILES FIRST SAMPLING



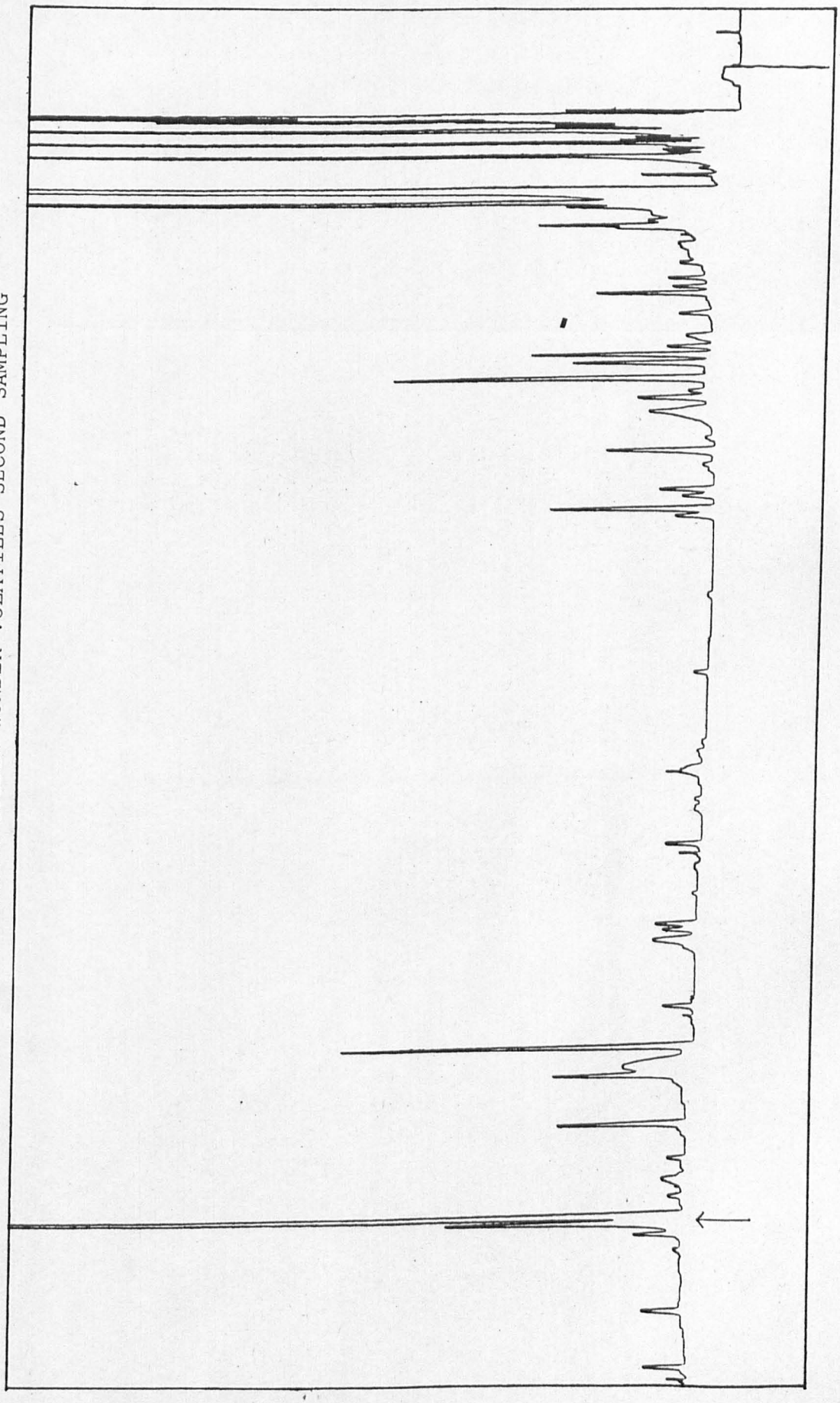

CHROMATOGRAM 9.2 (CONTINUED)



CHROMATOGRAM 9.3 DORMANT RECORD VOLATILES SECOND SAMPLING

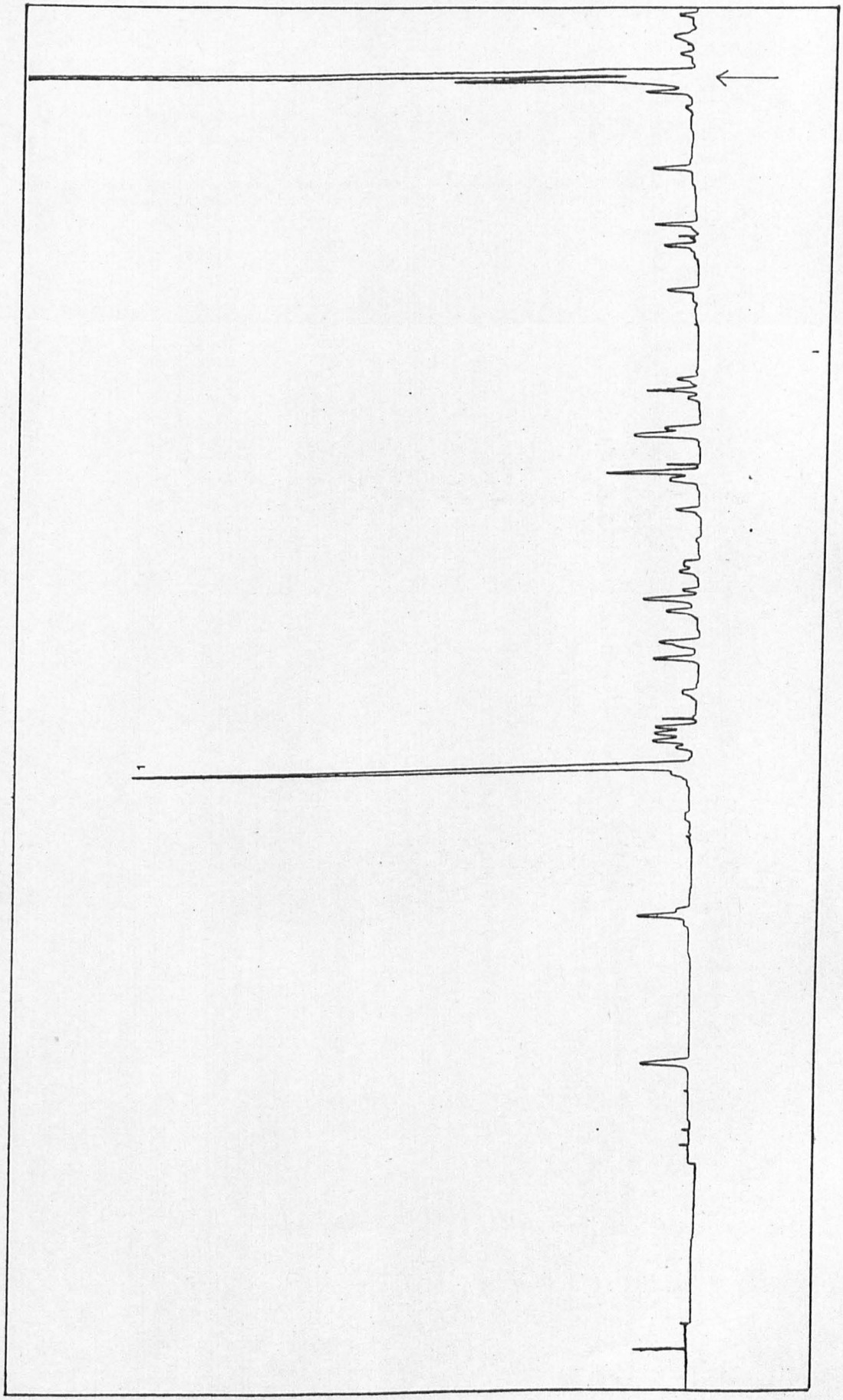



CHROMATOGRAM 9.3 (CONTINUED)



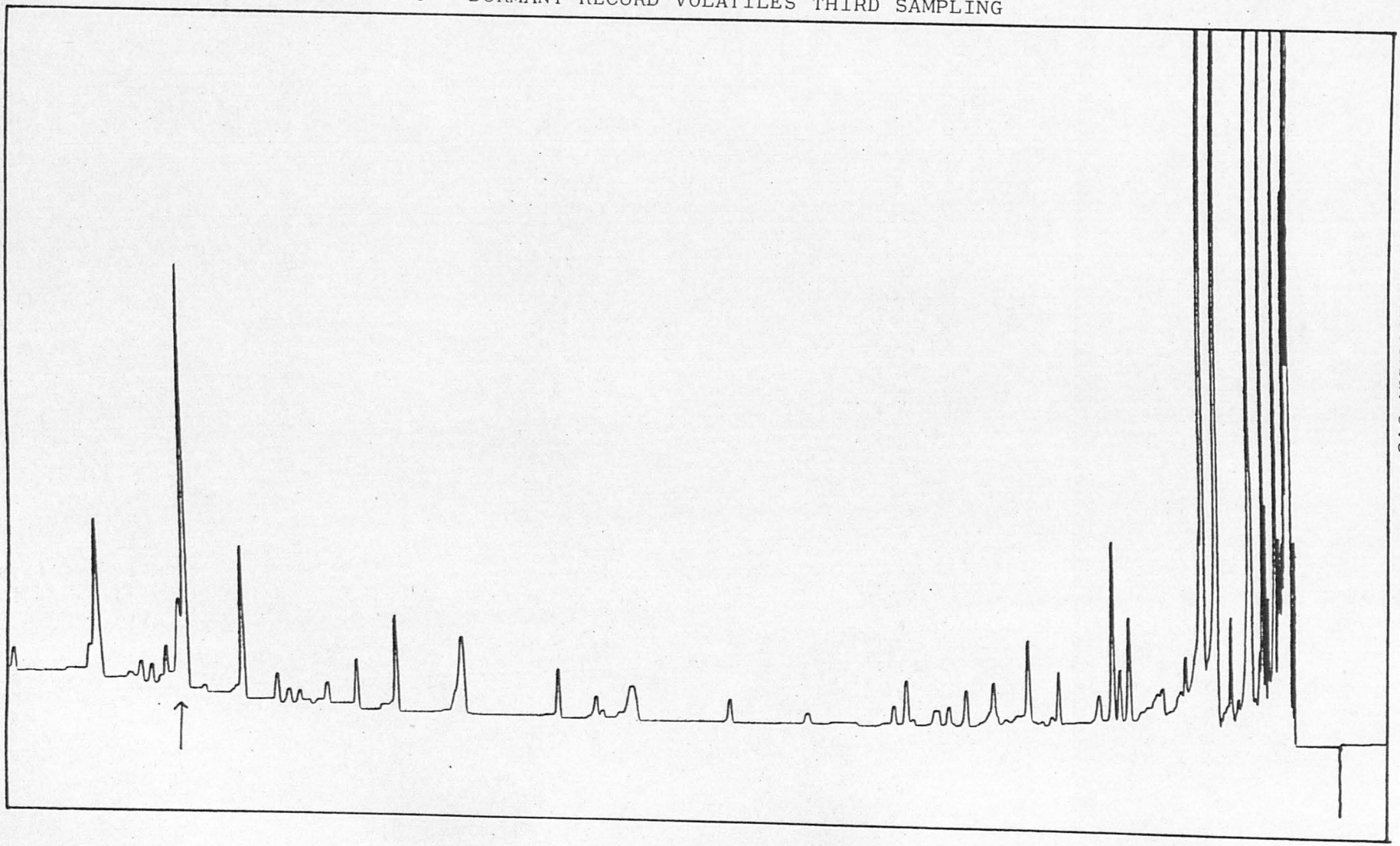
CHROMATOGRAM 9.4 DORMANT GOLDEN WONDER VOLATILES SECOND SAMPLING

CHROMATOGRAM 9.4 (CONTINUED)



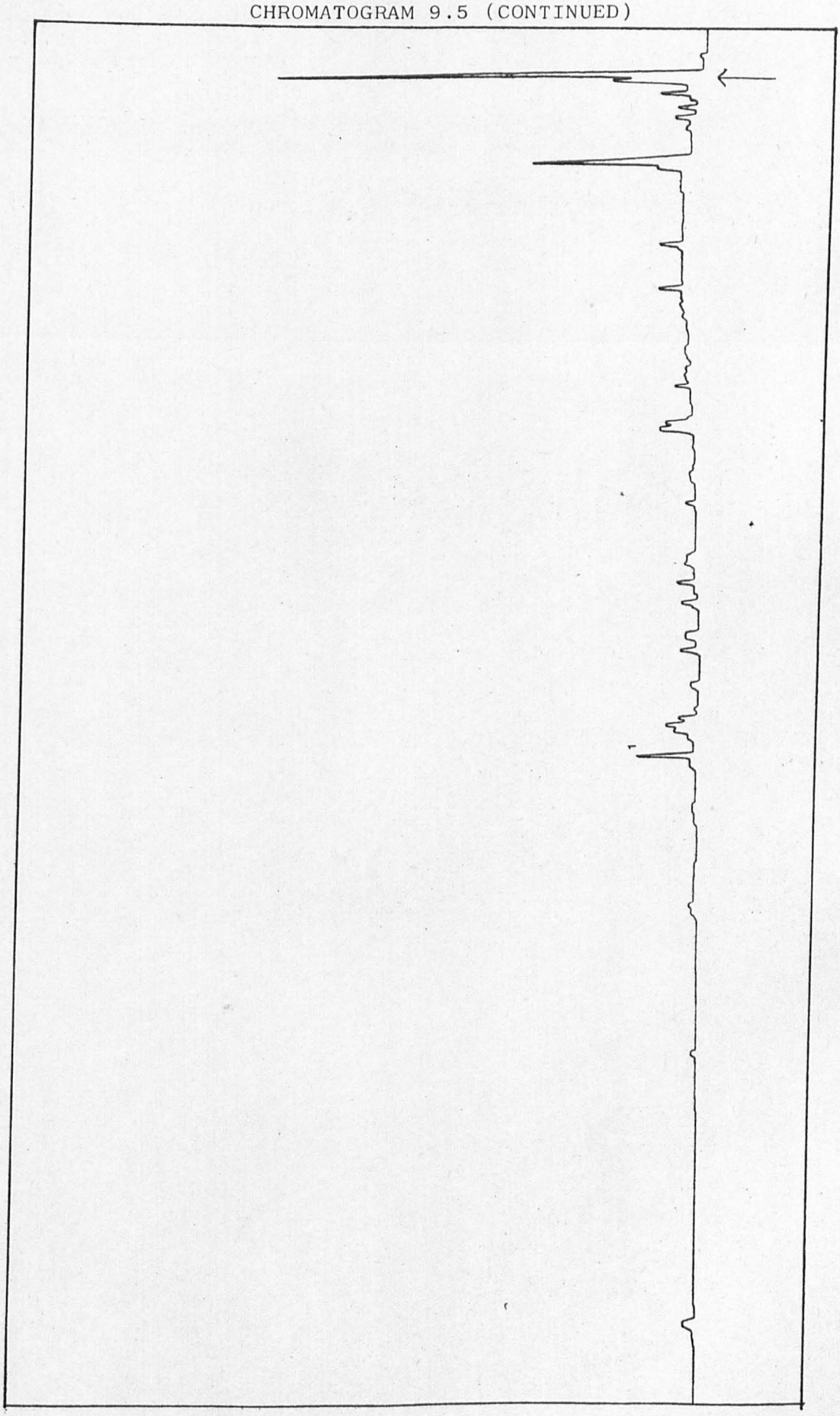

CHROMATOGRAM 9.4 (CONTINUED)

CHROMATOGRAM 9.5 DORMANT RECORD VOLATILES THIRD SAMPLING



CHROMATOGRAM 9.5

CHROMATOGRAM 9.5 (CONTINUED)



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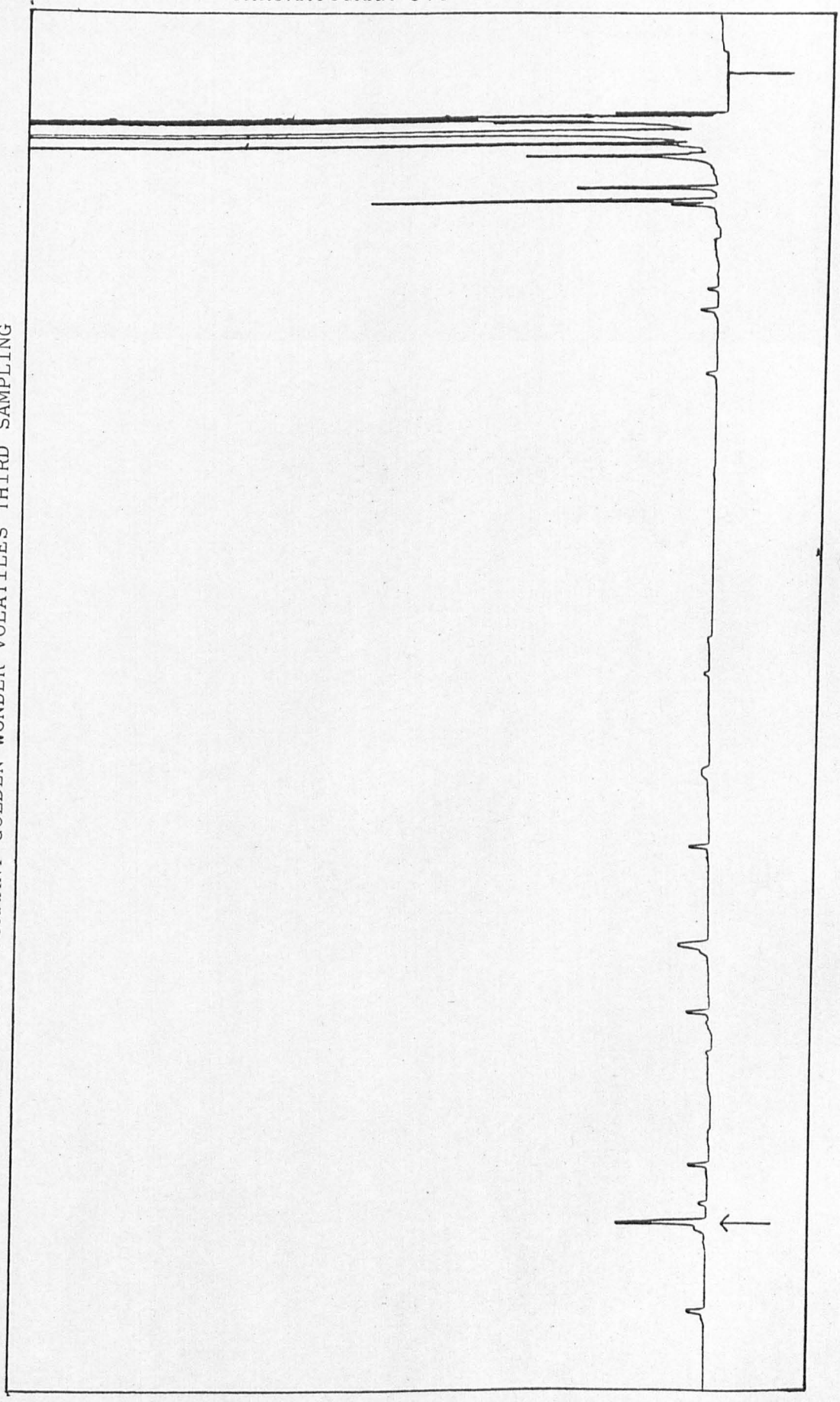
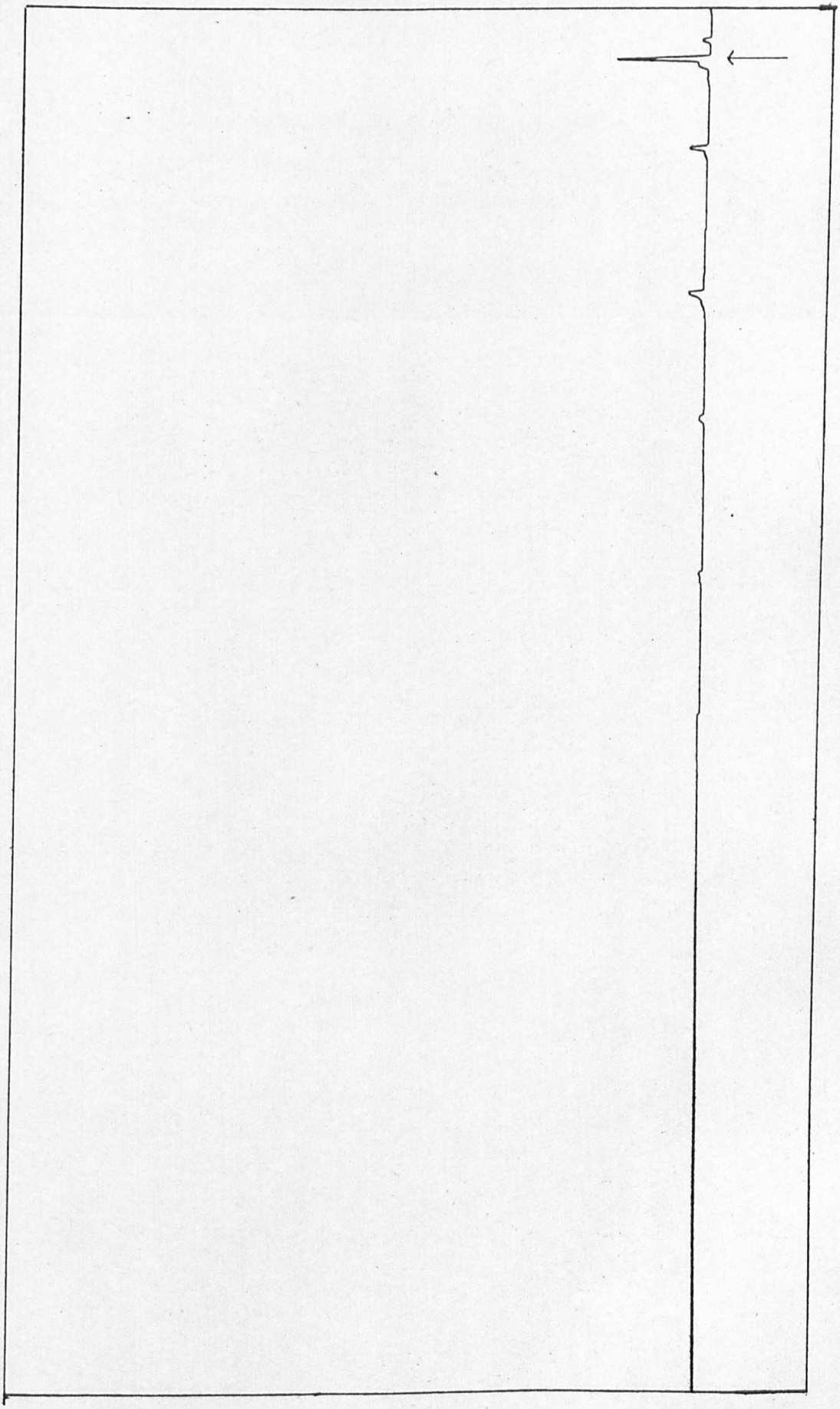


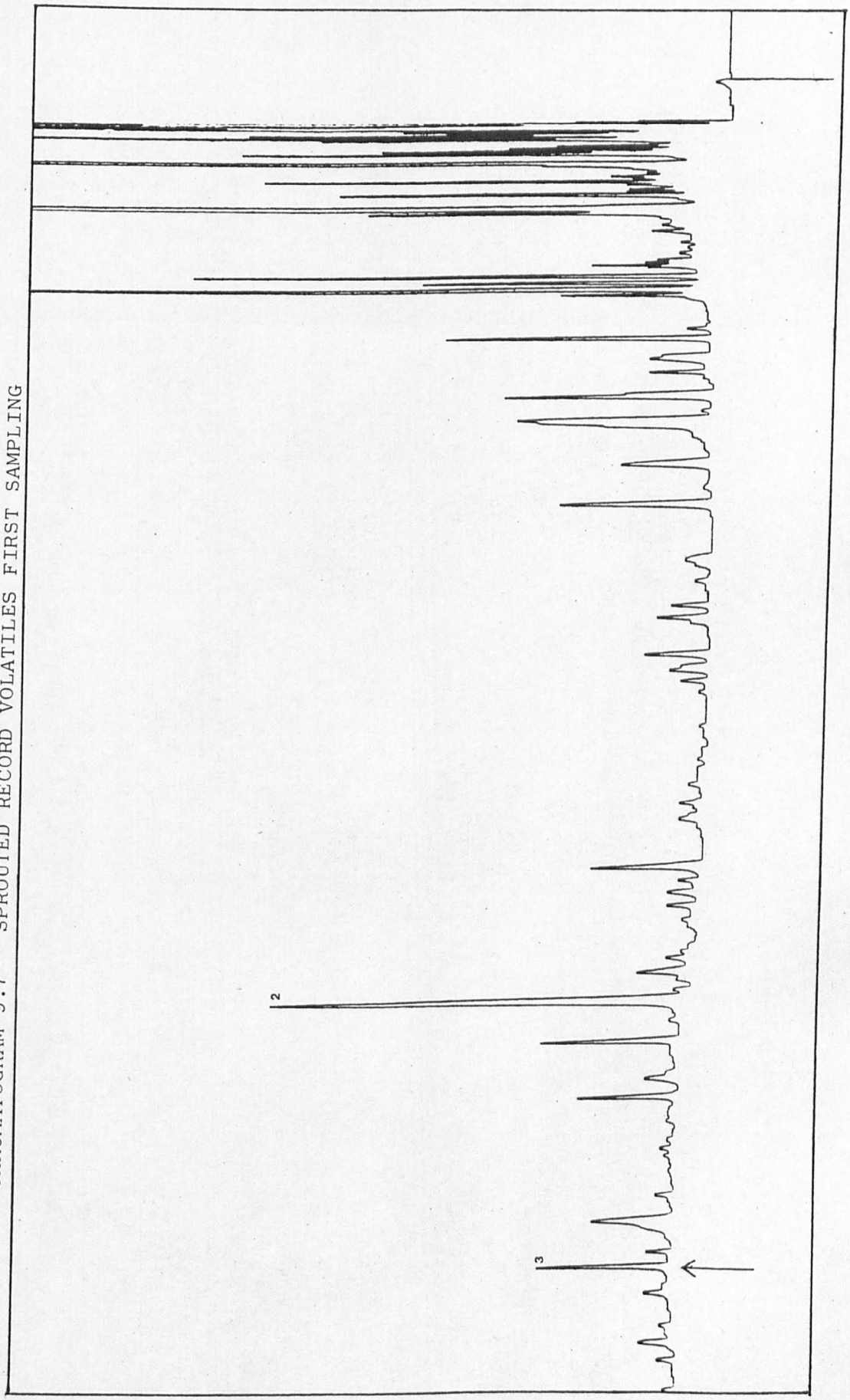
CHROMATOGRAM 9.6 DORMANT GOLDEN WONDER VOLATILES THIRD SAMPLING

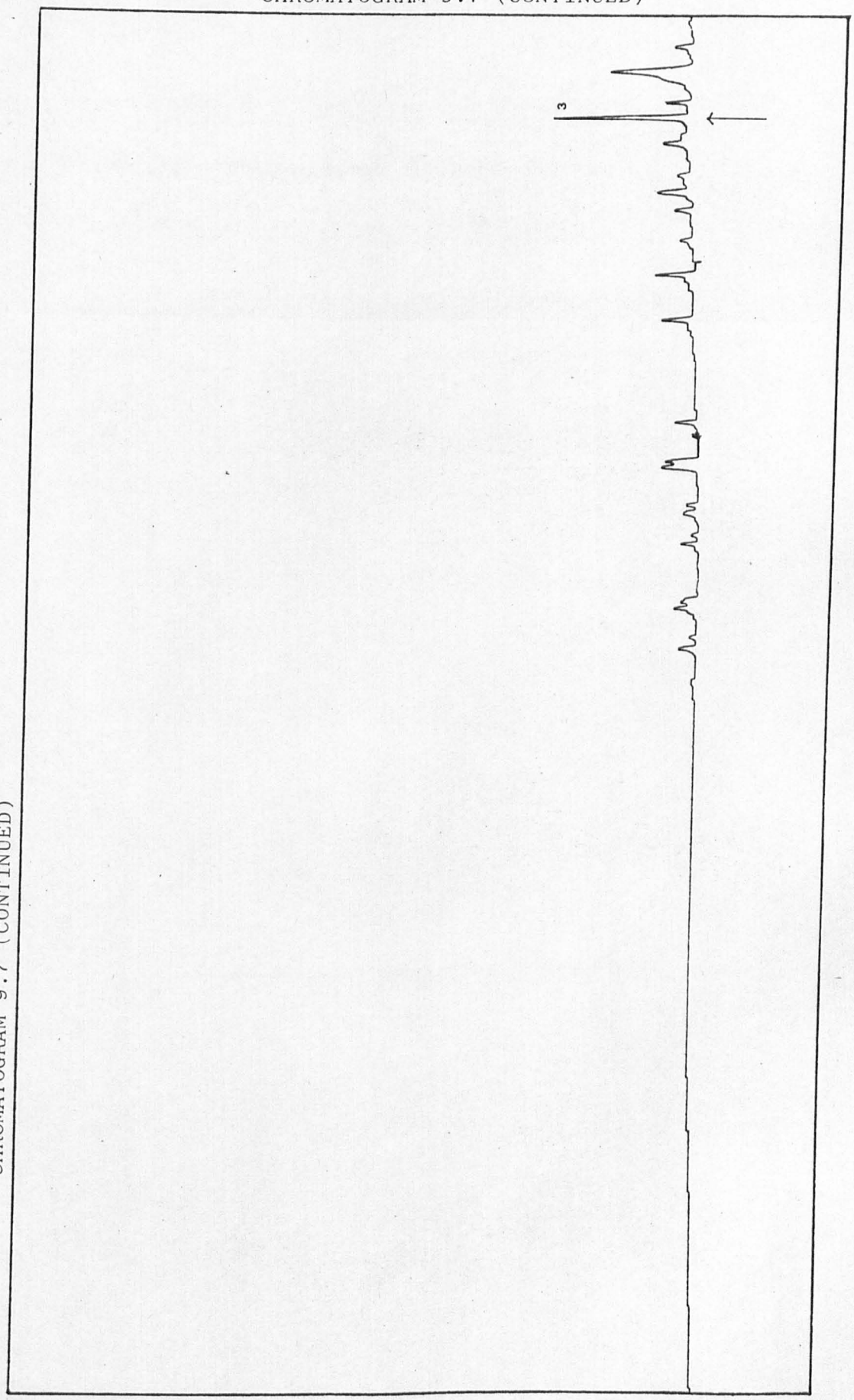
CHROMATOGRAM 9.6 (CONTINUED)



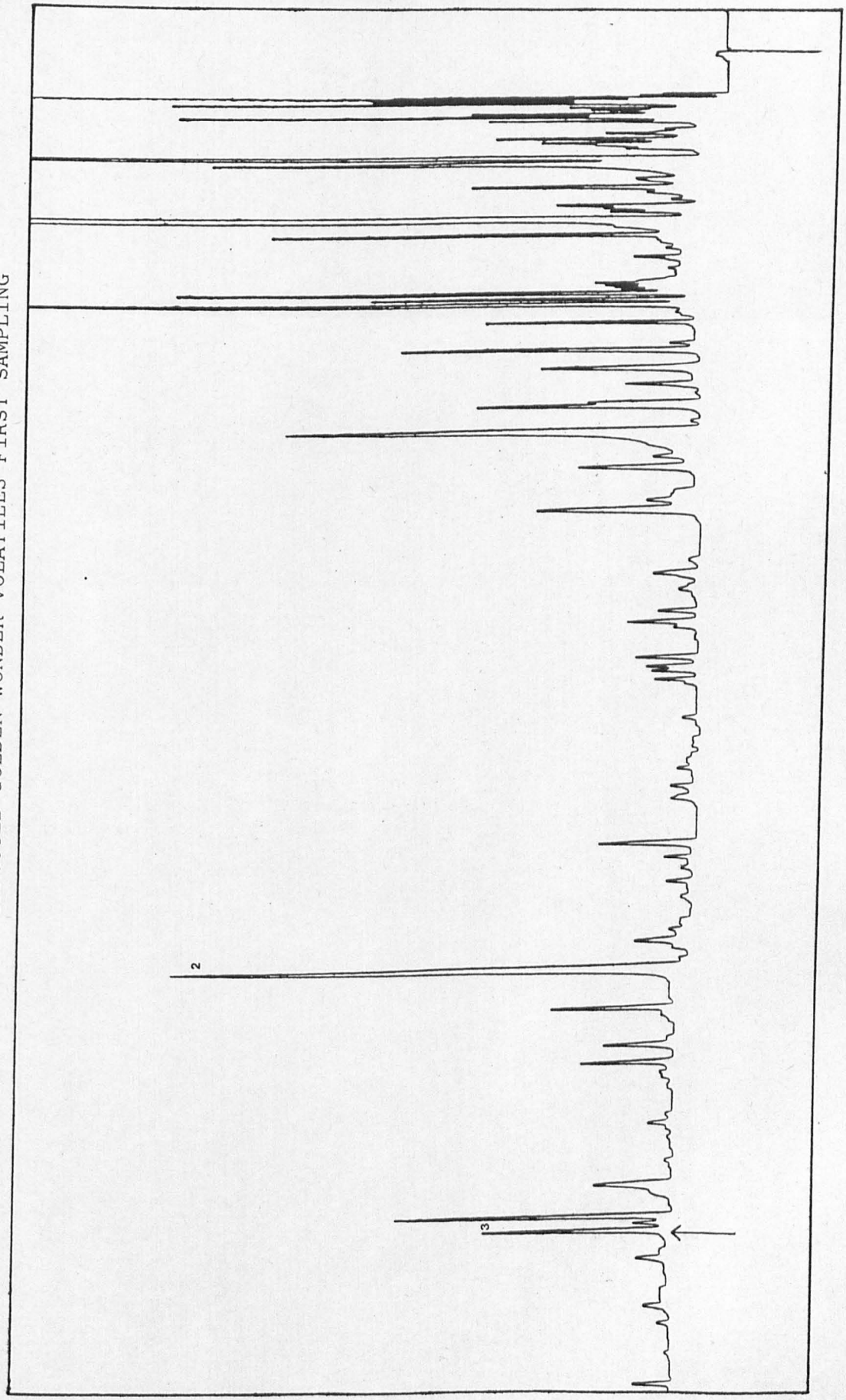
CHROMATOGRAM 9.6 (CONTINUED)

CHROMATOGRAM 9.7 SPROUTED RECORD VOLATILES FIRST SAMPLING

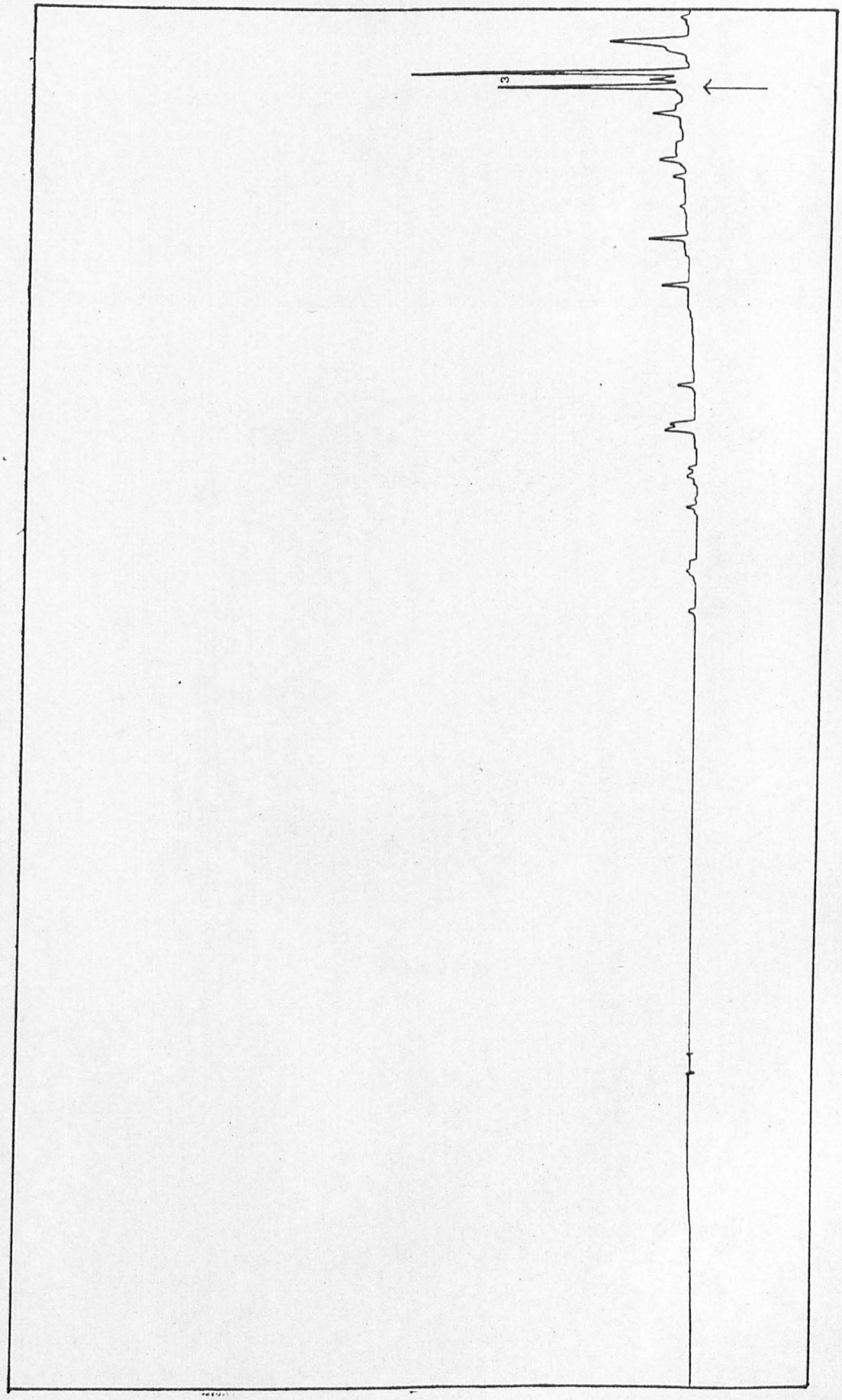





CHROMATOGRAM 9.7 (CONTINUED)

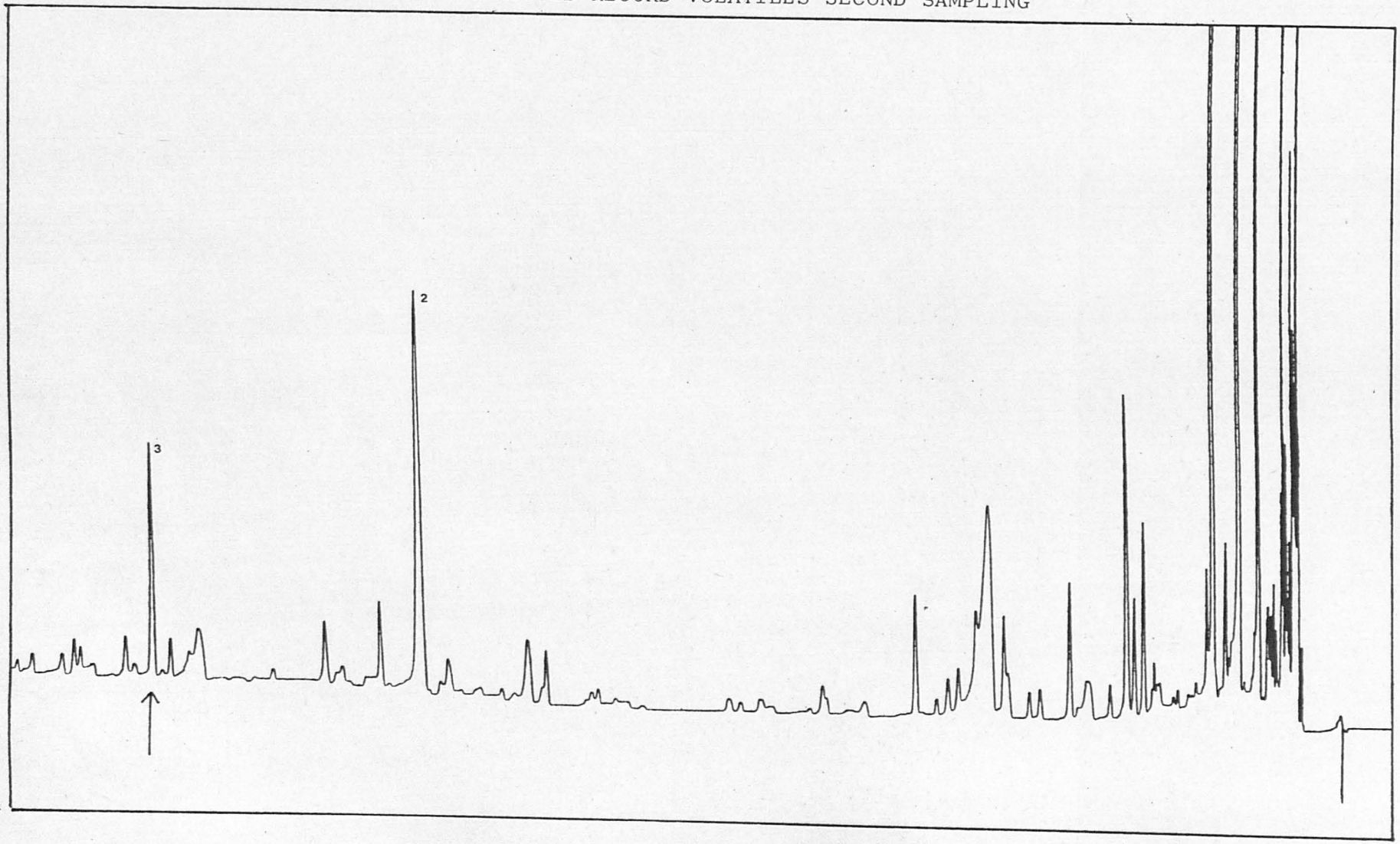

CHROMATOGRAM 9.8 SPROUTED GOLDEN WONDER VOLATILES FIRST SAMPLING
2
3

CHROMATOGRAM 9.8 (CONTINUED)

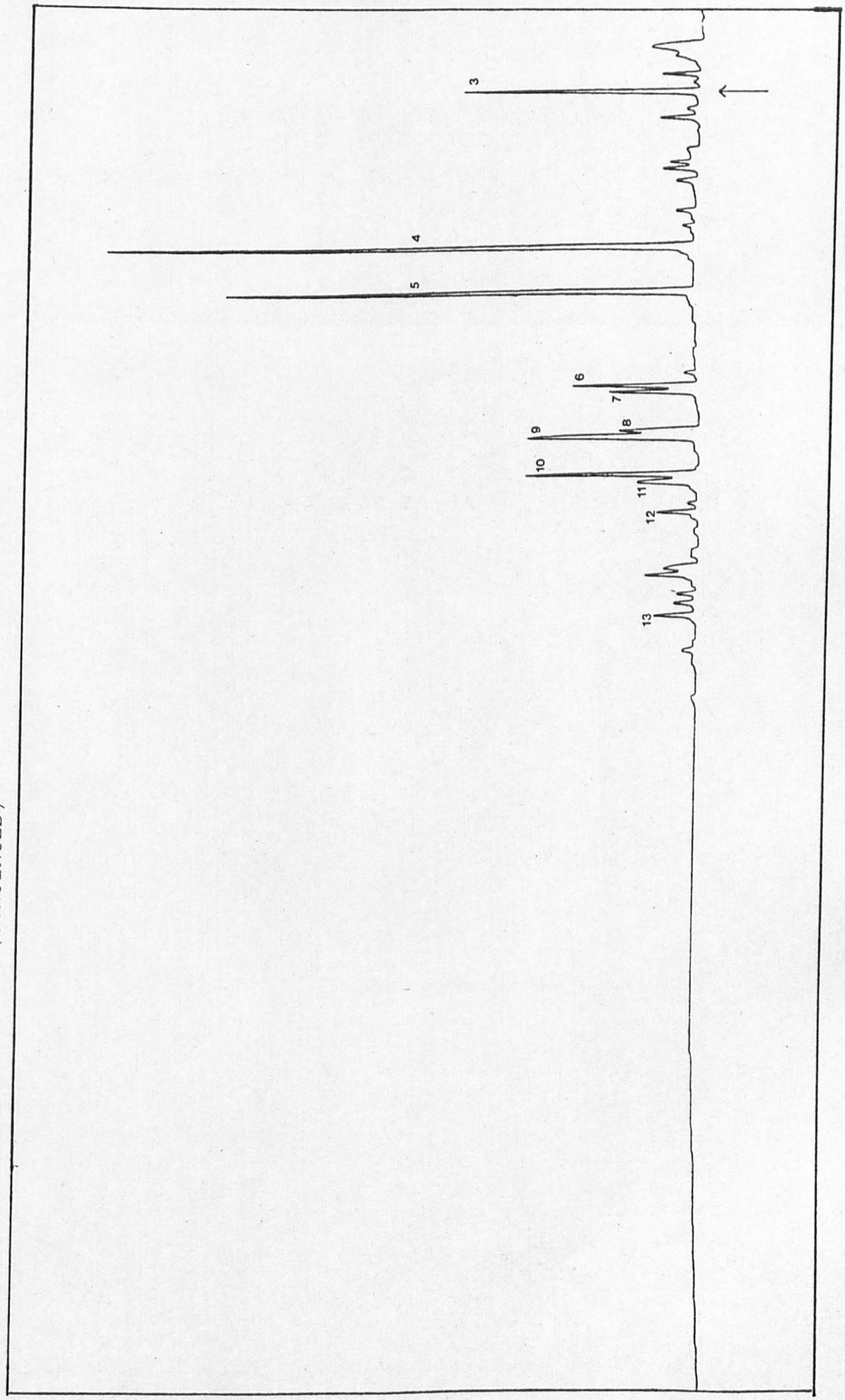



CHROMATOGRAM 9.8 (CONTINUED)

CHROMATOGRAM 9.9 SPROUTED RECORD VOLATILES SECOND SAMPLING

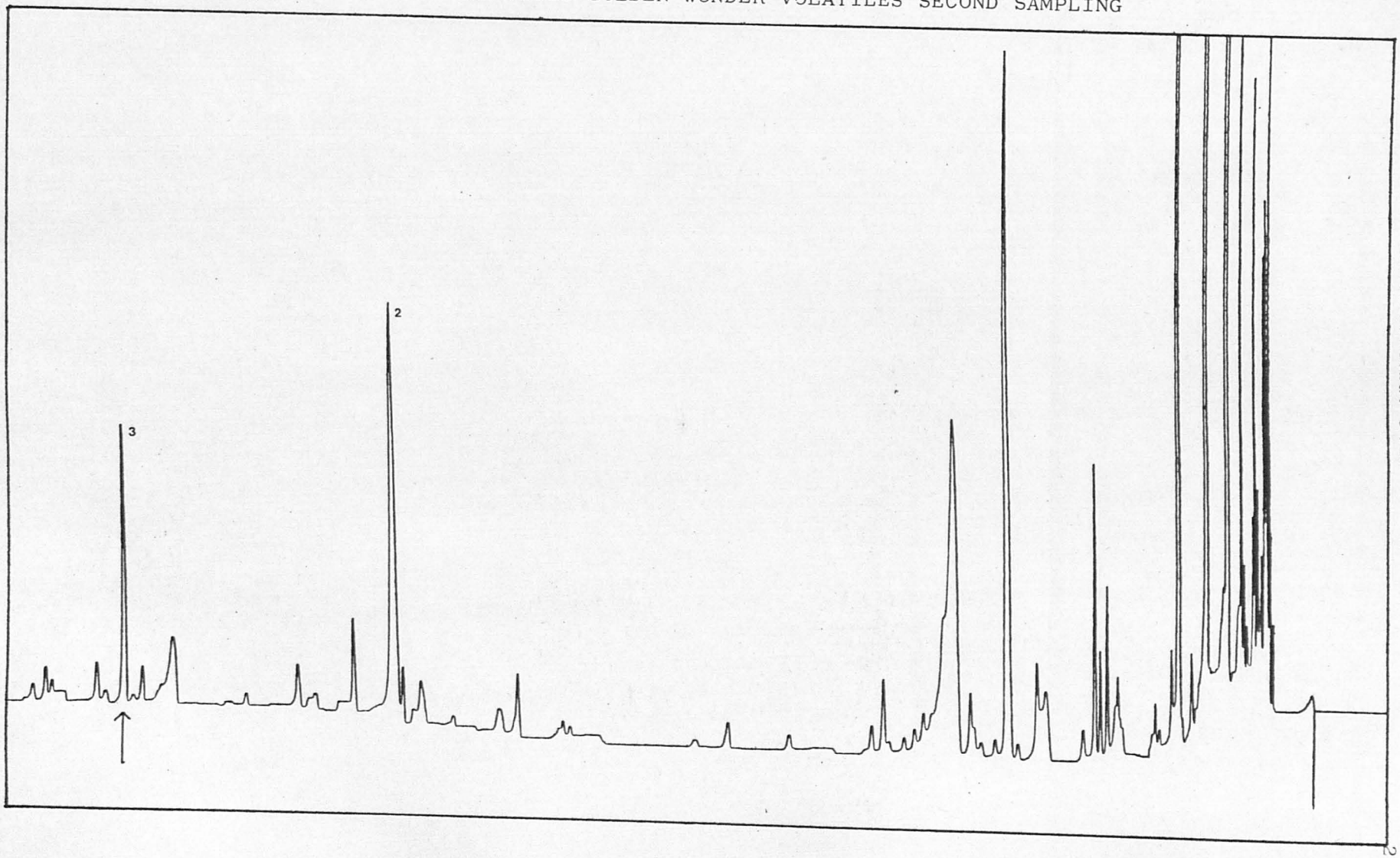



CHROMATOGRAM 9.9

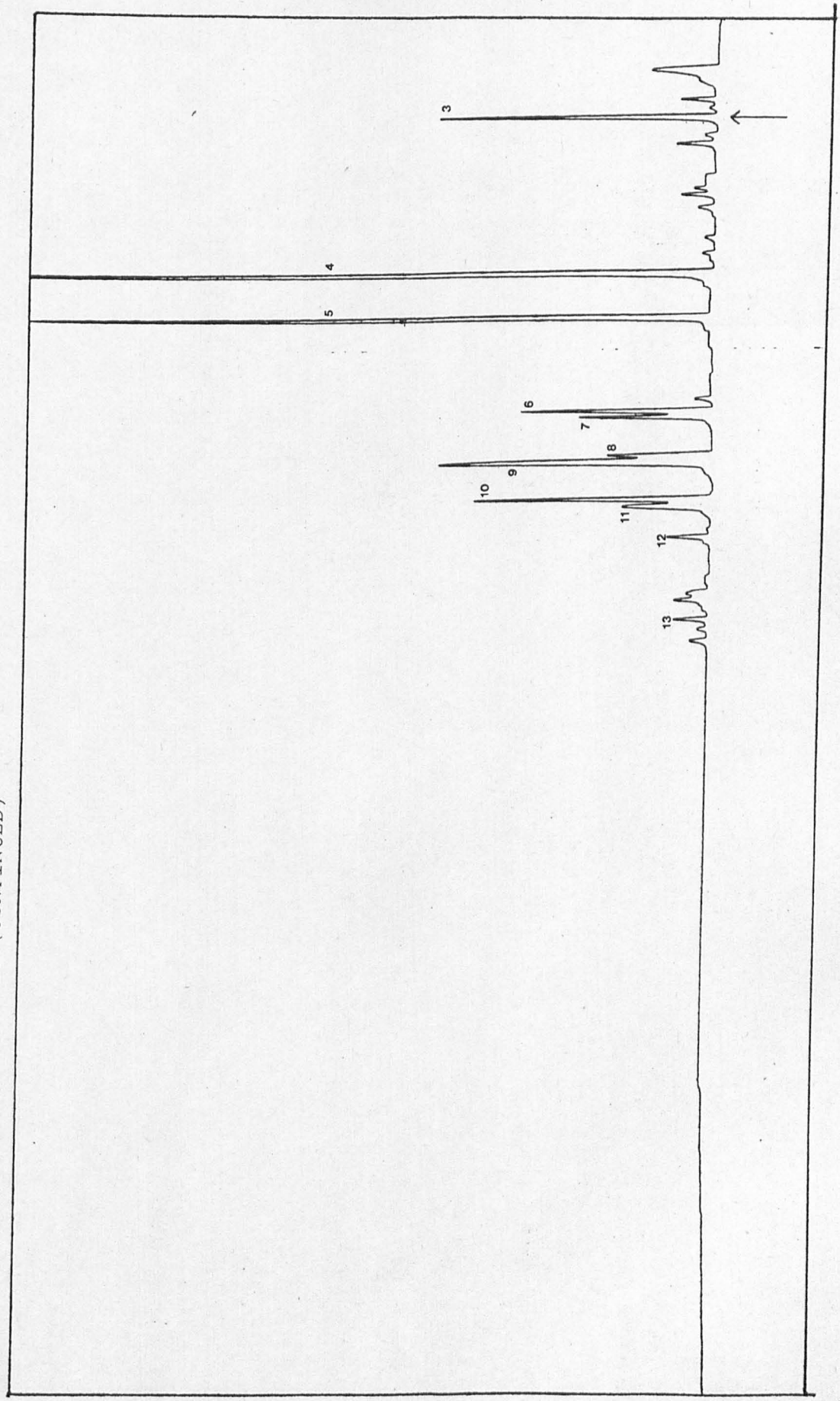



CHROMATOGRAM 9.9 (CONTINUED)

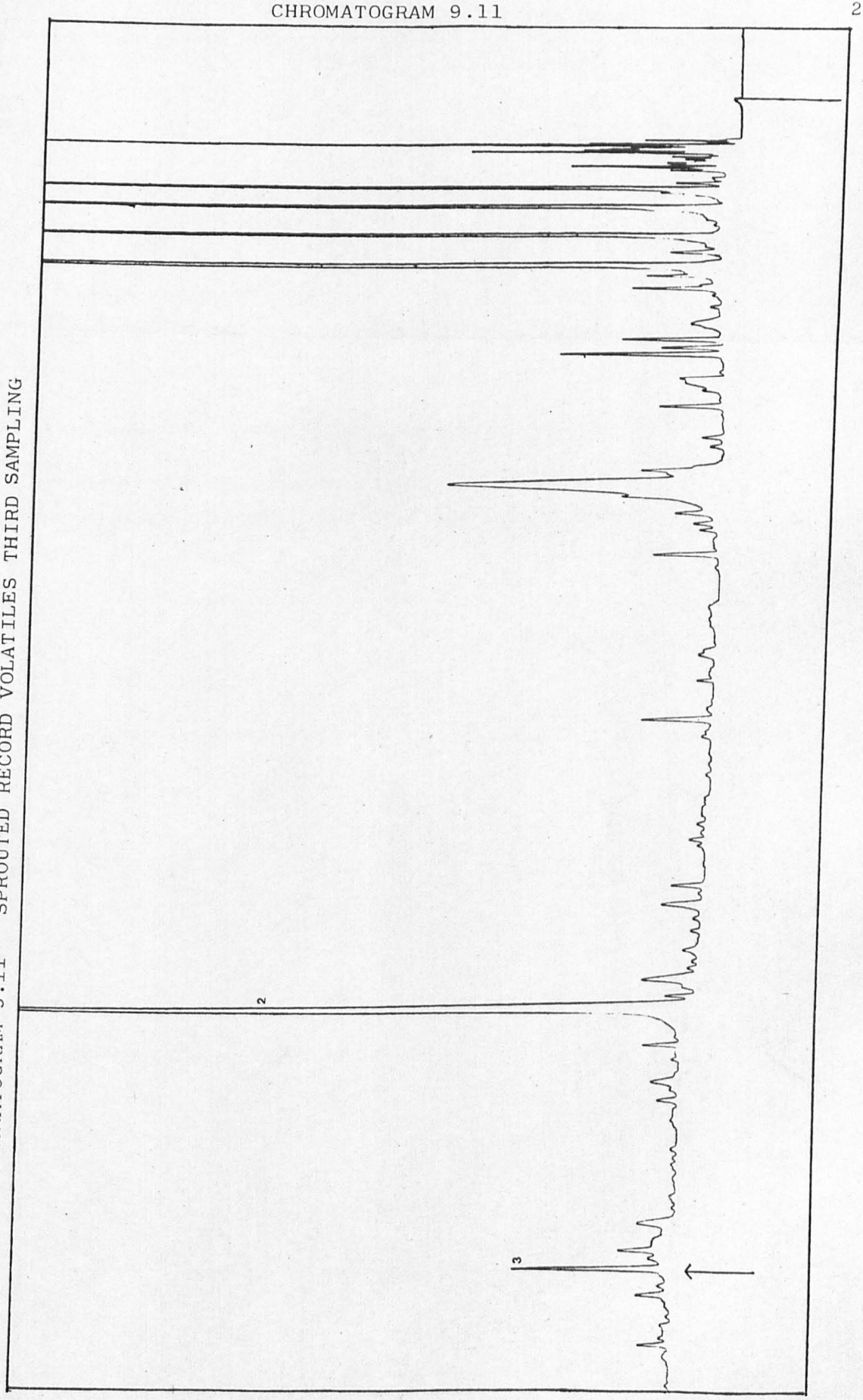
CHROMATOGRAM 9.10 SPROUTED GOLDEN WONDER VOLATILES SECOND SAMPLING



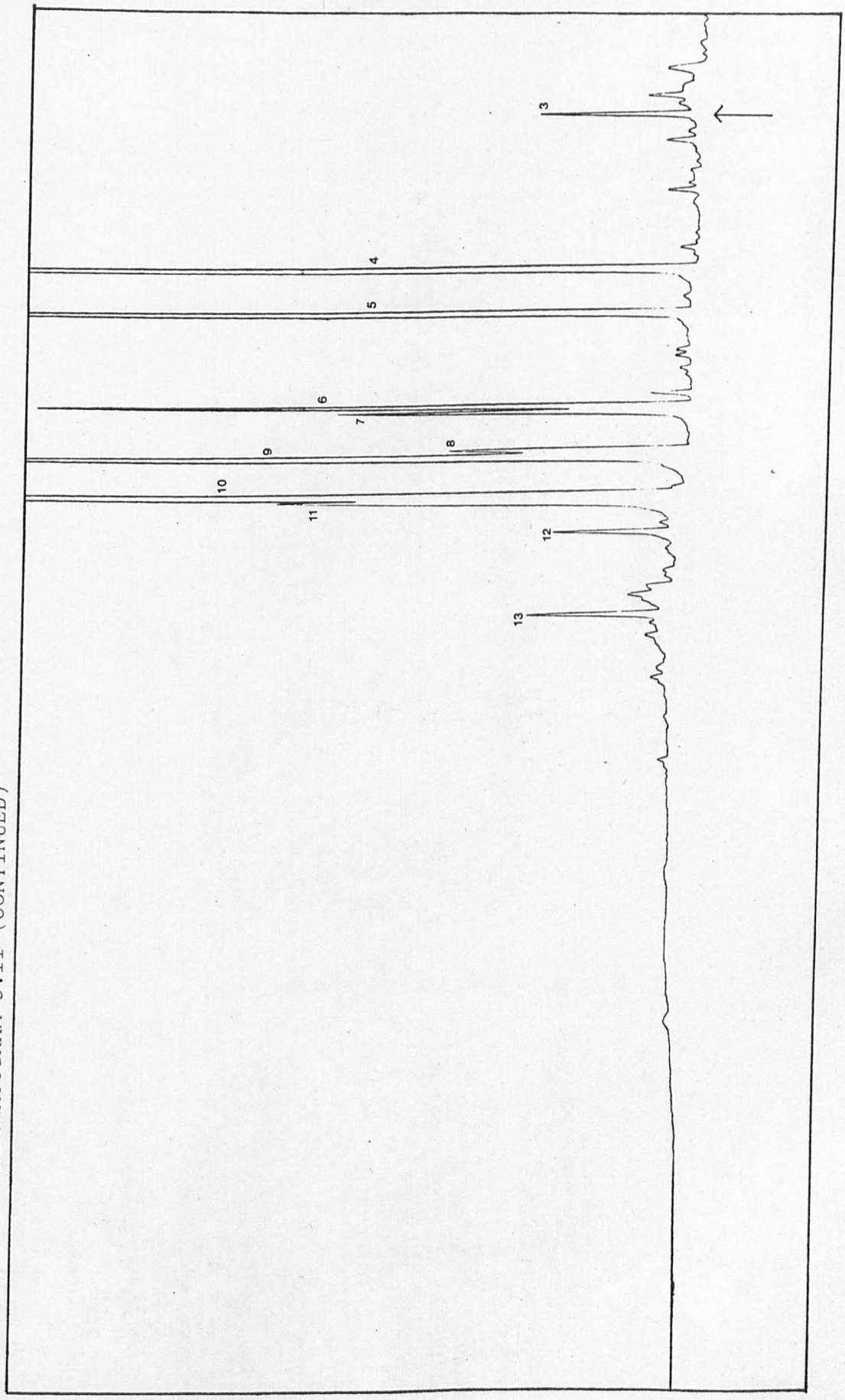

CHROMATOGRAM 9.10




CHROMATOGRAM 9.10 (CONTINUED)

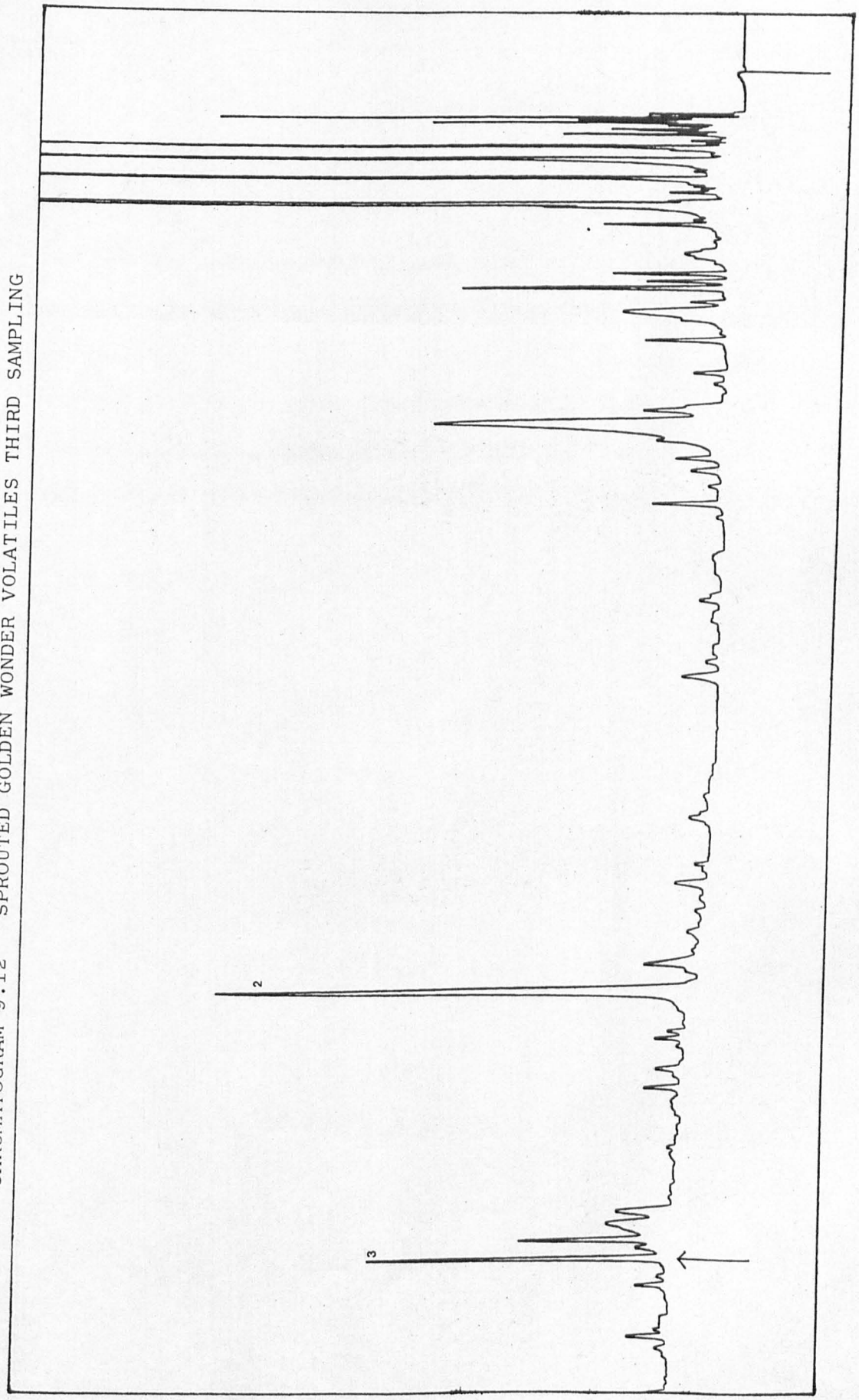

CHROMATOGRAM 9.11 SPROUTED RECORD VOLATILES THIRD SAMPLING
2
3

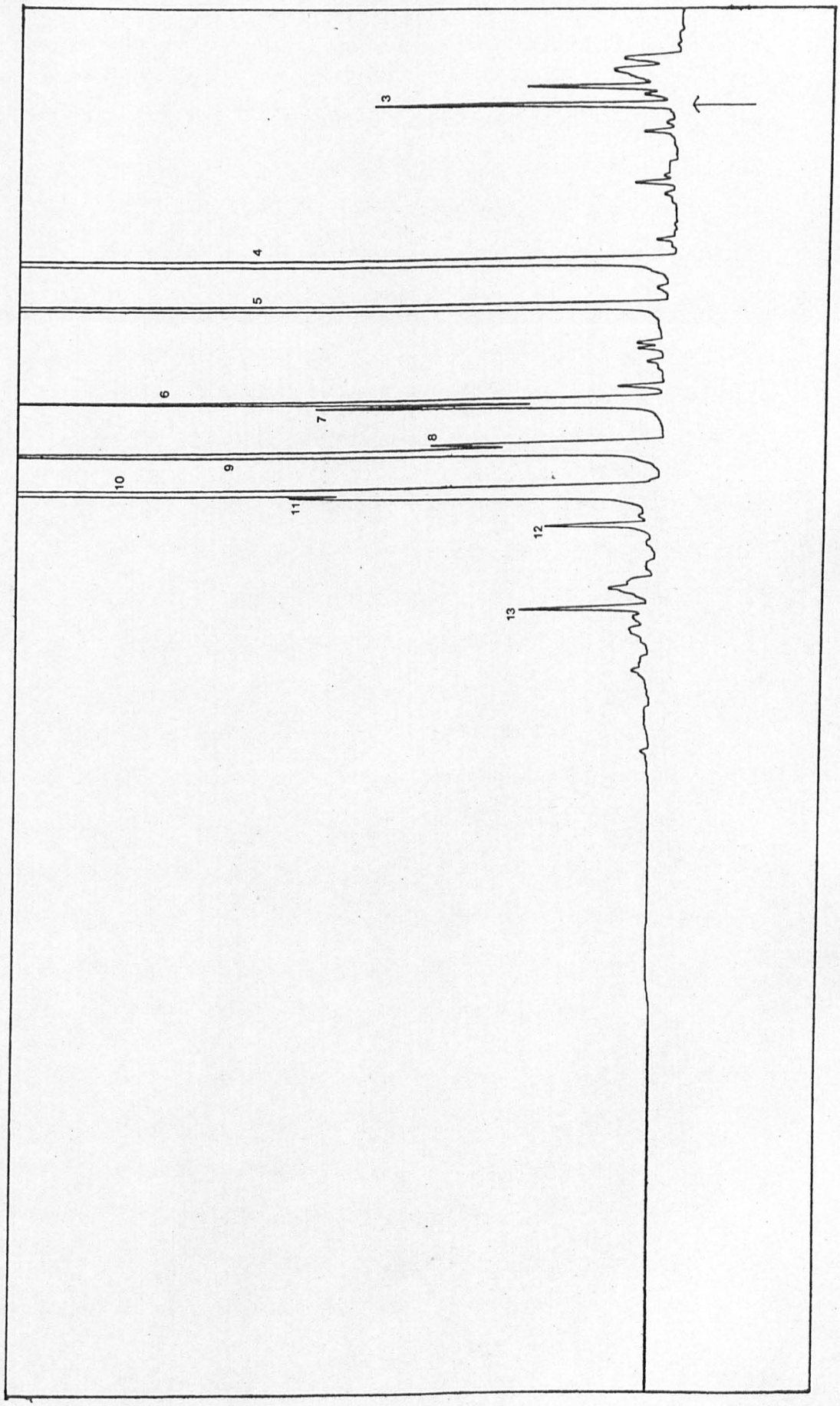
CHROMATOGRAM 9.11 (CONTINUED)




CHROMATOGRAM 9.11 (CONTINUED)

CHROMATOGRAM 9.12 SPROUTED GOLDEN WONDER VOLATILES THIRD SAMPLING






CHROMATOGRAM 9.12 (CONTINUED)

## 9.3 DISCUSSION

A comparison of the volatile profiles of the Record and Golden Wonder tubers showed that at the same sampling times the volatiles obtained were very similar with only a few differences between the varieties. The changes in volatile profiles with time were also very similar, it was concluded that in the varieties of potatoes investigated, the varietal and dormancy characteristics had no major effect on the volatiles produced.

The most noticeable difference between dormant and sprouted tubers for both varieties was in the decrease of peak 1 and the increase in peaks 2 - 13, when the potatoes broke dormancy. Apart from these, the most obvious changes, it is difficult to draw any further conclusions from the volatile profiles, any changes in the more volatile compounds that eluted within the first 15 min could have been due to small variations in the sampling conditions which would have affected the more volatile components to a greater extent. The reason for the lack of volatiles in Chromatogram 9.6 is not known, the levels of potato volatiles from a duplicate sample were the same as found in Chromatogram 9.6.

The decrease in the level of peak 1 as the potatoes broke dormancy suggested that either peak 1 decreased as a result of the tubers breaking dormancy or that peak 1 was involved in the tubers dormancy and its decrease was responsible for the breaking of dormancy. The increase in peaks 2 - 13 also appeared to be linked to the tubers dormancy.

It was hoped that these volatile samples would have been analysed by G.C.M.S., in particular for the identification of peaks 1 - 13. Their identification would have enabled experiments to be set up to assess the effect that these compounds had on the dormancy of potato tubers. However, G.C.M.S. facilities were not available. The conclusions that were drawn from the results as they stood were that the potato volatiles change in relation to dormant and sprouted tubers and that differences between Golden Wonder and Record potato volatiles were small under the sampling conditions used.

# CHAPTER 10

# G.C.M.S. ANALYSIS OF POTATO VOLATILES

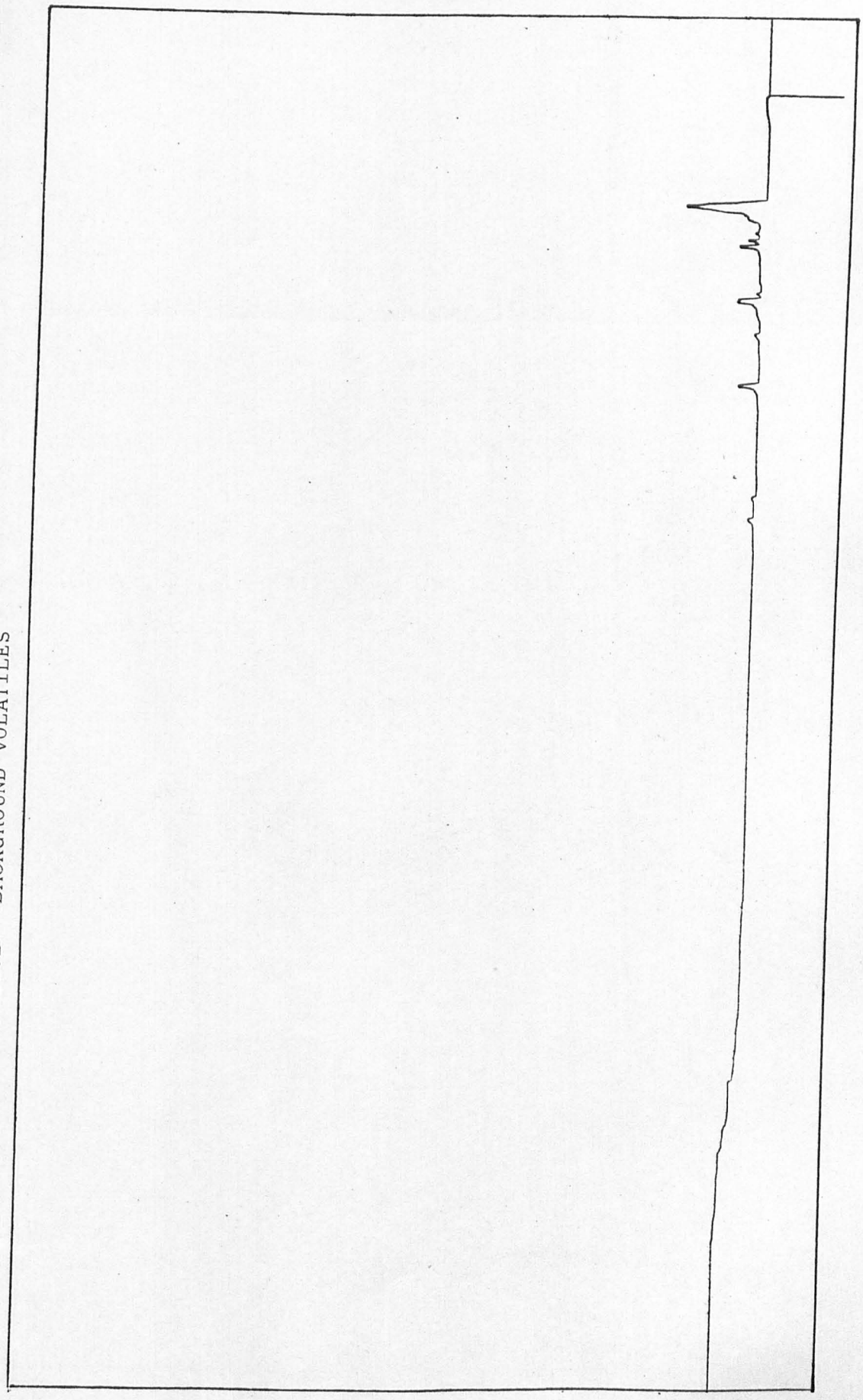
## 10.1 INTRODUCTION

This chapter is concerned with the G.C.M.S. analysis of the headspace volatiles produced by potato tubers that had broken dormancy.

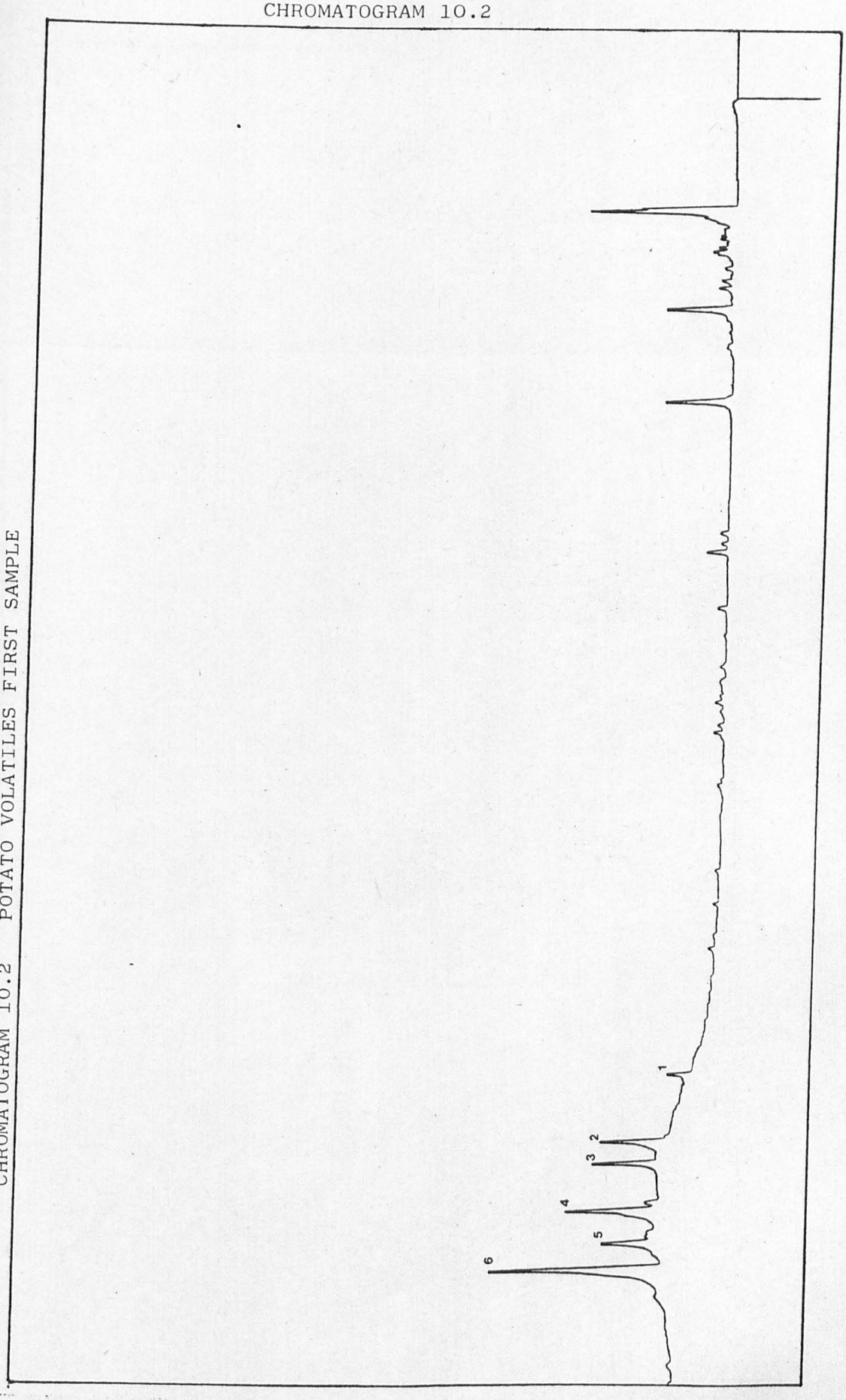
An opportunity had arisen for the analysis of the potato volatiles by G.C.M.S., unfortunately there were no dormant potato tubers available, it would have been preferable to analyse the headspace volatiles produced by dormant tubers. However, it was thought that an identification of the volatiles produced by non-dormant tubers would be worthwhile, as up to this point in the project a G.C.M.S. analysis of potato volatiles had not been performed.

## 10.2 EXPERIMENTAL

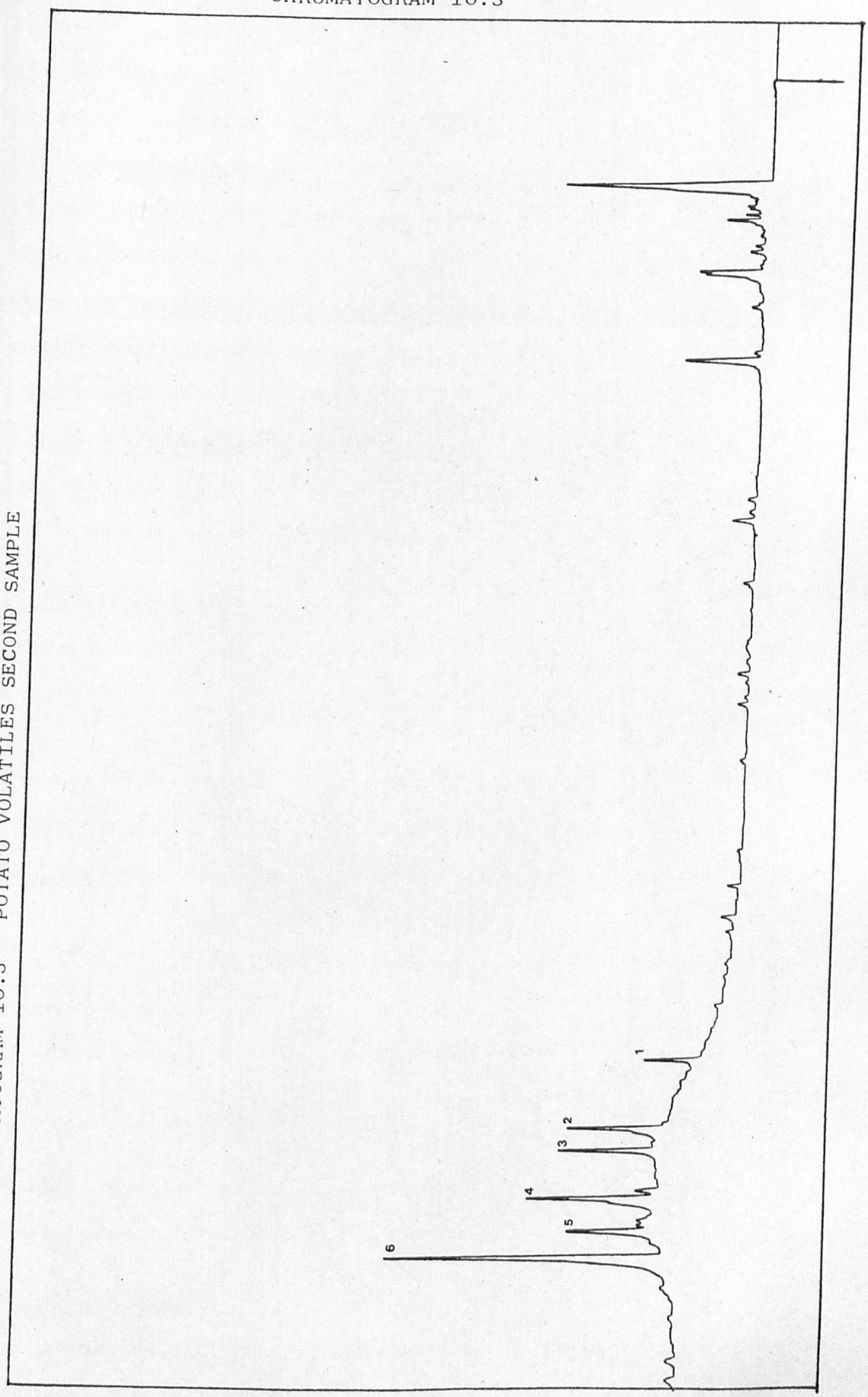
Potatoes c.v. King Edward were obtained from commercial sources, they were washed and graded to obtain 10 kg of healthy tubers. The potatoes were then stored in the dark at 10°C for 2 wk before use, during storage the potatoes broke dormancy, all of the tubers had their eyes open. The All Glass system as described in Chapter 5 was used for the collection of the potato volatiles. The All Glass system was assembled, the air mix flow rate set at 200 cc $min^{-1}$, and the apparatus flushed with the air mix for 4 hr to remove any laboratory air from the apparatus. A sample of the background volatiles from the system was then taken at 200 cc $min^{-1}$ for a sample volume of 10 $dm^3$, Chromatogram 10.1, the analysis was performed with System 3, described in Chapter 7. The background volatiles in the system were low, and their levels were considered to be acceptable.



CHROMATOGRAM 10.1 BACKGROUND VOLATILES




CHROMATOGRAM 10.2 POTATO VOLATILES FIRST SAMPLE




CHROMATOGRAM 10.3 POTATO VOLATILES SECOND SAMPLE

The containment vessel was opened and filled with 8 kg of potatoes, resealed and flushed with the air mix for 4 hr. Two consecutive samples of the potato volatiles were taken at 200 cc $min^{-1}$ for sample volumes of 10 $dm^3$, Chromatogram 10.2 and Chromatogram 10.3. The levels of potato volatiles were low in both chromatograms, however, peaks 1 - 6 were potato derived, and it was decided that it was worthwhile to attempt an identification of these potato volatiles. A further two samples of the potato volatiles were taken for analysis by G.C.M.S., both samples taken at 200 cc $min^{-1}$ for a sample volume of 10 $dm^3$.

Gas Chromatography

| | | |
|---|---|---|
| G.C.: | | Pye 104 (Pye Unicam Ltd). |
| Column: | | C.W.20M S.C.O.T. glass 43 m, 0.5 mm I.D., 1.0 mm O.D., (S.G.E., U.K. Ltd). |
| Carrier Gas: | | $H_2$ u = 20 cm $sec^{-1}$. |
| Temperature: | Initial | 50°C 10 min. |
| | Rise | 5°C $min^{-1}$. |
| | Hold | 185°C. |
| Detector: F.I.D. | | 270°C. |
| Detector Gasses: | | $H_2$ 15 cc $min^{-1}$. |
| | | $O_2$ 200 cc $min^{-1}$. |
| | | $N_2$ 10 cc $min^{-1}$. |
| Sensitivity: | | 5 x $10^{-10}$ A. |
| Chart Speed: | | 5 mm $min^{-1}$. |

G.C.M.S. Analysis

The potato volatiles were analysed by a G.C.M.S. which was equipped with a thermal desorption unit similar to the one

described in Chapter 7, System 3.

Gas Chromatography for Mass Spectrometry

| | | |
|---|---|---|
| G.C.: | | Sigma 3B (Perkin Elmer Ltd). |
| Column: | | C.W.20M S.C.O.T. glass 43 m, 0.5 mm I.D., 1 mm O.D., (S.G.E., U.K. Ltd). |
| Carrier Gas: | | He u = 15 cm $sec^{-1}$. |
| Temperature: | Initial | 50°C 10 min. |
| | Rise | 4°C $min^{-1}$. |
| | Hold | 185°C. |
| Desorption: | | The precolumn was desorbed at 250°C for 15 min into a liquid $N_2$ cooled stainless steel cold trap. The trap was heated ohmically to flush volatiles into the capillary column. |

Mass Spectrometry

| | |
|---|---|
| M.S.: | Micromass 16F (V.G. Analytical Ltd). |
| Interface Temperature: | 220°C. |
| Accelerating Potential: | 10 k.V. |
| Mass Range: | 20 - 350 amu. |
| Electron Energy: | 70 e.V. |
| Source Temperature: | 230°C. |
| Scan Time: | 1 sec $decade^{-1}$. |
| Cycle Time: | 3.2 sec. |
| Data System: | V.G. 2000 series (V.G. Analytical Ltd). |

One sample of the potato volatiles was analysed by G.C.M.S. and is shown in the form of a Total Ion Current (T.I.C.) chromatogram in Figure 10.1 and in Figure 10.2 (a), (b), (c) and (d), the latter having an expanded scan number or time axis.

It can be seen from Figure 10.1 that the T.I.C. rose sharply after scan 600, this was due to column bleed. The column bleed did not appear to be as severe on the F.I.D. chromatograms of the potato volatiles, however, it was severe on the T.I.C. chromatograms, this made the positive identification of the potato volatiles that eluted after scan 600 very difficult, despite computer assisted background subtraction techniques. The volatiles that eluted before scan 600, on comparison with the F.I.D. chromatograms were not thought to be potato derived. A tentative identification of 1,3 or 2,3 dimethylnaphthalene was made at scan 784 where the enhanced mass spectrum was as follows: 156(100), 141(67), 155(26), 115(10), 157(10), 153(9), 128(9), 142(6) this matches closely with published spectra: 1,3 dimethlnaphthalene 156(100), 141(70), 155(23), 115(13), 157(13), 153(9), 128(9), 142(8); 2,3 dimethlnaphthalene 156(100), 141(70), 155(24), 157(14), 115(13), 76(10), 153(9), 128(8), (Eight Peak Index of Mass Spectra, Mass Spectroscopy Data Centre, A.W.R.E., Reading), these dimethylnaphthalene isomers were expected to be eluted within this region of the chromatogram. Apart from a tentative identification of dimethylnaphthalene, a known component of potato volatiles (Meigh, D.F., et al, 1973), no potato volatiles were identified as

the region in which they eluted was subject to excessive column bleed. It was decided that before more time was spent on G.C.M.S. analysis of potato volatiles, a more satisfactory column would have to be employed that was capable of resolving the potato volatiles but did not contribute excessive bleed, especially during temperature programmed use.

FIGURE 10.1

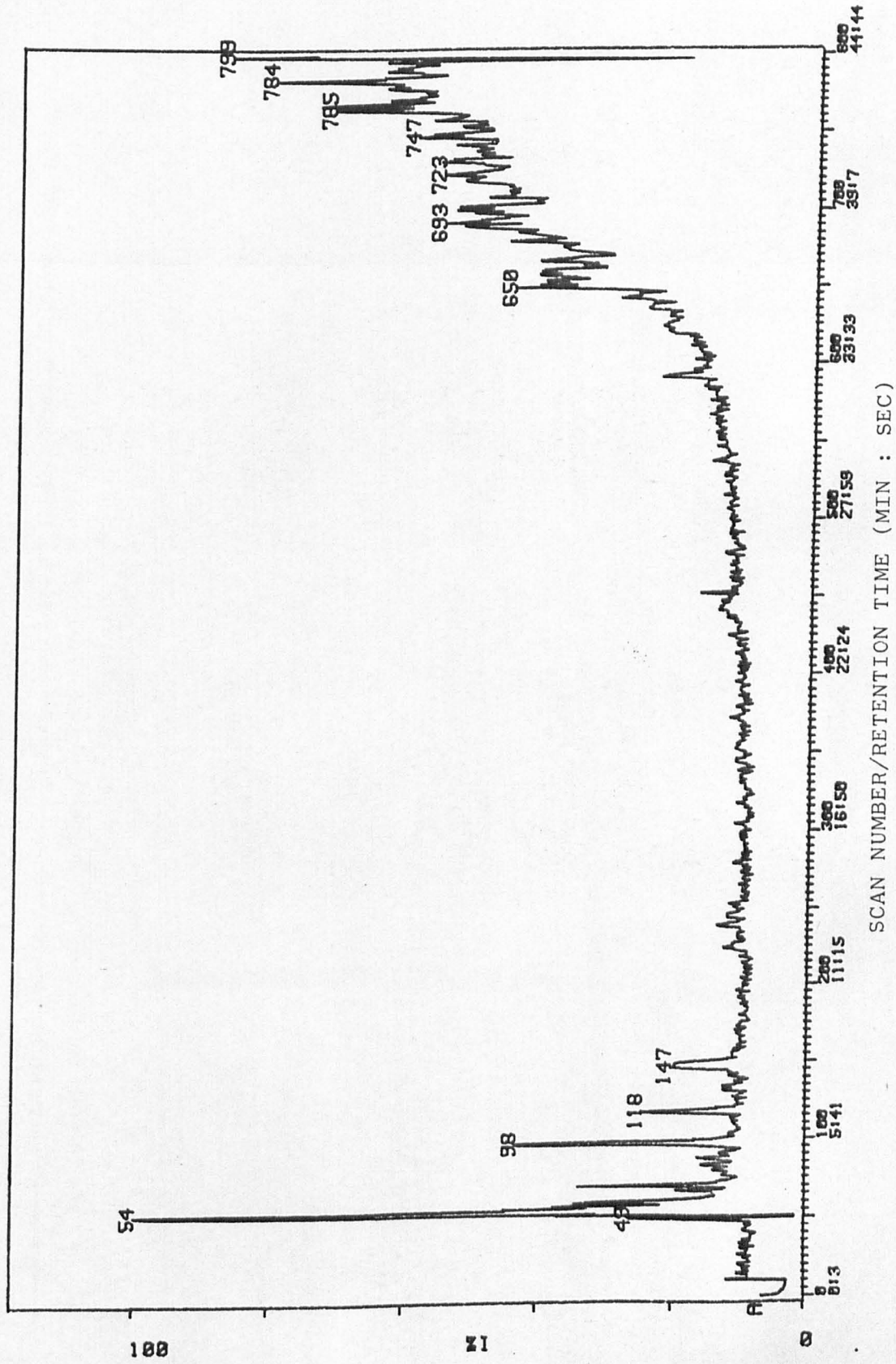



FIGURE 10.1
T.I.C. CHROMATOGRAM SCAN No. 0 - 800

FIGURE 10.2 (a)

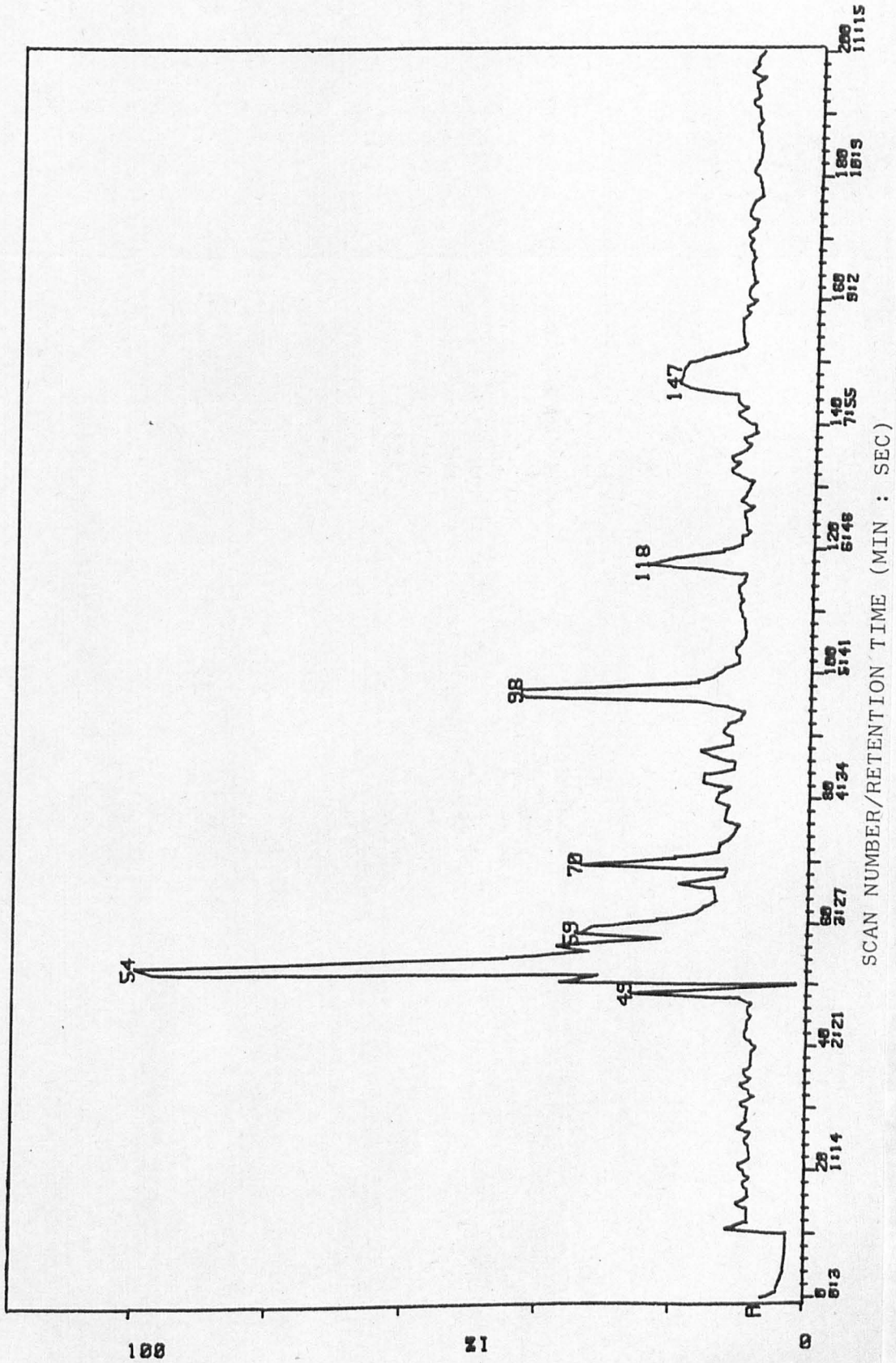



FIGURE 10.2 (a)

T.I.C. CHROMATOGRAM SCAN No. 0 - 200

FIGURE 10.2 (b)

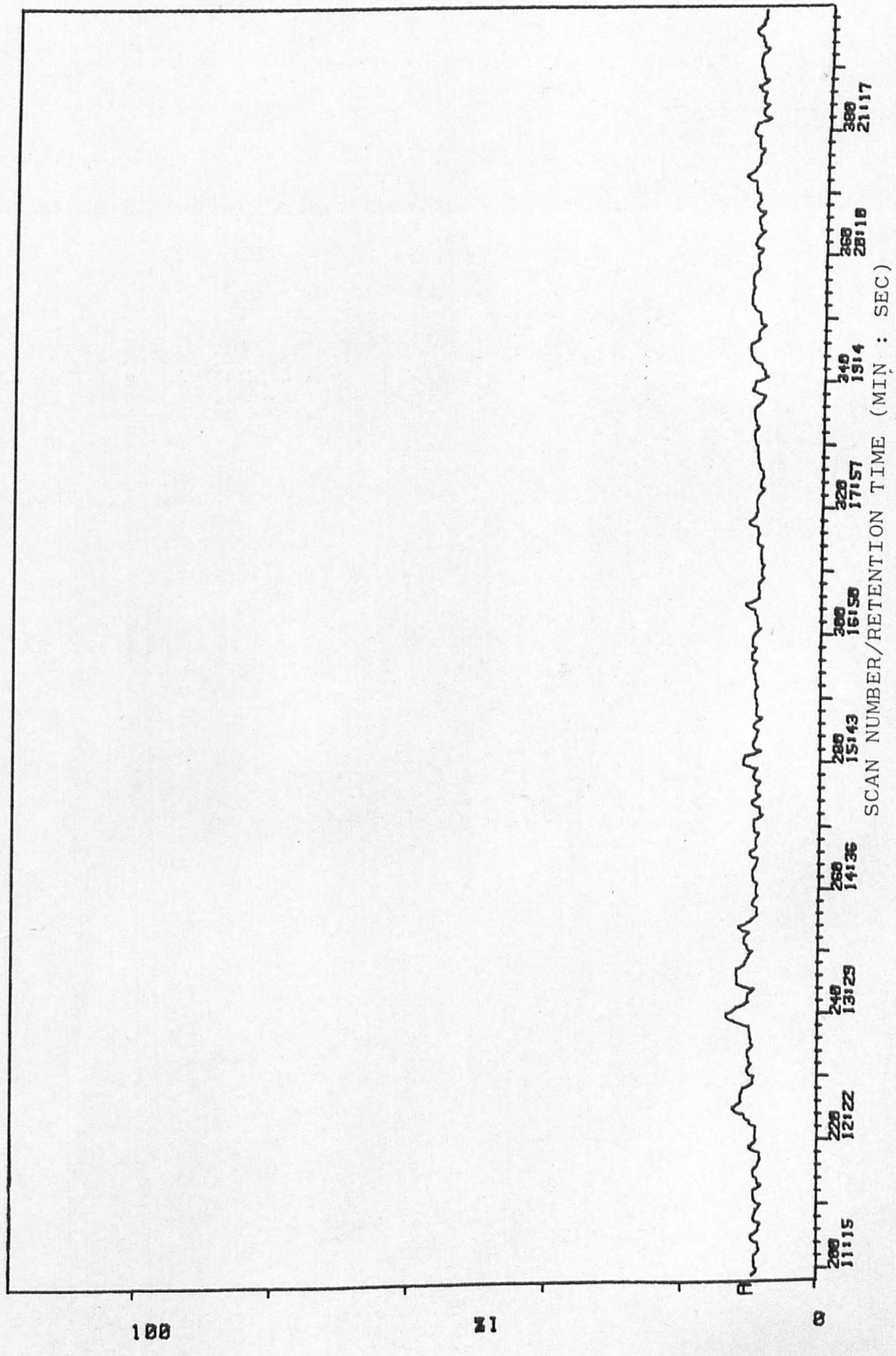



FIGURE 10.2 (b)
T.I.C. CHROMATOGRAM SCAN No. 199 - 400

FIGURE 10.2 (c)

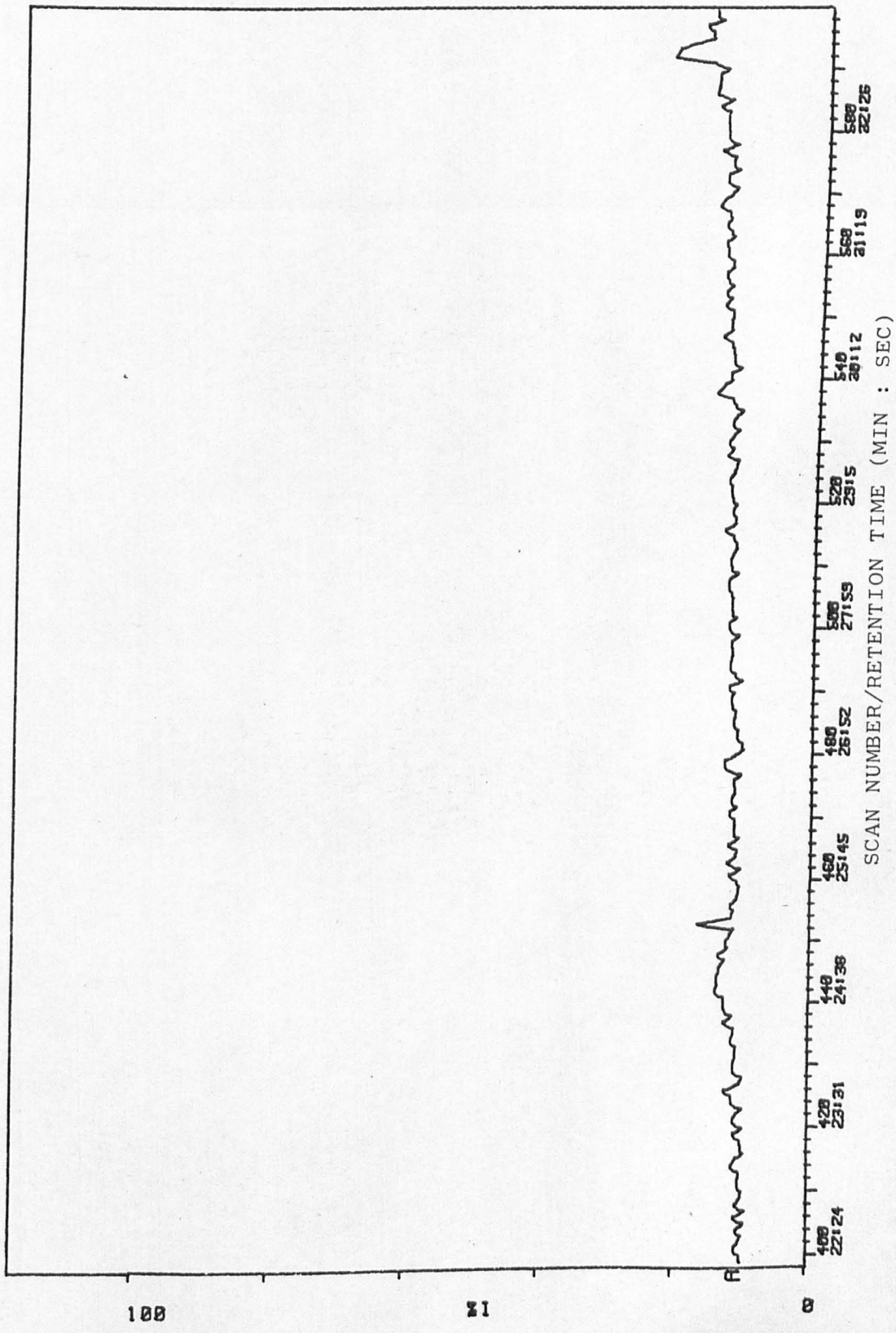



FIGURE 10.2 (c)
T.I.C. CHROMATOGRAM SCAN No. 399 - 600

FIGURE 10.2 (d)

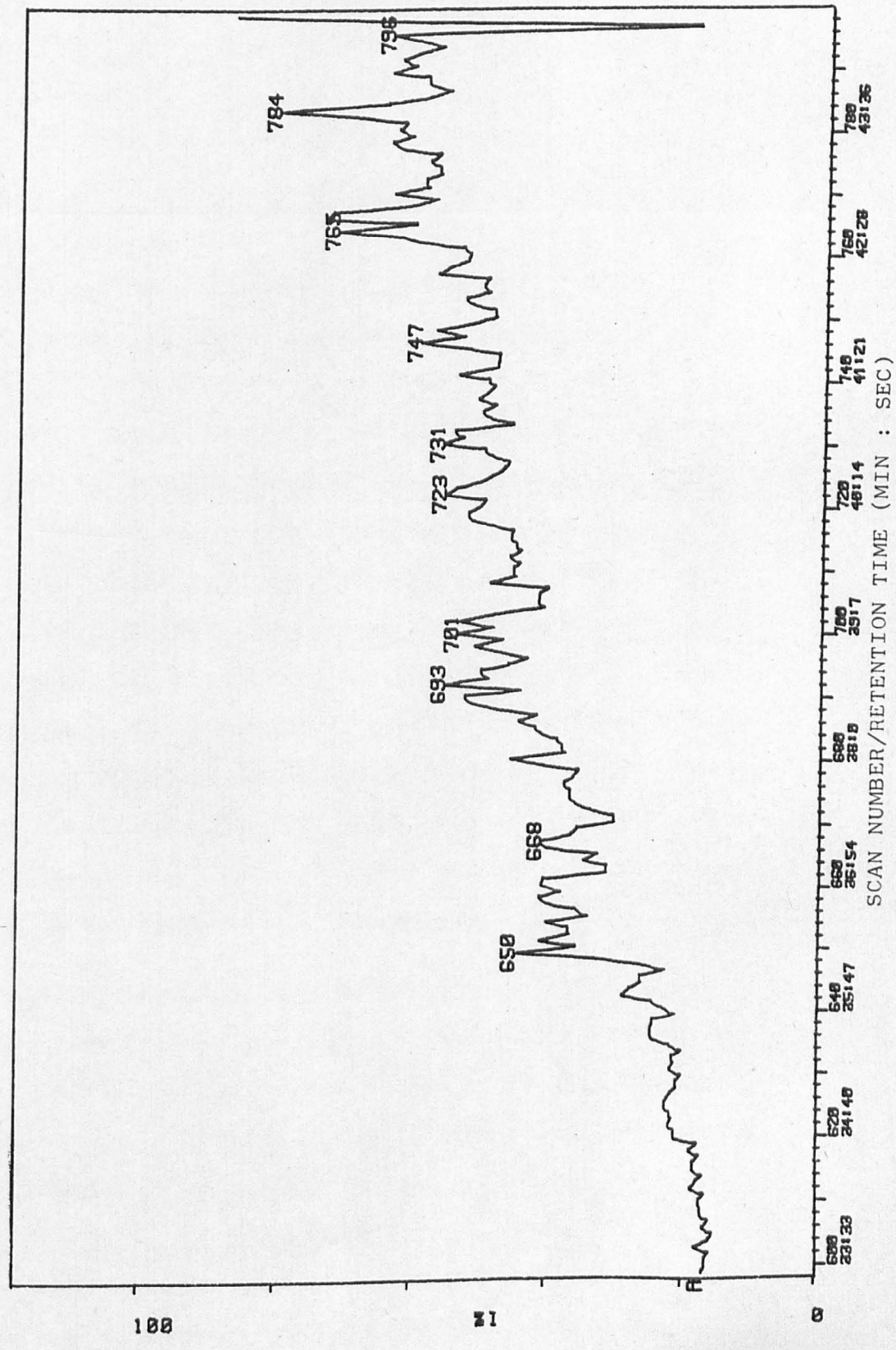


FIGURE 10.2 (d)
T.I.C. CHROMATOGRAM SCAN No. 599 - 800
RELATIVE INTENSITY
100
%
0
650
668
693
701
723
731
747
765
784
796
SCAN NUMBER/RETENTION TIME (MIN : SEC)

## 10.3 CONCLUSIONS

The project at this point had reached the stage where it was not possible to continue the work on the analysis of potato tuber volatiles due to a lack of time. It is unfortunate that the project had not reached a satisfactory conclusion in that a successful analysis of potato volatiles by G.C.M.S. was not completed. It was hoped that the presence of dimethylnaphthalenes in the headspace of dormant potatoes would be confirmed. Further to this, the isolation and identification of dormant potato volatiles other than the dimethylnaphthalenes would have enabled the project to be taken a stage further where these compounds could be assessed for their effect upon tuber dormancy, either by themselves or together with dimethylnaphthalene. The volatiles isolated from the dormant potato tubers described in Chapter 9 would have been candidates for this form of assessment. However, despite the absence of suitable G.C.M.S. data, it is thought that the G.C.M.S. analysis of potato volatiles using the techniques described in this project is practical, the only point requiring further modification is the type of G.C. column used.

In terms of the equipment and facilities that were within the complete control of the project, the developmental work was considered to be successful. The collection systems, in particular the All Glass system, ensured that the potato volatiles were of a known high purity, in addition the All Glass system proved to be a quick and simple system to operate and maintain. Similarly, the final system

adopted for the transfer of the desorbed volatiles from the precolumn to the G.C. column, System 3 described in Chapter 7, was operated successfully and easily.

In a broader perspective the techniques and equipment specified in this project could have a wider range of applicability. The extreme sensitivity of the headspace sampling technique means that it could be applied to the headspace analysis of a much broader range of biological materials other than potatoes, the primary considerations are the facts that: headspace volatiles which are present at extremely low concentrations can be concentrated to detectable amounts, the All Glass system in particular can accomodate a large sample of material, the headspace volatiles are contained in a high purity environment, the sample can be maintained aerobically in the system for long term periods, this point being important to the quality of the sample during the sampling period.

Finally, the future of the work on potato volatiles can be considered. Obviously a complete identification of potato headspace volatiles by G.C.M.S. is the next stage, followed by the assessment of these volatiles for sprout suppressant activity. The basic principles for the isolation and separation of potato volatiles developed in this project are satisfactory, however, some recommendations can be made in terms of the equipment used.

The collection systems, All Glass and Vacuum Distillation, are satisfactory, however, the precolumns could be modified

to contain larger amounts of Tenax G.C. which would ensure complete trapping of the potato volatiles. The most significant improvements could be made in the G.C. system. Firstly, satisfactory analysis of volatiles by capillary G.C. can only be achieved using a capillary gas chromatograph with an oven designed to meet the demands of precise and accurate temperature control for analysis to analysis R.T. reproducability. The Pye 104 gas chromatograph used in this project did not fulfill these demands. Secondly, the recently developed "bonded phase" flexible silica capillary columns would be suitable for separation of the potato volatiles, these columns provide superior resolution compared to the glass S.C.O.T. columns used for most of the separations in this project. Additionally the "bonded phase" columns are much less prone to damage by sample overload than their "coated" phase counterparts.

# Section 2.

# Potato Store Headspace Analysis

## CHAPTER 11

## INTRODUCTION AND EXPERIMENTAL

## 11.1 INTRODUCTION

This section of the project is primarily concerned with the headspace analysis of two volatile potato sprout suppressants, Tecnazene and Chlorpropham, which are widely used in large scale commercial potato stores.

Potatoes are now extensively used for the manufacture of processed potato products, such as potato crisps, potato granules and potato chips. The bulk of the potato crop is available to the manufacturer in the period from September to October, at times outwith this main crop season the potato supply is either limited or more expensive. Therefore, main crop potatoes are stored for periods of up to eight months to ensure a continuity of supply for the potato processor. The potatoes are stored in large scale potato stores of capacities ranging from 1000 - 5000 tonnes, there are two main forms of potato store:-

Bulk Store - The potatoes are simply piled loose and are ventilated with a series of ducts buried in the pile:

Box Store - The potatoes are stored in 1 tonne wooden boxes which are stacked, stored and ventilated in a controlled environment building.

The air used for ventilation in both forms of store is cooled, humidified and extensively recirculated. Under these storage conditions, the potatoes sprout if untreated with a sprout suppressant. Sprouting of potatoes is considered to be undesirable as this entails mobilisation of tuber energy reserves into the sprout which is of no manufacturing value, also the sprout increases water loss from

the tuber with subsequent decrease in tuber quality. Therefore it is common practice for the potatoes to be treated with a chemical sprout suppressant for the purposes of long term storage. The two most commonly used sprout suppressants are Chlorpropham (Figure 11.1) and Tecnazene (Figure 11.2). Henceforth, Chlorpropham will be referred to as CIPC and Tecnazene will be referred to as TCNB. Both CIPC and TCNB are active as sprout suppressants in the vapour phase (Vlief, W.F. Van, Sparenberg, H., 1970), (Brown, W., & Reavill, M.J., 1954), and both are applied to the potatoes just before or during storage. CIPC is usually applied as a thermal fog, where a solution of CIPC in a suitable solvent such as Methanol or Dichloromethane is heated and vapourised, the resultant "fog" of particulate CIPC is directed onto the stored potato tubers, where it deposits on the tubers. TCNB is usually applied to the tubers as a granular formulation, where the TCNB is adsorbed onto a solid support, the granules are applied to the tubers as they are being loaded into the bulk store or into the 1 tonne boxes.

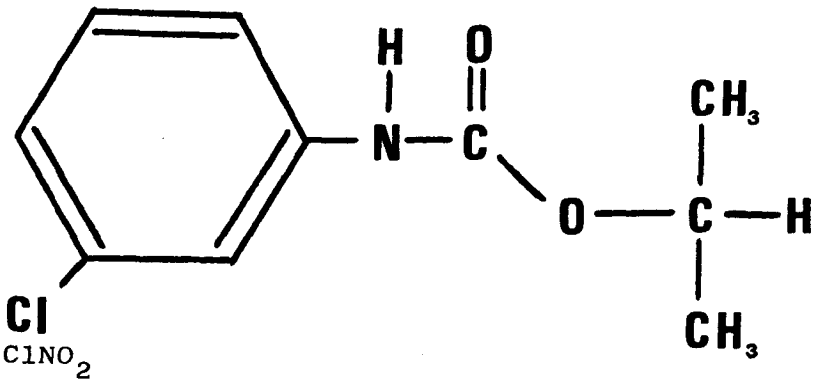
The "fogging" method of application as used for CIPC, has the advantage that as the fog is very penetrating, the CIPC can be reapplied to the potatoes as necessary after they have been stacked, the TCNB granular formulation requires a major disturbance of the stacked potatoes to be reapplied successfully, which is not consistent with good store management.

CIPC and TCNB act as sprout suppressants in different ways, TCNB prevents dormant tubers from sprouting, but will not

FIGURE 11.1

Chlorpropham: CIPC

(3-chlorophenyl)carbamic acid - 1 methyl ethyl ester




$C_{10}H_{12}ClNO_2$

Mol. Wt. 213.68

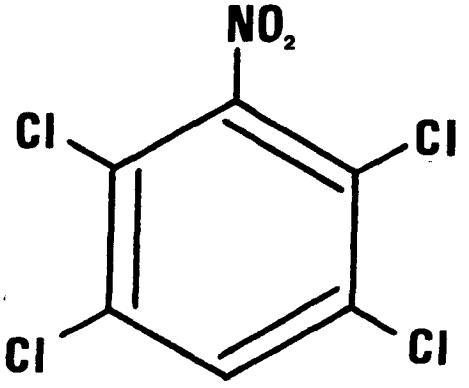
mp 40.7 - 41.1°C

bp 229°C

FIGURE 11.2

Tecnazene: TCNB

1,2,4,6-tetrachloro - 3 nitro benzene




$C_6HCl_4NO_2$

Mol. Wt. 260.89

mp 98 - 101°C

bp 304°C

stop sprout growth after it has commenced and is therefore applied as sprout suppressant to dormant potato tubers. CIPC acts as a mitotic poison and stops active sprout growth by killing the growing sprout tip, it is therefore used in the latter stages of potato storage where some potato sprout growth inevitably occurs.

As both TCNB and CIPC act in the vapour phase and therefore are present in a potato store headspace after their application, a study of the headspace concentration of both these chemicals in large scale commercial potato stores was undertaken. The primary aim was the investigation of the distribution of headspace TCNB and CIPC within box potato stores. It was thought that these investigations would show how the headspace concentrations of both sprout suppressants were influenced by the storage conditions. Furthermore as both TCNB and CIPC are transported to the eye of the tuber in the vapour phase where they then act to suppress or kill sprout growth. The headspace TCNB and CIPC could be regarded as a measure of the "active" levels of both these chemicals. It was hoped that the results of these investigations would be useful in terms of improved store management through a greater understanding of the behaviour of headspace TCNB and CIPC under commercial storage conditions.

## 11.2 ANALYTICAL

### 11.2.1 Introduction

The headspace sampling techniques developed in Section 1 of this project were drawn upon for the development of the store headspace analysis system.

From the outset, the Tenax G.C. precolumn was considered to be the most suitable method for the collection of the headspace CIPC/TCNB from the potato store. From the results of the work in Section 1 and other researchers as discussed in Chapter 3, the Tenax G.C. precolumn sampling technique has been shown to be a simple, quick and efficient method of collecting trace organic headspace volatiles. Tenax G.C. precolumns have been used for the study of headspace CIPC and other volatile substances in large scale potato stores (Filmer, A.A.E., Land, D.G., 1978), (Beveridge, J., 1980).

### 11.2.2 Headspace Sampling

A portable headspace sampling apparatus was constructed. The apparatus was designed in such a way that it could be operated at any point within the store and that at each sampling point triplicate samples of the store headspace were obtained. The apparatus was constructed as follows:

Three 100cc soap film flow meters were connected in parallel to one neck of a 500cc three neck Wolf bottle (Corning Ltd). To another neck of the Wolf bottle a mercury manometer was attached. A small diaphragm air pump ((Capex Mk2) (Charles Austen Pumps Ltd, Byfleet, England)) was connected to the remaining neck of the Wolf bottle via an air leak which

served as a flow controller. The headspace samples were obtained by connecting three precolumns to the three soap film bubble meters, and then drawing the store headspace through the precolumns with the air pump. The Wolf bottle served as an expansion chamber to damp and smooth the air flow pulses from the pump. The mercury manometer measured the air pressure in the sampling system which operated at a negative pressure due to the flow restriction of the precolumns. The soap film bubble meters measured the air flow rate, from which the air sample volume was calculated. A schematic diagram of the system is shown in Figure 11.3, and the system mounted on a board in Plate 14. This was the first design of the system and it was found to be suitable for store headspace sampling without further modification.

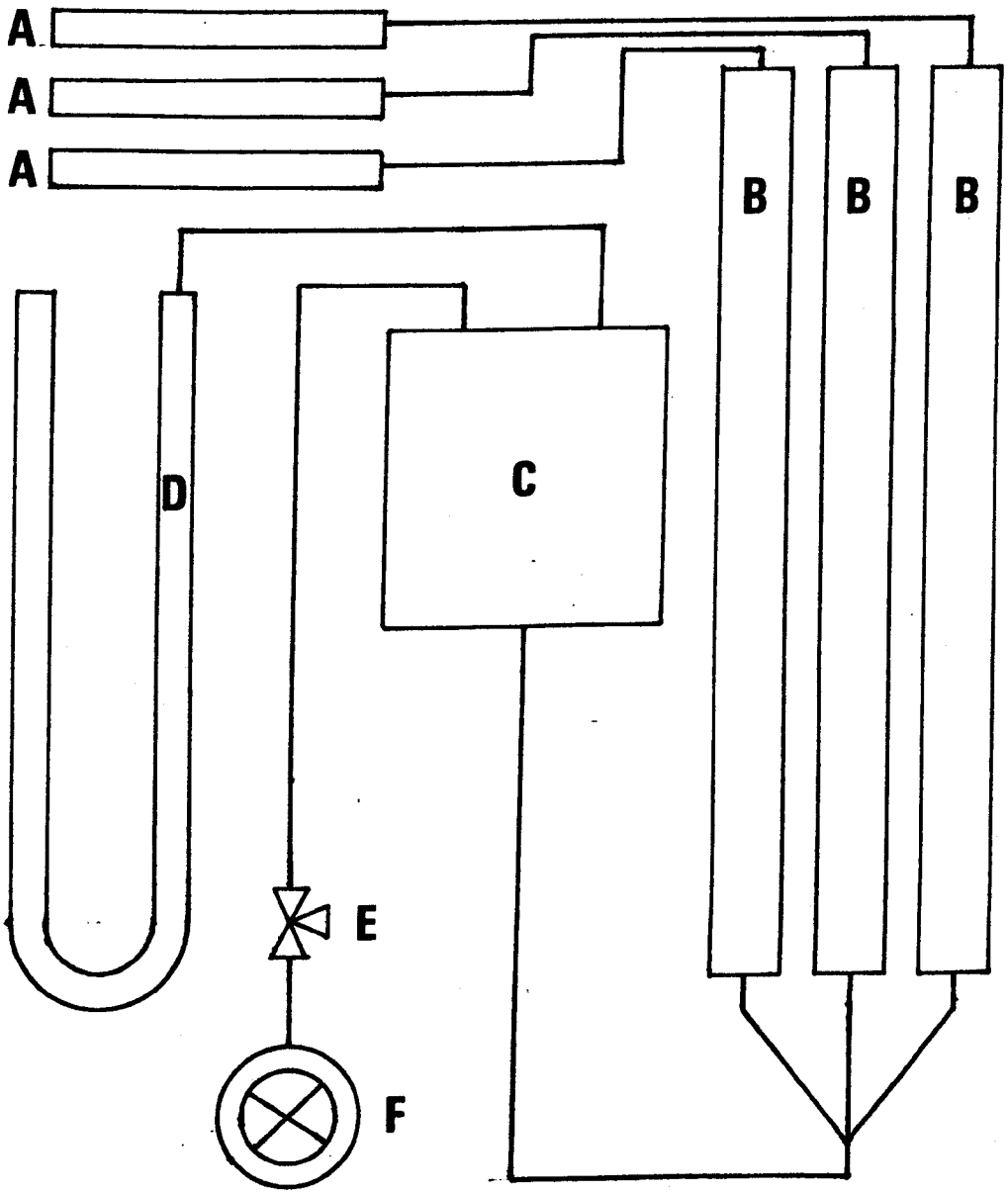
The precolumns were constructed as described in Chapter 4 and conditioned before use as described in Chapter 7. Thirty precolumns were made and were used solely for potato store headspace sampling, before and after sampling the precolumns were sealed with PTFE caps and stored for a period of not more than four days.

The system was operated as follows. The pump was run for 30 min before sampling commenced, as it was found that the flow rates were variable during the first 15 min of the pumps operation, thereafter the flow rate was found to be stable. The precolumns were then connected to the soap film flow meters with lengths of plastic tubing (Portex Ltd, Hythe, England), the precolumns were placed at a distance of at least 50mm from any surface during sampling. The sample

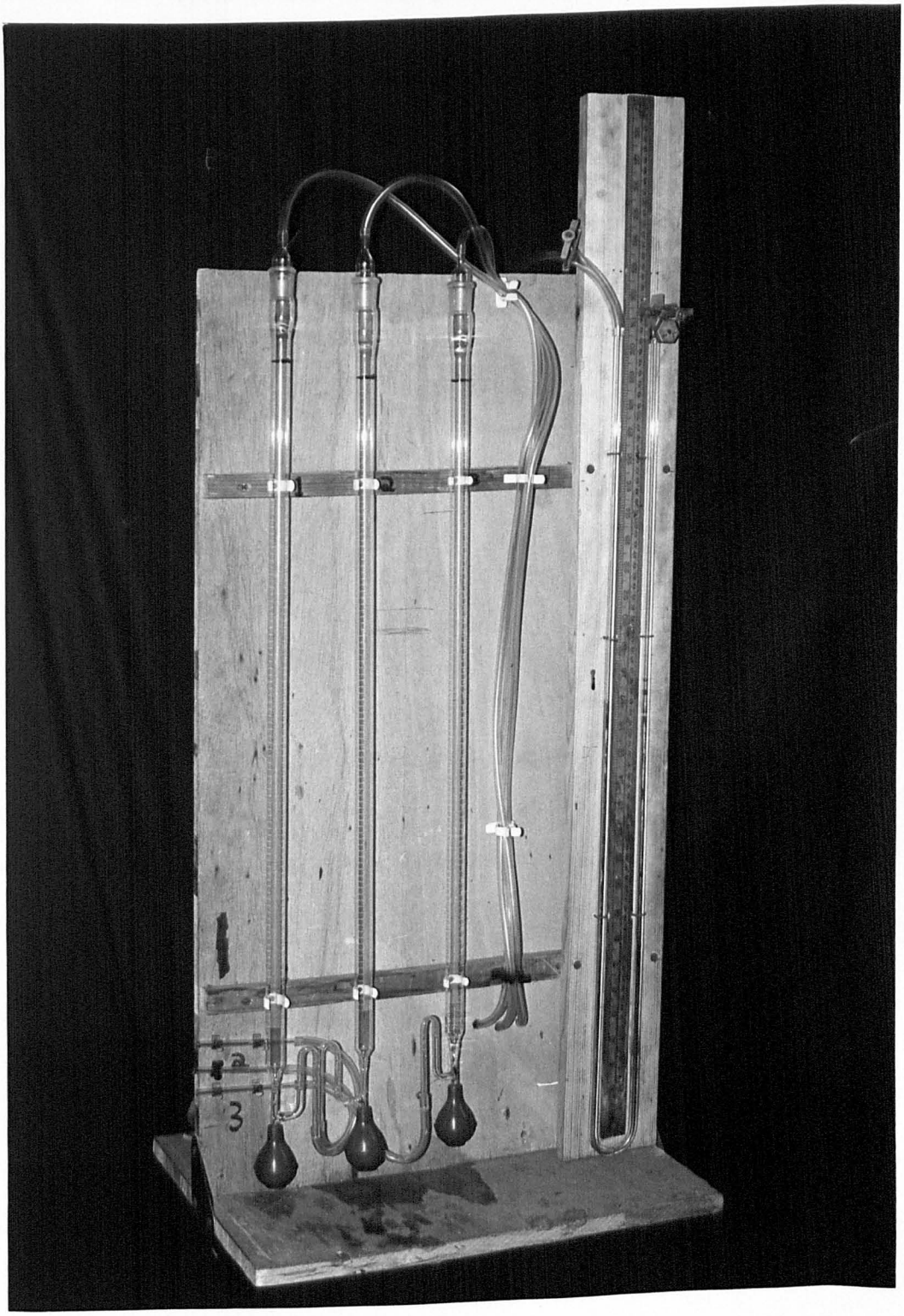
FIGURE 11.3

STORE HEADSPACE SAMPLING APPARATUS

| | | | |
|---|---|---|---|
| A | PRECOLUMN | D | MANOMETER |
| B | FLOWMETER | E | AIR LEAK/FLOW CONTROLLER |
| C | EXPANSION CHAMBER | F | AIR PUMP |



# PLATE 14



flow rates were 200cc $min^{-1}$ - 1000cc $min^{-1}$, and the sample volumes were 5 $dm^3$ - 15 $dm^3$. The sample flow rates were measured at the beginning, middle and end of the sampling period, and were found to vary by not more than 4% for each precolumn during the sampling period. As the system operated under reduced pressure, the sample volumes calculated from the flow rate and the sampling time, reflected the sample volume at the reduced pressure. The mercury manometer was included in the system so that the sample volumes could be corrected to the atmospheric pressure outside the system. All the headspace sample volumes quoted in this section of the thesis are corrected to atmospheric pressure.

### 11.2.3 Gas Chromatography

The analysis problems in this section of the project are much simplified compared to those encountered in Section 1. Only two compounds were of interest, these being TCNB and CIPC, and therefore it was not essential that all the headspace components were completely resolved, a separation of TCNB and/or CIPC from each other and any other components was sufficient. Also the identification of CIPC and TCNB could be performed with confidence on an RT basis, without the need for GCMS facilities. Therefore the analysis system used was less sophistocated than those developed for Section 1. A conventional packed column resolved the CIPC and TCNB from the other headspace components, the CIPC and TCNB were

identified and quantified by comparison with authentic CIPC and TCNB standards. The precolumns were coupled directly onto the top of the analytical column, without the use of a switching valve or cold trap, the precolumns were thermally desorbed using a heating block. The details of the analysis sytem are as follows:-

Desorption Block - A desorption block was constructed from a block of aluminium, 30mm in diameter and 70mm long, the centre of the block was drilled with a 7mm diameter hole, the block was heated with a 150W cartridge heater (Hedin Ltd), which was inserted into the block. The block was maintained at 230°C for desorption;

Desorption - The precolumn was connected to the top of the packed column with a drilled out ¼ inch coupling (Dralim), (Phase Separations Ltd), the seals were made with ¼ inch Viton "O" rings (Pye Unicam Ltd), the coupling was drilled out so that the precolumn could be butted directly onto the top of the packed column providing an all glass flow path.

The analysis system was operated as follows. The precolumn was connected to the top of the packed column with the ¼ inch coupling, the desorption block was placed round the precolumn and the carrier gas was connected to the top of the precolumn. All these operations were carried out as quickly as possible, usually within 30 sec, as during these operations the carrier gas flow to the column was interrupted. A cross sectional diagram of the precolumn, heating block and packed column is

shown in Figure 11.4, and they are illustrated in Plate 15 and Plate 16.

Gas Chromatography

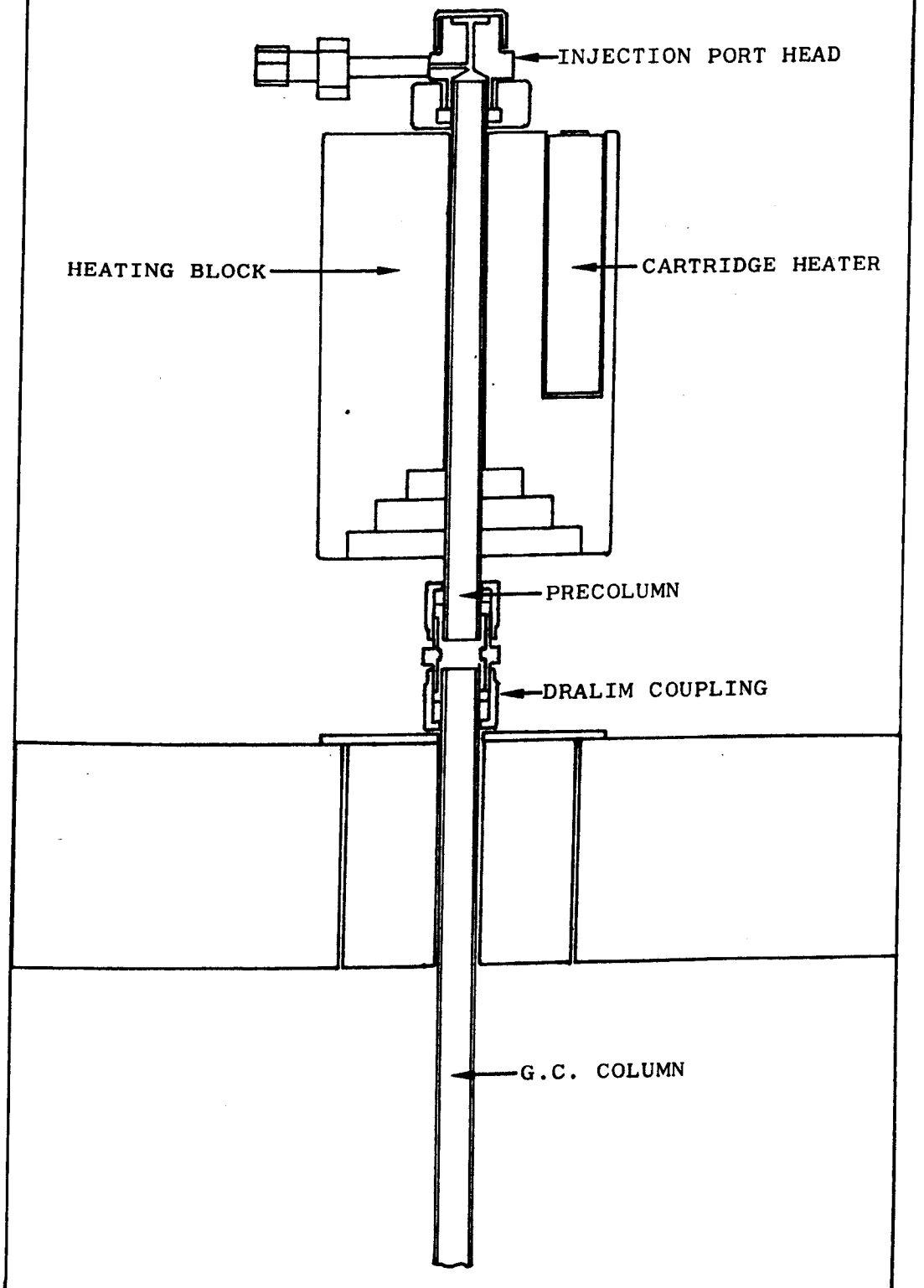
| | |
|---|---|
| G.C: | Pye 104 (Pye Unicam Ltd). |
| Column: | 2 meter glass column 6mm O.D., 4mm I.D., packed with 5% OV.101 (Phase Separations Ltd) on Gas Chrom Q 100/120 mesh (Phase Separations Ltd). |
| Temperature: | Initial: 120°C 10 min |
| | Rise: 16°C $min^{-1}$ |
| | Hold: 220°C 20 min |
| | Injection Port: 240°C |
| | Detector F.I.D.: 250°C |
| Detector: | $H_2$ 45cc $min^{-1}$ |
| | $O_2$ 210cc $min^{-1}$ |
| Carrier Gas: | $N_2$ 43cc $min^{-1}$. |

These conditions were used for all of the analyses in this section of the project.

The CIPC and TCNB were identified and quantified by injecting aliquots of standard solutions of TCNB (Aldrich Chemical Co. Ltd) and CIPC (Sigma London Chemical Co. Ltd) made up in glass distilled hexane (Rathburn Chemicals Ltd) onto an empty glass precolumn which was heated with the heating block and connected to the top of the column. The standard solutions were injected with a $5mm^3$ syringe((Hamilton 700 series), (Phase Separations Ltd)).

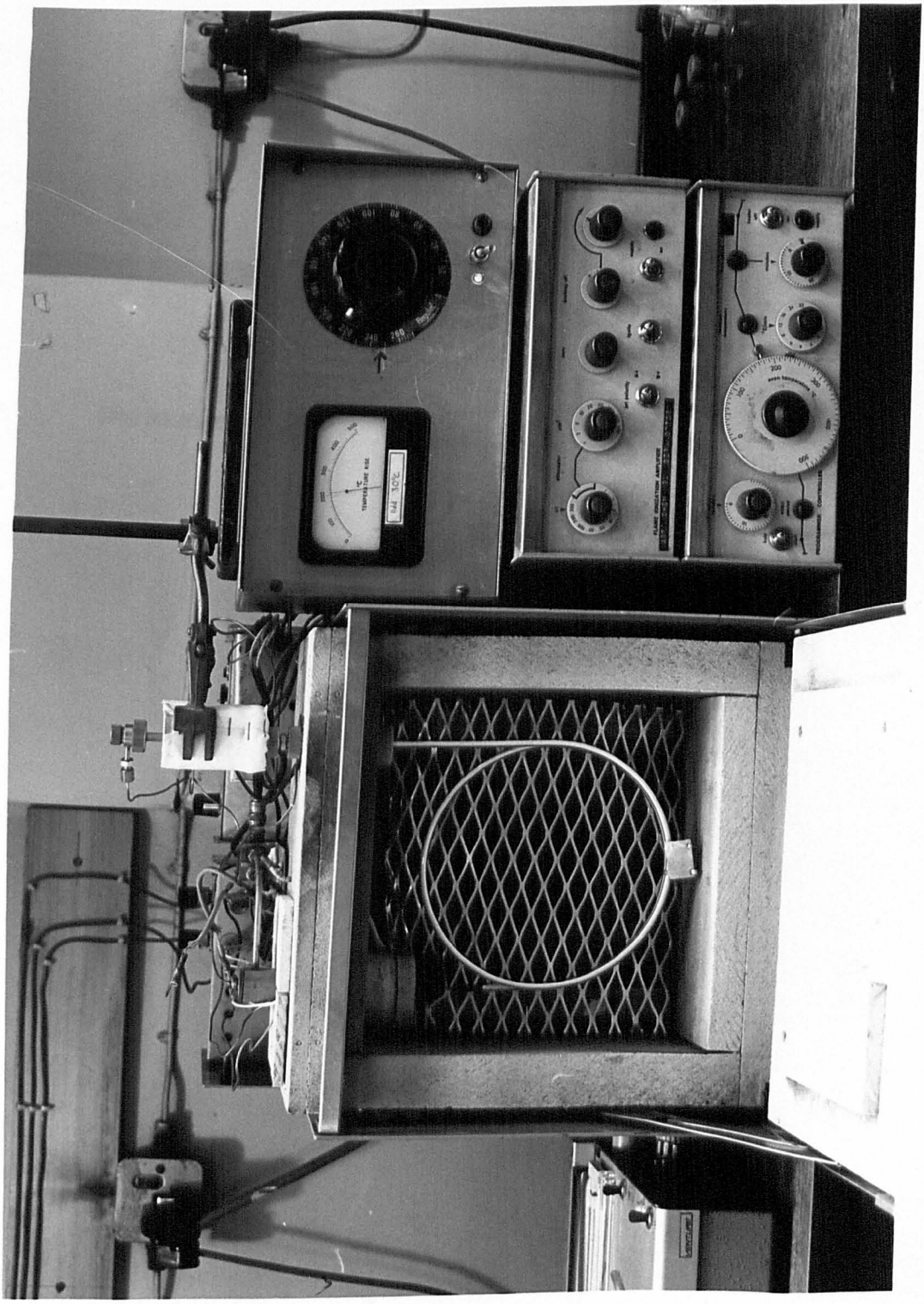
FIGURE 11.4

DESORPTION APPARATUS



# PLATE 15

# PLATE 16



11.2.4 Assessments

A series of experiments were carried out to assess the desorption characteristics of CIPC and TCNB from the Tenax G.C. precolumns. The factors assessed were as follows:-

The time taken to desorb CIPC/TCNB from a precolumn.

The sampling and analysis orientation of the precolumn, whether the amount of CIPC/TCNB desorbed was dependant upon the precolumn being coupled to the G.C. column so that the end closest to the store headspace was coupled to the top of the G.C. column.

The storage life of precolumns sampled with CIPC/TCNB before and appreciable loss of CIPC/TCNB occurred.

The peak areas for CIPC/TCNB when desorbed from a precolumn onto the G.C. column and when directly injected onto the G.C. column.

For these assessments, a series of TCNB and CIPC standards were made up in glass distilled hexane (Rathburn Chemicals Ltd) using authentic TCNB (Aldrich Chemical Co Ltd) and authentic CIPC (Sigma London Chemical Co Ltd). Both TCNB and CIPC standards were in the concentrations, 100ug $cc^{-1}$, 500ug $cc^{-1}$, 1000ug $cc^{-1}$, 5000ug $cc^{-1}$ and 10,000ug $cc^{-1}$, all the standard solutions were kept in stoppered flasks at 0°C when not in use. The standards were injected onto the pre-columns or G.C. column to give the desired amount of CIPC/TCNB using a $5mm^3$ syringe (Hamilton 700 series), (Phase Separations Ltd), the injection volumes were $1mm^3$.
The peak areas for the CIPC and TCNB were calculated by multiplying the peak height by the width of the peak at half peak height.

Peak Areas Comparison of Injection/Desorption

This experiment was carried out to assess whether the CIPC/TCNB were quantiatively and reproducibly desorbed from a Tenax G.C. precolumn in comparison to a conventional direct injection of the CIPC/TCNB made up in a solvent onto the top of the G.C. column. The experiment was performed at three CIPC/TCNB concentrations.

The following procedures were used:-

1. Ten freshly conditioned precolumns were injected with $1mm^3$ of 1000ug $cc^{-1}$ CIPC in hexane, which gave lug of CIPC injected onto the precolumn. The injections were made so that the CIPC was injected onto the Tenax G.C. in the middle of the precolumn, after the injection was made the precolumn was sealed with PTFE caps and allowed to equilibrate for 30 min before analysis. These operations were performed sequentially, one precolumn was injected with CIPC and analysed at a time.

Stage 1. was repeated using:

$1mm^3$ of 5000ug $cc^{-1}$ CIPC; 5ug CIPC injected,

$1mm^3$ of 10,000ug $cc^{-1}$ CIPC; 10ug CIPC injected,

$1mm^3$ of 1000ug $cc^{-1}$ TCNB; lug TCNB injected,

$1mm^3$ of 5000ug $cc^{-1}$ TCNB; 5ug TCNB injected,

$1mm^3$ of 10,000ug $cc^{-1}$ TCNB; 10ug TCNB injected.

2. Ten $1mm^3$ injections were made of each of the standard CIPC/TCNB solutions used in step 1, directly onto the G.C. column. Each particular standard solution was injected and analysed so that it was performed in alternation with the equivalent standard solution injected by desorption in step 1.

The peak areas for CIPC and TCNB were calculated for all six standard solutions used and for both injection methods, these are given in Table 11.1.

TABLE 11.1

Peak area for CIPC/TCNB desorbed and injected

| | TCNB Peak Area ($mm^3$) | | | | | | CIPC Peak Area ($mm^3$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 ug | | 5 ug | | 10 ug | | 1 ug | | 5 ug | | 10 ug | |
| Inj. | I | D | I | D | I | D | I | D | I | D | I | D |
| 1 | 384 | 375 | 484 | 442 | 511 | 501 | 602 | 638 | 641 | 683 | 849 | 830 |
| 2 | 397 | 373 | 451 | 484 | 543 | 483 | 605 | 624 | 684 | 621 | 866 | 744 |
| 3 | 371 | 361 | 492 | 483 | 582 | 522 | 625 | 584 | 797 | 656 | 850 | 759 |
| 4 | 375 | 354 | 461 | 493 | 555 | 607 | 591 | 604 | 701 | 708 | 856 | 759 |
| 5 | 362 | 382 | 473 | 473 | 537 | 546 | 692 | 619 | 726 | 745 | 705 | 853 |
| 6 | 379 | 377 | 445 | 462 | 535 | 505 | 680 | 577 | 733 | 699 | 740 | 802 |
| 7 | 390 | 379 | 479 | 467 | 576 | 554 | 667 | 541 | 705 | 649 | 864 | 866 |
| 8 | 382 | 371 | 487 | 439 | 545 | 467 | 638 | 610 | 728 | 733 | 735 | 714 |
| 9 | 384 | 361 | 481 | 476 | 594 | 554 | 649 | 633 | 713 | 777 | 833 | 797 |
| 10 | 369 | 383 | 449 | 455 | 559 | 499 | 654 | 596 | 676 | 662 | 881 | 824 |
| $\bar{x}$ | 379 | 371 | 470 | 464 | 553 | 514 | 640 | 602 | 710 | 692 | 818 | 801 |
| S.D. | 10 | 10 | 17 | 18 | 25 | 30 | 34 | 29 | 41 | 50 | 65 | 49 |
| C.V. | 3 | 3 | 4 | 4 | 4 | 6 | 5 | 5 | 6 | 7 | 8 | 8 |

I : Injected on column

D : Desorbed from precolumn

The results of Table 11.1 show that the direct injection and thermal desorption methods of injection were equivalent in terms of peak area for the eluted CIPC and TCNB. The error increased with increasing levels of CIPC and TCNB, at the three concentrations used. The error was higher for CIPC than TCNB at all three concentrations, this was probably due to the thermal labile nature of CIPC (Romagnoli, R.J., Bailey, J.P., 1966). CIPC decomposes on contact with metal surfaces at temperatures over 230°C, however, the variability was equivalent for both the injection and desorption techniques for CIPC.

It was concluded that there was no significant difference between the thermal desorption and direct injection of CIPC and TCNB in terms of peak area.

Desorption Time for CIPC and TCNB

The time taken to desorb the CIPC/TCNB from a Tenax G.C. precolumn at the desorption temperature, 230°C, and carrier gas flow rate, 43cc $min^{-1}$, was assessed.

Five freshly conditioned precolumns were injected with $1mm^3$ of a 5000ug $cc^{-1}$ CIPC in hexane, which gave 5ug of CIPC injected onto the precolumn. The injections were made so that the CIPC was injected onto the Tenax G.C. in the middle of the precolumn, after the injection was made the precolumn was sealed with PTFE caps and allowed to equilibrate for 30 min before analysis. These operations were performed sequentially, one precolumn was injected with CIPC and analysed at a time. After the equilibration period, the precolumns were desorbed and the mean of the

five peak areas for the CIPC was taken to be equivalent to the peak area for 5ug of CIPC. A further five freshly conditioned precolumns were injected with $1mm^3$ of 5000ug $cc^{-1}$ CIPC in hexane, after equilibration these precolumns were connected to a nitrogen gas flow, 43 cc $min^{-1}$, and inserted into the heating block for 1 min and then removed, cooled and sealed with PTFE caps. The heating block was maintained at 230°C, the CIPC desorbed from the precolumn during the 1 min heating period was vented to the atmosphere and not onto the G.C. column. After cooling the precolumns were then coupled to the top of the G.C. column and desorbed. The resultant CIPC peak was proportional to the amount of CIPC left on the precolumn after a 1 min desorption. This step was repeated with heating periods of 2, 4 and 8 minutes prior to the precolumns analysis by desorption. The experiment was repeated using a $1mm^3$ injection of 5000ug $cc^{-1}$ TCNB, all the other conditions being the same.

The results of the analysis of the precolumns are given in Table 11.2, the peak areas for CIPC and TCNB desorbed 30 min after their injection with the CIPC/TCNB standard solutions were taken to be equivalent to 5ug of TCNB and CIPC respectively. The results of the analyses of the precolumns which were heated prior to the analysis are given in terms of ug CIPC/TCNB left on the precolumn.

TABLE 11.2

Desorption time for CIPC/TCNB

TCNB

| | TCNB left on precolumn (ug) | | | | |
|---|---|---|---|---|---|
| | Time at 230°C (min) | | | | |
| Precolumn | 0 | 1 | 2 | 4 | 8 |
| 1 | 5.21 | 0.91 | 0.27 | < 0.01 | < 0.01 |
| 2 | 4.93 | 0.80 | 0.25 | < 0.01 | < 0.01 |
| 3 | 4.80 | 0.74 | 0.22 | < 0.01 | < 0.01 |
| 4 | 5.21 | 0.93 | 0.31 | < 0.01 | < 0.01 |
| 5 | 4.85 | 0.87 | 0.26 | < 0.01 | < 0.01 |
| $\bar{x}$ | 5.00 | 0.85 | 0.26 | < 0.01 | < 0.01 |
| S.D. | 0.20 | 0.08 | 0.03 | - | - |

CIPC

| | CIPC left on precolumn (ug) | | | | |
|---|---|---|---|---|---|
| | Time at 230°C (min) | | | | |
| Precolumn | 0 | 1 | 2 | 4 | 8 |
| 1 | 4.81 | 1.10 | 0.34 | < 0.01 | < 0.01 |
| 2 | 5.17 | 0.64 | 0.21 | < 0.01 | < 0.01 |
| 3 | 4.72 | 1.24 | 0.09 | < 0.01 | < 0.01 |
| 4 | 4.90 | 0.97 | 0.15 | < 0.01 | < 0.01 |
| 5 | 5.43 | 0.83 | 0.20 | < 0.01 | < 0.01 |
| $\bar{x}$ | 5.00 | 0.97 | 0.21 | < 0.01 | < 0.01 |
| S.D. | 0.29 | 0.23 | 0.12 | - | - |

The results of Table 11.2 show that 99.8% of the CIPC and TCNB was desorbed from the precolumn within the first 4 min of desorption, at the CIPC and TCNB concentrations used. The large standard deviations for the 1 min and 2 min desorption periods, especially in the case of CIPC, were thought to be due to errors in the periods at which the precolumns were heated. As the CIPC and TCNB were desorbed very rapidly during the 1 and 2 min periods, small variations in the time that the precolumns were maintained at 230°C would cause large variations in the amount of CIPC and TCNB left on the precolumn.

Precolumn Orientation for Desorption

An investigation was made of the orientation of the precolumn relative to its sampling position and desorption position. It was thought that during the sampling of a potato store headspace the CIPC/TCNB could be trapped on the first few mm of the Tenax G.C. Therefore, it might have been advantageous to desorb the precolumn so that the end of the precolumn through which the store headspace was initially drawn was coupled to the top of the G.C. column.

Ten freshly conditioned precolumns were injected with $1mm^3$ of 5000ug $cc^{-1}$ CIPC in hexane, which gave 5ug of CIPC injected onto the precolumn. The injections were made so that the CIPC in hexane was injected onto the first few mm of the Tenax G.C. at one end of the precolumn, after the injection was made the precolumn was sealed with PTFE caps and allowed to equilibrate for 30 min before analysis. These operations were performed sequentially, one precolumn

was injected with CIPC and analysed at a time.

Five of the precolumns were desorbed so that the end of the precolumn that was injected with CIPC in hexane was facing the top of the G.C. column, the other five precolumns were coupled to the G.C. column so that the end injected with CIPC in hexane was facing away from the top of the G.C. column.

The above procedure was repeated except $1mm^3$ injection of $5000ug\ cc^{-1}$ TCNB in hexane was used.

The results of the analyses of the CIPC and TCNB desorbed from the precolumns are given in Table 11.3.

TABLE 11.3

Desorption orientation of the precolumn

| Precolumn | TCNB | | CIPC | |
|---|---|---|---|---|
| | Peak Area ($mm^3$) | | Peak Area ($mm^3$) | |
| | A | B | A | B |
| 1 | 485 | 451 | 734 | 673 |
| 2 | 454 | 473 | 687 | 780 |
| 3 | 459 | 490 | 750 | 689 |
| 4 | 431 | 426 | 731 | 757 |
| 5 | 460 | 445 | 752 | 732 |
| $\bar{x}$ | 458 | 457 | 710 | 726 |
| S.D. | 19 | 25 | 40 | 45 |

A : Precolumn desorbed with end injected with CIPC/TCNB facing the top of the GC column

B : Precolumn desorbed with end injected with CIPC/TCNB facing away from the top of the GC column

The results of Table 11.3 show that the orientation of the precolumn relative to the G.C. column did not make a significant difference to the peak areas of the CIPC/TCNB desorbed from the precolumn.

Storage Life of Sampled Precolumns

This experiment was performed to determine the maximum storage period for sampled precolumns before any appreciable loss of CIPC/TCNB occurred from the precolumns.

Fifteen freshly conditioned precolumns were injected with $1mm^3$ of 5000ug $cc^{-1}$ CIPC in hexane, which gave 5ug of CIPC injected onto the precolumn. The injections were made so that the CIPC was injected onto the middle of the precolumn. After the injection was made, the precolumns were sealed with PTFE caps. Five of the precolumns were desorbed within 30 min of injection, of the remaining ten precolumns, five were desorbed five days after injection with CIPC and the final five were desorbed ten days after injection with CIPC.

The above procedure was repeated except that the precolumns were injected with $1mm^3$ of 5000ug $cc^{-1}$ TCNB in hexane.

The results of the analyses are given in Table 11.4, the amount of CIPC/TCNB left on the precolumn was calculated by comparing the peak areas for the CIPC/TCNB desorbed from the stored precolumns to the peak areas of CIPC/TCNB desorbed from three freshly conditioned precolumns injected with $1mm^3$ of 5000ug $cc^{-1}$ CIPC/TCNB, which were desorbed 30 min after injection.

TABLE 11.4

Storage life of sampled precolumns

| Precolumn | CIPC (μg) | | | TCNB (μg) | | |
|---|---|---|---|---|---|---|
| | TIME (days) | | | TIME (days) | | |
| | 0 | 5 | 10 | 0 | 5 | 10 |
| 1 | 4.91 | 4.84 | 4.50 | 4.93 | 4.82 | 4.83 |
| 2 | 5.03 | 4.86 | 4.48 | 5.00 | 4.80 | 4.90 |
| 3 | 5.11 | 5.05 | 4.33 | 5.07 | 4.76 | 4.97 |
| 4 | 4.88 | 4.93 | 4.52 | 4.92 | 4.98 | 4.84 |
| 5 | 4.97 | 4.98 | 4.74 | 5.02 | 5.01 | 4.90 |
| $\bar{x}$ | 4.98 | 4.93 | 4.51 | 4.99 | 4.87 | 4.88 |
| S.D. | 0.09 | 0.08 | 0.15 | 0.06 | 0.11 | 0.06 |

The results of Table 11.4 show that precolumns sampled with CIPC can be stored for up to five days with no appreciable sample loss, precolumns sampled with TCNB could be stored for up to ten days with no appreciable sample loss.

## 11.3 CONCLUSIONS

From these assessments it was concluded that the gas chromatographic/desorption system was satisfactory. It has been shown from the comparison of the desorption and injection methods that both CIPC and TCNB were quantitatively introduced into the column using the desorption method, and that they were introduced within 2 - 4 min. Furthermore the orientation of the precolumn during desorption made no difference to the levels of CIPC/TCNB that were determined. Finally, the sampled precolumns could be stored for up to five days with no appreciable sample loss, which permitted the sampling of potato stores at a considerable geographical distance and journey time from the laboratory.

# CHAPTER 12

## HEADSPACE ANALYSIS OF TCNB AND CIPC AT DALTON POTATO STORE 1980

## 12.1 INTRODUCTION

This chapter is concerned with the headspace analysis of TCNB and CIPC in a box potato store. The work was carried out to assess the headspace concentration of TCNB after it had been applied to stored potatoes as an experimental formulation. TCNB is usually applied to potatoes when they are loaded into 1 tonne boxes, in the form of a granular formulation. However, in this case the TCNB was applied to the potatoes, after they had been boxed and loaded into the store, in the form of a thermal fog which is normally used for the application of CIPC.

The aim of the work was to sample the headspace TCNB in the potato store for a period of 3 months - 4 months after the TCNB had been applied. However, 16 days after the TCNB was applied, an application of CIPC was made as the initial application of TCNB had not suppressed sprout growth, 46 days after this the potatoes were removed from the store as their storage condition had seriously deteriorated.

Therefore, a long term analysis of the TCNB headspace in the potato store was not possible. Two headspace samplings were taken, the first 3 days after the TCNB thermal fog had been applied, the second 5 days after the CIPC thermal fog application.

The potato store was a 2150 tonne box potato store at Dalton, Yorkshire, the potatoes c.v. Record were stored in 1 tonne wooden boxes, these were stacked along the sides of the store. The store temperature was 10°C - 11°C. The application and sampling dates are as follows:-

25th January 1980 - Store treated with TCNB.

28th January 1980 - Store sampled for headspace TCNB.

13th February 1980 - Store treated with CIPC.

28th February 1980 - Store sampled for headspace TCNB/CIPC.

31st March 1980 - Store emptied and potatoes taken for processing.

The TCNB was applied as a thermal fog to the stacked potatoes in the store. The TCNB was made up in a formulation called Tecnafog 30 (30% TCNB in dichloromethane) and was applied at the level of 30g TCNB tonne$^{-1}$ of potatoes. The CIPC was applied as a thermal fog to the stacked potatoes in the store. The CIPC formulation, unspecified, was applied at the level of 20g CIPC tonne$^{-1}$ of potatoes.

## 12.2 EXPERIMENTAL

The headspace samples of TCNB and CIPC were obtained with the headspace sampling apparatus described in Chapter 11, the headspace samples were taken at flow rates of 300 cc $min^{-1}$ - 600 cc $min^{-1}$ for sample volumes of 7 $dm^3$ - 12 $dm^3$. Before and after sampling the precolumns were sealed with PTFE caps and stored in glass screw top jars, the precolumns were analysed within 3 days of being sampled.

The sampled precolumns were analysed using the method and conditions described in Chapter 11. The headspace results are expressed in terms of ug TCNB/CIPC in 10 $dm^3$ of store headspace. Headspace samples were taken at eight locations within the store on each of the two sampling dates. These locations are referred to as Site 1 - Site 8, each site was sampled in triplicate. A diagram of the store, Figure 12.1, shows the location of the sampling sites.

The headspace concentrations of TCNB and CIPC for both sampling dates are given in Tables 12.1 - 12.4.

FIGURE 12.1

DALTON STORE DIAGRAM

SCALE 1 cm = 2 METRES

S1 - S8 CORRESPOND TO SITES 1 - SITES 8

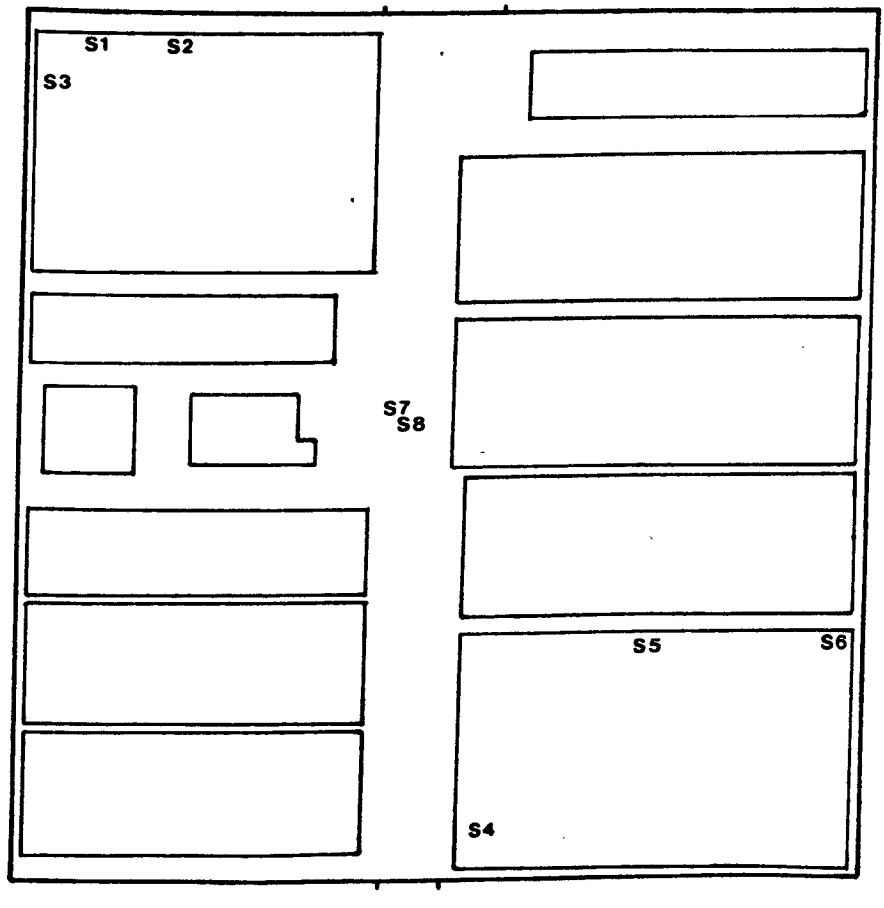


TABLE 12.1

TCNB Headspace, First Sampling 28/1/80

| Site | Height | TCNB (ug 10 $dm^{-3}$) | | | | |
|---|---|---|---|---|---|---|
| | | SAMPLE | | | $\bar{x}$ | S.D. |
| | | 1 | 2 | 3 | | |
| 1 | Top | 8.3 | 7.5 | 9.9 | 8.6 | 1.2 |
| 2 | Middle | 9.1 | 8.0 | 8.8 | 8.6 | 0.5 |
| 3 | Bottom | 6.9 | 7.5 | 6.9 | 7.1 | 0.3 |
| 4 | Top | U | 6.0 | 7.6 | 6.8 | 1.1 |
| 5 | Middle | 5.8 | 6.3 | U | 6.0 | 0.4 |
| 6 | Bottom | 8.3 | 6.8 | U | 7.5 | 1.1 |
| 7 | Top | 6.5 | 7.4 | 7.0 | 7.0 | 0.4 |
| 8 | Bottom | 7.4 | 6.1 | 5.3 | 6.3 | 1.1 |

TABLE 12.2

TCNB Headspace, Second Sampling 28/2/80

| Site | Height | TCNB (ug 10 $dm^{-3}$) | | | | |
|---|---|---|---|---|---|---|
| | | SAMPLE | | | $\bar{x}$ | S.D. |
| | | 1 | 2 | 3 | | |
| 1 | Top | U | 11.9 | 9.3 | 10.6 | 1.8 |
| 2 | Middle | 11.0 | 13.3 | 11.5 | 11.9 | 1.2 |
| 3 | Bottom | 12.2 | 11.2 | 10.6 | 11.3 | 0.8 |
| 4 | Top | 6.0 | 7.3 | 7.5 | 6.9 | 0.8 |
| 5 | Middle | 6.4 | 7.3 | 7.0 | 6.9 | 0.5 |
| 6 | Bottom | 6.2 | 6.2 | 6.5 | 6.3 | 0.2 |
| 7 | Top | 6.9 | 9.2 | 4.5 | 6.9 | 2.3 |
| 8 | Bottom | 7.2 | 6.9 | 8.3 | 7.5 | 0.7 |

TABLE 12.3

CIPC Headspace, Second Sampling 28/2/80

| Site | Height | CIPC (ug 10 $dm^{-3}$) | | | | |
|---|---|---|---|---|---|---|
| | | SAMPLE | | | | |
| | | 1 | 2 | 3 | $\bar{x}$ | S.D. |
| 1 | Top | U | 0.62 | 0.61 | 0.61 | 0.01 |
| 2 | Middle | 0.92 | 0.99 | 1.13 | 1.01 | 0.11 |
| 3 | Bottom | 1.10 | 0.78 | 0.85 | 0.91 | 0.17 |
| 4 | Top | 0.47 | 0.24 | 0.32 | 0.34 | 0.12 |
| 5 | Middle | 0.25 | 0.36 | 0.30 | 0.30 | 0.05 |
| 6 | Bottom | 0.88 | 0.78 | 0.93 | 0.86 | 0.08 |
| 7 | Top | 0.51 | 0.57 | 0.45 | 0.51 | 0.06 |
| 8 | Bottom | 0.50 | 0.67 | 0.83 | 0.67 | 0.16 |

TABLE 12.4

Mean headspace concentrations of TCNB and CIPC on both sampling dates

| Site | Height | HEADSPACE CONCENTRATION (ug 10 $dm^{-3}$) | | |
|---|---|---|---|---|
| | | TCNB 1st Sampling | TCNB 2nd Sampling | CIPC 2nd Sampling |
| 1 | Top | 8.6 | 10.6 | 0.61 |
| 2 | Middle | 8.6 | 11.9 | 1.01 |
| 3 | Bottom | 7.1 | 11.3 | 0.91 |
| 4 | Top | 6.8 | 6.9 | 0.34 |
| 5 | Middle | 6.0 | 6.9 | 0.30 |
| 6 | Bottom | 7.5 | 6.3 | 0.86 |
| 7 | Top | 7.0 | 6.9 | 0.51 |
| 8 | Bottom | 6.3 | 7.5 | 0.67 |
| | * $\bar{x}$ | 7.3 | 8.4 | 0.66 |
| | * S.D. | 1.1 | 2.4 | 0.27 |

Notes on Tables 12.1 - 12.4

Height - refers to the sampling position on the stack of boxed potatoes, the boxes were stacked 5 high.

Top : sample taken at top box of stack.

Middle : sample taken from third box of stack.

Bottom : sample taken from bottom box of stack.

Sites 7 and 8 were in the middle of the central corridor.

Site 7 : 4m off the floor.

Site 8 : 200mm off the floor.

Table 12.4 * $\bar{x}$ - mean of all results for each sampling.

* S.D. - standard deviation of all the results for each sampling.

U - precolumn partially unpacked during sampling due to suction from the sampling pump, therefore no results available.

## 12.3 DISCUSSION

The most noticeable feature of the results was that there was no decrease in the levels of headspace TCNB between the first and the second sampling dates, the mean headspace concentration of TCNB was slightly higher on the second sampling, however, this may not be significant as the S.D. was large for these results, due to the high levels of TCNB at Site 1 - Site 3. It was thought that the headspace TCNB levels would fall during the four week period due to irreversable adsorption of the TCNB into the potatoes and fabric of the store, and loss of TCNB by ventilation to the environment outside the store. However, it appeared that there

was a sufficient reservoir of residue TCNB that could generate a constant headspace level of TCNB, it was not known whether the headspace was saturated with vapour phase TCNB and that eventually after the source of residue TCNB had been depleted, the headspace TCNB would fall.

The next feature of the results was the difference in the levels of headspace TCNB and CIPC on the second sampling date, the CIPC was present in the headspace at an order of one tenth of the TCNB. During the four week period when only the headspace TCNB was present in the store atmosphere, the potatoes sprouted vigorously, the CIPC when applied immediately arrested the sprout growth and subsequently the sprouts were killed within 3 days. These observations in combination with the relative headspace concentrations of CIPC and TCNB show that CIPC is a more active sprout suppressant than TCNB and that it is active at headspace concentrations of 0.3 ug 10 $dm^{-3}$ - 1.0 ug 10 $dm^{-3}$.

At Site 7 and Site 8 the headspace samples were taken at a distance of 4m from any of the treated tubers, and at Site 8 the headspace was sampled 4m away from any surface. The headspace levels of CIPC and TCNB at both sites were similar to the levels present above the treated tubers, Site 1 - Site 6, this indicated that both chemicals were well distributed throughout the store headspace.

The large S.D. for the headspace CIPC results was due to the method used to quantify the CIPC and TCNB, the TCNB and CIPC were quantified by multiplying the peak height by

the peak width at half peak height to obtain the area for the eluted CIPC or TCNB. As each headspace sample when desorbed only permitted one analytical run to be made, the sensitivity of the FID amplifier was set so that the TCNB peak was on scale. Therefore, as the CIPC was present at a tenth of the concentration of TCNB, the CIPC peak was very small on the chart recorder trace, and the quantification of the small CIPC peak was difficult and subject to greater error. However, despite this a relative measure of the headspace concentrations of CIPC and TCNB was obtained.

# CHAPTER 13

## HEADSPACE ANALYSIS OF CIPC AT GRIMSBY POTATO STORE 1980

## 13.1 INTRODUCTION

This chapter relates to the headspace and residue analysis of CIPC in a large scale commercial potato store. The store had been in continuous use for four years prior to this experiment and several applications of CIPC had been made to potatoes in the store over the last four storage seasons. It was thought that the fabric of the store itself as it had been in contact with the CIPC when applied to the previous seasons potatoes would also contain some residue CIPC, and that this residue CIPC could act as a source for headspace CIPC in the store.

The headspace CIPC was sampled on two occasions. The first sample was taken just after the new season potatoes had been loaded into the store, four months after the last application of CIPC to the previous seasons potatoes and two weeks prior to the initial application of CIPC to the current new seasons potatoes. The second set of headspace samples were taken five weeks after the initial application of CIPC to the new seasons potatoes. Several samples of the walls, floor and crates used to store the potatoes were taken at the same time as the first headspace samples.

The analysis of the residue and headspace CIPC samples would show the levels of CIPC that were carried over from one storage season to the next, and the levels of CIPC present in the store headspace after the initial application of CIPC to the new seasons potatoes.

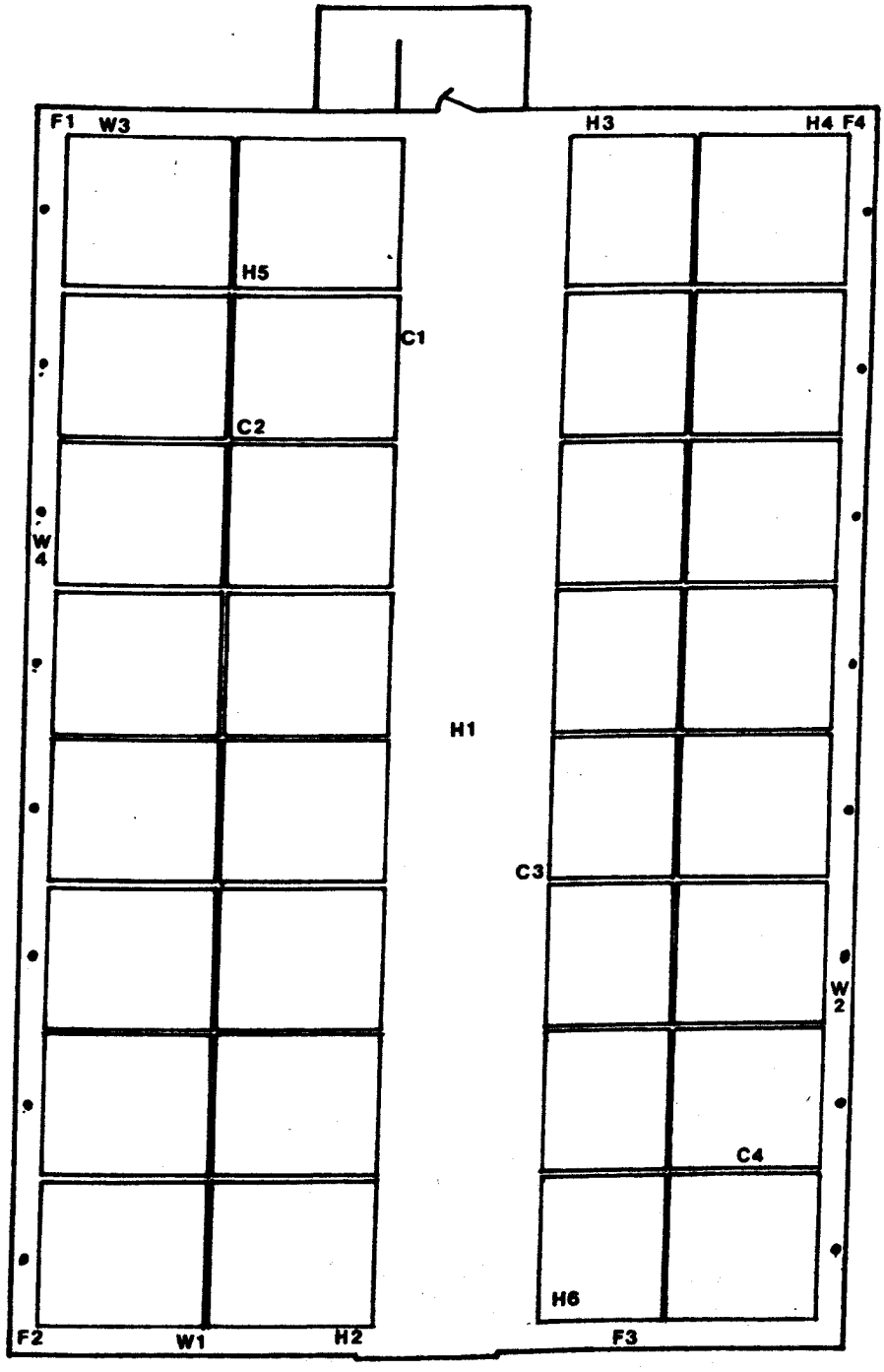
A diagram of the store is shown in Figure 13.1 and the central corridor and top of the potato crates are illustrated in Plates 17 and 18, respectively.

FIGURE 13.1

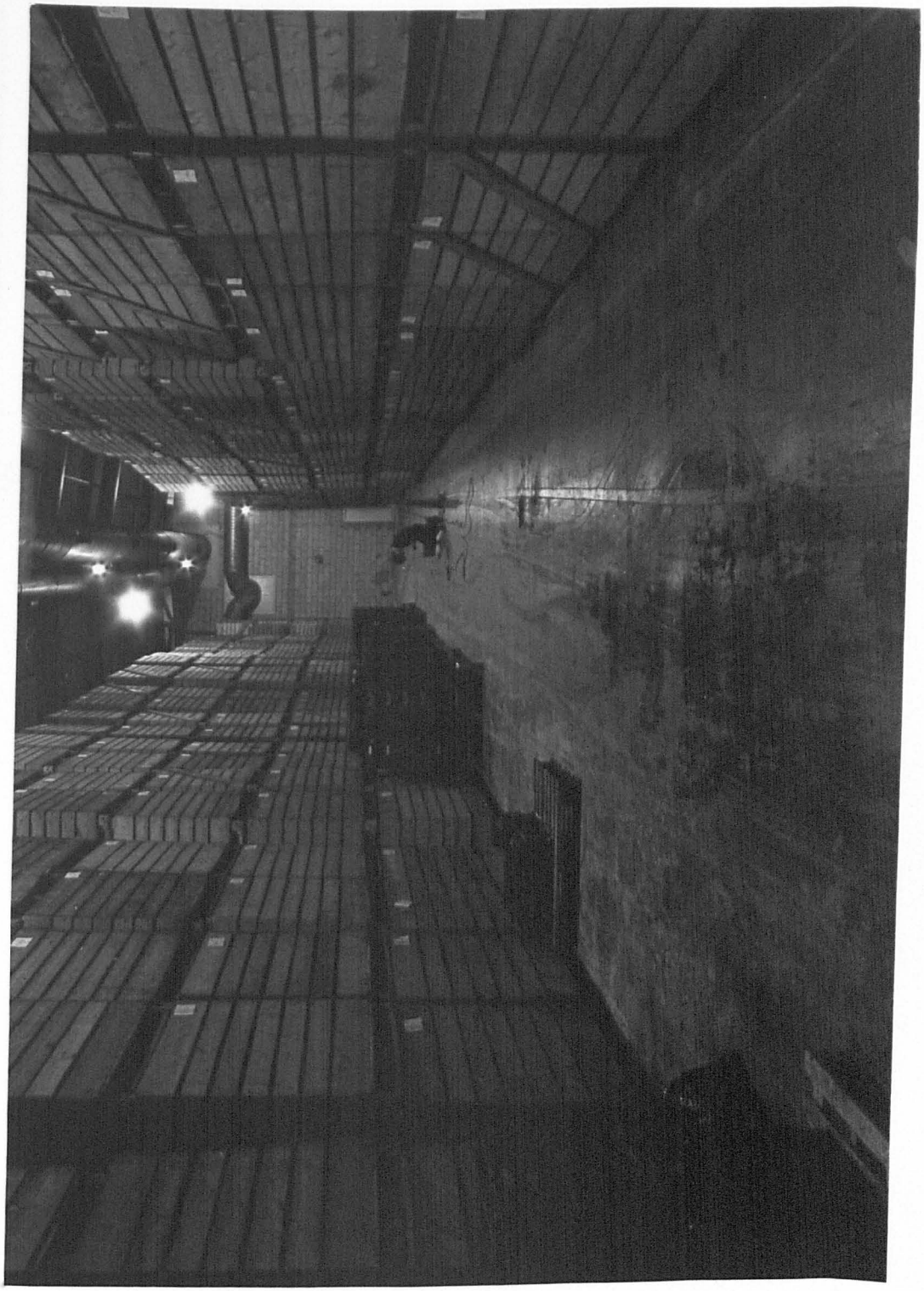
GRIMSBY STORE A DIAGRAM

H1 - H6 HEADSPACE SAMPLING SITES
C1 - C6 CRATE SAMPLING SITES
W1 - W4 WALL SAMPLING SITES
F1 - F4 FLOOR SAMPLING SITES

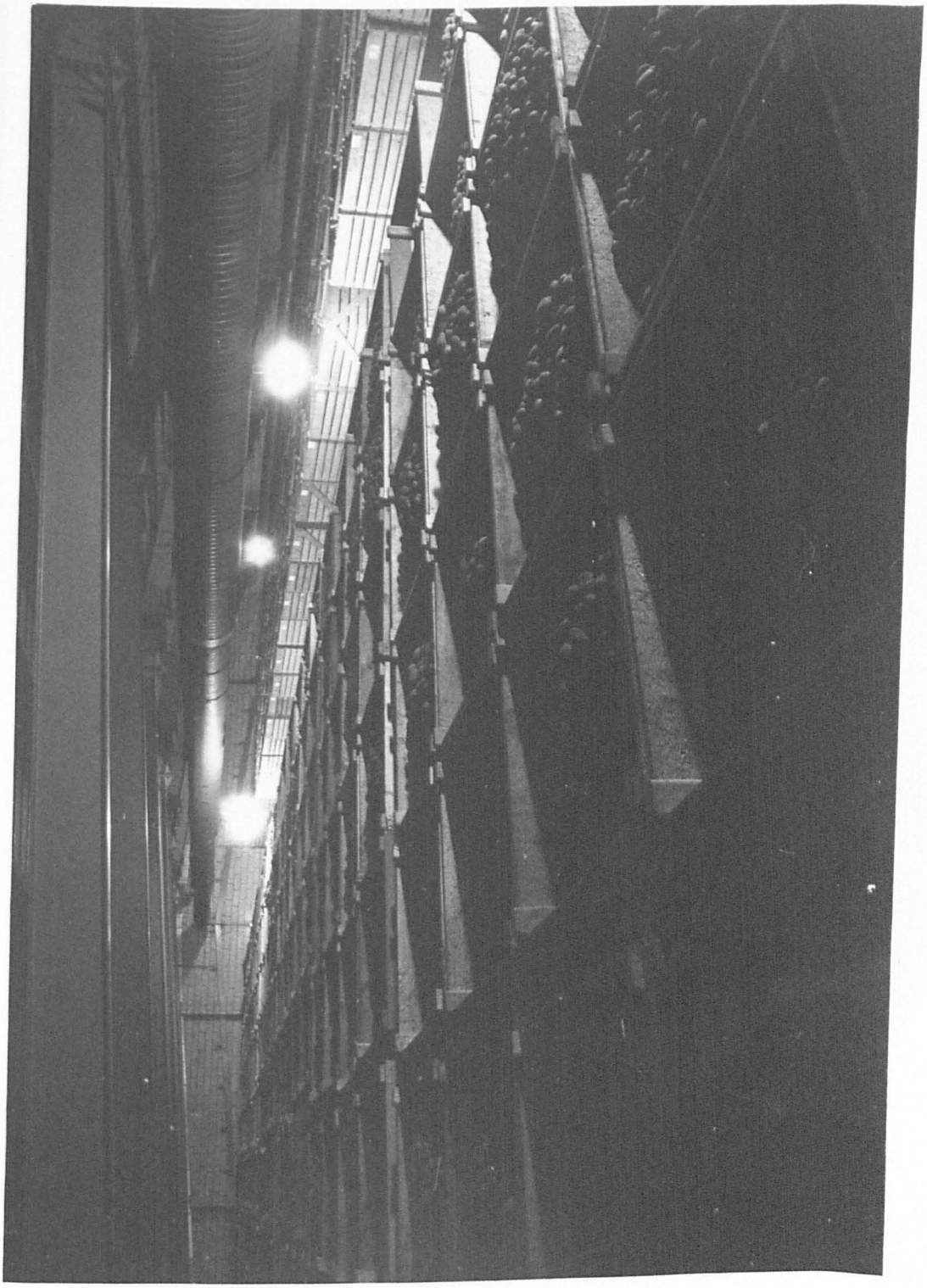
SCALE: 1 cm = 2 METRES



# PLATE 17



# PLATE 18



The store used for this experiment was Store A, a 3,500 tonne box potato store which was part of United Biscuits potato processing complex at Grimsby, Yorkshire. The sampling and application of CIPC dates are as follows:-

1. June 1980 — Store treated with CIPC, store emptied of potatoes.
2. July - October 1980: — Store empty.
3. October 14th 1980: — Store loaded with potatoes. Headspace samples taken from the store, residue samples taken from the fabric of the store.
4. October 28th 1980: — Store treated with a thermal fog of CIPC applied at a rate of 20mg CIPC $kg^{-1}$ potatoes.
5. December 3rd 1980: — Headspace samples taken from the store.

## 13.2 EXPERIMENTAL

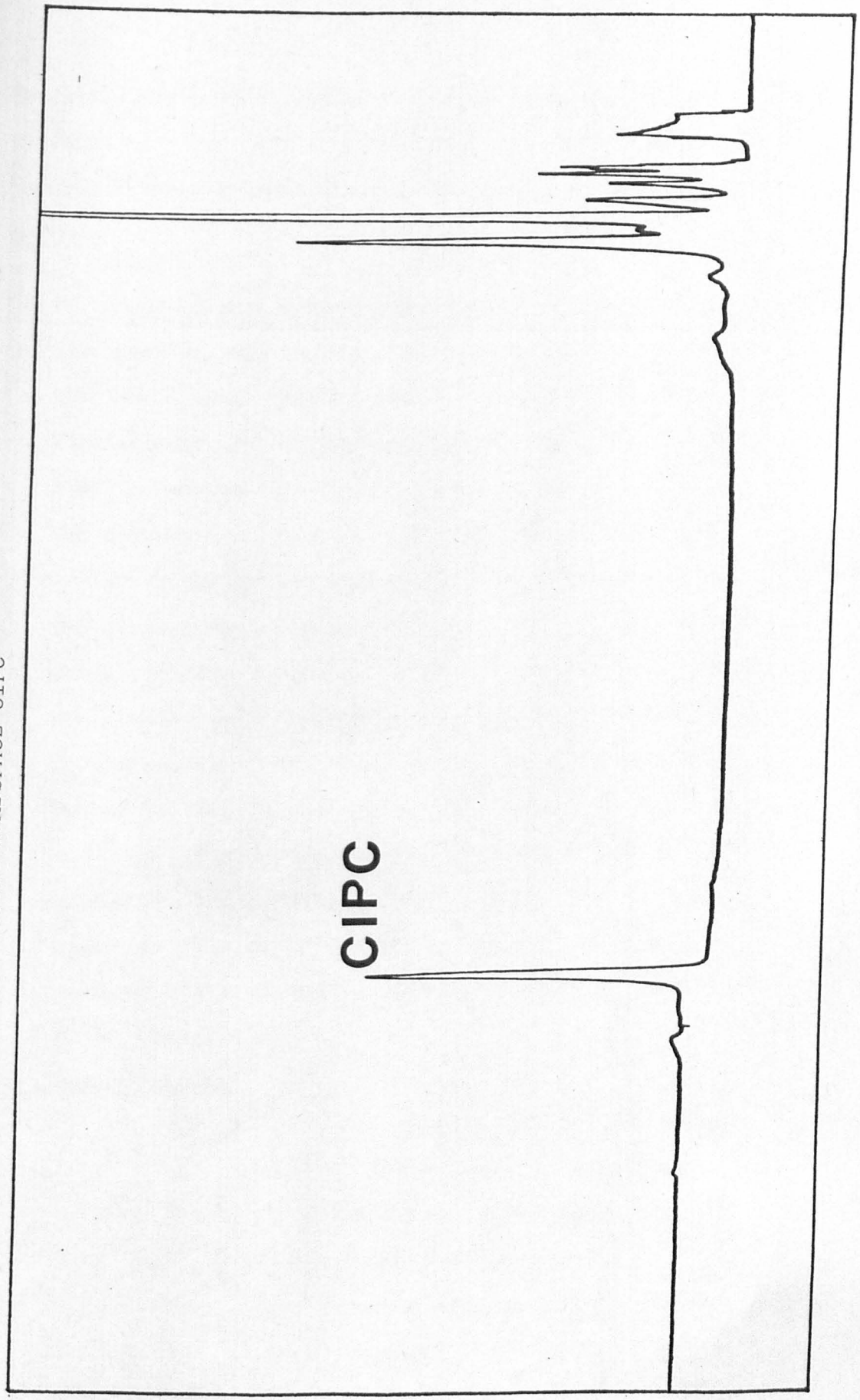
### 13.2.1 Headspace Analysis

The store was sampled in triplicate at six sites on both sampling dates, the headspace sampling sites are H1 - H6 as shown in Figure 13.1. The headspace samples were obtained using the headspace sampling apparatus described in Chapter 11, the samples were taken at flow rates of 200 cc $min^{-1}$ - 400 cc $min^{-1}$ for sample volumes of 7 $dm^3$ - 10 $dm^3$. The precolumns were sealed with PTFE caps and stored in glass screw top jars before and after sampling, and were analysed within three days of being sampled. A representative chromatogram of a CIPC headspace analysis is shown in Chromatogram 13.1.

### 13.2.2 Residue Analysis

Samples of the floor, wall and potato crates were taken on the first sampling date. Before the samples were taken, the surfaces were brushed and the top 1 - 2 mm removed and discarded, (thereafter the samples were taken to a depth of 5 - 10 mm).

The floor samples were taken at four sites in the store, F1 - F4 as shown in Figure 13.1, the samples weights were 100g - 200g and contained a mixture of cement and stones. The wall samples were taken at four sites in the store, W1 - W4 as shown in Figure 13.1, the samples weights were 40g - 60g. The crate samples were taken at four sites, C1 - C4 as shown in Figure 13.1, the samples weights were 20g - 40g.




CHROMATOGRAM 13.1 STORE HEADSPACE CIPC

The precolumns were analysed using the analysis method and conditions as described in Chapter 11. The headspace results are expressed as ug CIPC in 10 $dm^3$ of store headspace.

The floor, wall and crate samples were analysed as follows:-

1. The samples were milled to a fine powder in the case of the crate wood samples, or mechanically crushed to a fine powder in the case of the concrete floor samples and the breize block wall samples.
2. Two portions of each sample were then continuously extracted with acetone (Analar) as follows -
   The samples were placed in a cellulose extraction thimble, which was then placed in a soxhlet extraction apparatus, the samples were then continuously extracted with 100 cc of acetone for 16 hr - 18 hr. The sample weights placed in the thimble were 4g - 5g for the crate samples, 8g - 10g for the wall samples and 25g - 30g for the floor samples. The acetone extracts were then reduced in volume to 10cc and stored in vials at 0°C till analysis. The extracts were analysed by G.C., the G.C. conditions are as follows:

Gas Chromatography

G.C.: Pye 104 (Pye Unicam Ltd, U.K.)

Column: 2 metre glass column 6mm O.D., 4mm I.D., packed with 5% OV101 (Phase Separations Ltd) on Gas Chrom Q 100/120 mesh (Phase Separations Ltd).

Temperature:

Column 170°C.

| | |
|---|---|
| Injection Port: | 240°C. |
| Detector F.I.D.: | 250°C. |
| Detector: | $H_2$ 45 cc $min^{-1}$. |
| | $O_2$ 210 cc $min^{-1}$. |
| Carrier Gas: | $N_2$ 43 cc $min^{-1}$. |

The injections of the acetone extracts were $1mm^3$ - $5mm^3$ in volume and were made with a $10mm^3$ syringe (700 series Hamilton, Phase Separations Ltd). The CIPC was quantified by comparison with authentic CIPC standards run under the same chromatographic conditions. The CIPC residues are expressed as mg CIPC in 1g of sample.

The results of the first CIPC headspace analysis are shown in Table 13.1, and the results of the second CIPC headspace analysis are shown in Table 13.2. The results of the CIPC residue analyses are shown in Table 13.3.

TABLE 13.1

CIPC Headspace, First Sampling 14/10/80

| Site | Height | CIPC (ug 10 $dm^{-3}$) | | | | |
|---|---|---|---|---|---|---|
| | | SAMPLE | | | $\bar{x}$ | S.D. |
| | | 1 | 2 | 3 | | |
| H1 | Bottom | 1.27 | 1.39 | 1.10 | 1.25 | 0.14 |
| H2 | Bottom | 0.73 | 0.62 | 0.66 | 0.67 | 0.05 |
| H3 | Bottom | 0.99 | 0.91 | 0.95 | 0.95 | 0.04 |
| H4 | Top | 0.21 | 0.40 | 0.37 | 0.32 | 0.10 |
| H5 | Middle | 0.52 | 0.49 | 0.37 | 0.46 | 0.07 |
| H6 | Top | 0.41 | 0.51 | 0.50 | 0.47 | 0.05 |

TABLE 13.2

CIPC Headspace, Second Sampling 3/12/80

| Site | Height | CIPC (ug 10 $dm^{-3}$) | | | | |
|---|---|---|---|---|---|---|
| | | SAMPLE | | | $\bar{x}$ | S.D. |
| | | 1 | 2 | 3 | | |
| H1 | Bottom | 0.94 | 1.08 | 0.87 | 0.96 | 0.11 |
| H2 | Bottom | 0.62 | 0.62 | 0.75 | 0.66 | 0.07 |
| H3 | Bottom | 0.68 | 0.50 | 0.52 | 0.56 | 0.10 |
| H4 | Top | 0.24 | 0.34 | 0.11 | 0.23 | 0.11 |
| H5 | Middle | 0.44 | 0.28 | 0.32 | 0.34 | 0.08 |
| H6 | Top | 0.33 | 0.49 | 0.21 | 0.34 | 0.14 |

Notes on Table 13.1 and 13.2

Height - refers to the sampling position on the stack of boxed potatoes, the boxes were stacked 5 high.

Top : sample taken from top box of stack.

Middle : sample taken from third box of stack.

Bottom : sample taken from bottom box of stack.

Site H1 - in the middle of the central corridor, 1m off the floor.

TABLE 13.3

CIPC Residue in Floors, Walls and Crates of Store

| Material | Site | CIPC (mg CIPC/g sample) | | |
|---|---|---|---|---|
| | | SAMPLE | | |
| | | 1 | 2 | $\bar{x}$ |
| Floor | F1 | 9.53 | 9.41 | 9.47 |
| Floor | F2 | 1.98 | 2.07 | 2.02 |
| Floor | F3 | 2.47 | 2.30 | 2.38 |
| Floor | F4 | 8.96 | 8.77 | 8.87 |
| Wall | W1 | 0.23 | 0.20 | 0.21 |
| Wall | W2 | 0.14 | 0.17 | 0.15 |
| Wall | W3 | 0.13 | 0.12 | 0.13 |
| Wall | W4 | 0.30 | 0.28 | 0.29 |
| Crate | C1 | 0.84 | 0.79 | 0.81 |
| Crate | C2 | 0.10 | 0.13 | 0.11 |
| Crate | C3 | 0.07 | 0.06 | 0.06 |
| Crate | C4 | 0.11 | 0.11 | 0.11 |

## 13.3 DISCUSSION

A comparison of the headspace CIPC on the first and second samplings showed that the levels of CIPC were higher on the first sampling. This was unexpected bearing in mind the dates on which CIPC was applied tc the store, a period of four months had elapsed between an application of CIPC and the first headspace sampling, and a period of only five weeks between an application of CIPC and the second headspace sampling. It was thought that the most recent application of CIPC would have given higher headspace levels of CIPC, however, as shown by the results this was not the case. Further investigations of the storage conditions showed that the store temperature was 12°C on the first sampling and 9°C on the second sampling, the difference in the CIPC levels could be explained in terms of this, in that the higher temperature promoted increased volatilsation of CIPC.

The CIPC residue analysis showed that the store was heavily contaminated with CIPC, the floor by far was the most heavily contaminated, the sample taken at site F1 contained 9.47 mg CIPC/g sample, almost 1% of the sample was CIPC. This could have been due to spillage of the CIPC formulation at this site during application, however, similarly high levels of CIPC were found at the other three floor sites, F2 - F4, indicating that this result was not due to sampling an unusually heavily contaminated site. The method of application of CIPC was considered to be relevant to these high floor residues, when applied as a thermal fog the spray of volatilised CIPC mist was directed between the stacked potato boxes, and a high amount of CIPC has been observed

to "fall out" and deposit in the areas where the samples were taken. Therefore an accumulation of CIPC and solvent vapour occurred at these sites and the CIPC would be adsorbed into the floor. The residue levels of CIPC in the walls and crates although not as high as in the floor, did indicate a heavy contamination, the wall samples were taken at sites which would not have been subject to a direct spray from the application of a CIPC "fog", the location of the crates that were sampled during previous applications of CIPC was not known. It should be pointed out that when the residue samples were taken, the surface of the material was cleaned, and that the residue levels reported reflect the levels of CIPC adsorbed into the material and not the levels of CIPC that were deposited on the surface.

The source of the CIPC determined in the first headspace sampling was most probably from CIPC that was released from the residue present in the fabric of the store. On both the first and second headspace samplings the highest levels of headspace CIPC were encountered at site H1, in the middle of the corridor, this could have been due to high residue CIPC levels in the floor at that site. Overall the levels of headspace CIPC were in the range 0.3 ug 10 $dm^{-3}$ - 1.3 ug 10 $dm^{-3}$, which were similar to those enccuntered for headspace CIPC in the store at Dalton, Chapter 12, the differences in headspace CIPC at any particular site may be due to the temperature and levels of residue CIPC at that site.

Of particular interest was the residue and headspace CIPC relationship, and the extent to which the levels of residue CIPC had built up over the previous storage seasons.

It would seem from results of the first headspace sampling that the residue CIPC in the fabric of the store was a major factor in the levels of headspace CIPC, and that the levels of CIPC deposited on the tubers was of secondary importance.

It was decided that it was necessary to conduct further investigations into the distribution of both residue and headspace CIPC in the potato stores at Grimsby. These investigations are described in the next chapter.

# CHAPTER 14

# HEADSPACE ANALYSIS OF CIPC AT GRIMSBY POTATO STORE 1981 - 1982

## 14.1 INTRODUCTION

This chapter is concerned with the determination of the headspace and residue levels of CIPC in two large scale commercial potato stores. The two stores used in the work were A and G stores, part of a potato storage complex at Grimsby, Yorkshire. A store had been in use as a potato store for five years, and had been treated with CIPC on several occasions, G store had been constructed five months prior to the work and had not been treated with CIPC.

This, therefore, provided an opportunity for the determination of residue and headspace CIPC in A store, that over a period of time had become contaminated with CIPC, as shown in the results of the residue analysis performed on A store, Chapter 13. This was compared to the residue and headspace levels of CIPC in G store that at the start of the experiment had not been treated with CIPC.

The residue levels of CIPC in both A and G stores were determined on two sampling dates, the first before the initial application of CIPC to the new storage seasons potatoes, the second after two applications of CIPC had been made to both stores.

The headspace CIPC in A and G stores was sampled before the initial application of CIPC to both stores, and over a seven month period after the initial application of CIPC.

## 14.2 EXPERIMENTAL

The potato stores used for this experiment were Store A and Store G both 3,500 tonne box potato stores, they were part of a six store complex at the United Biscuits factor Grimsby, Yorkshire. Both stores were exactly the same in design and construction, and were loaded with 1 tonne crates of potatoes as shown in Figure 14.1.

The CIPC was applied and sampled onthe folowing dates:-

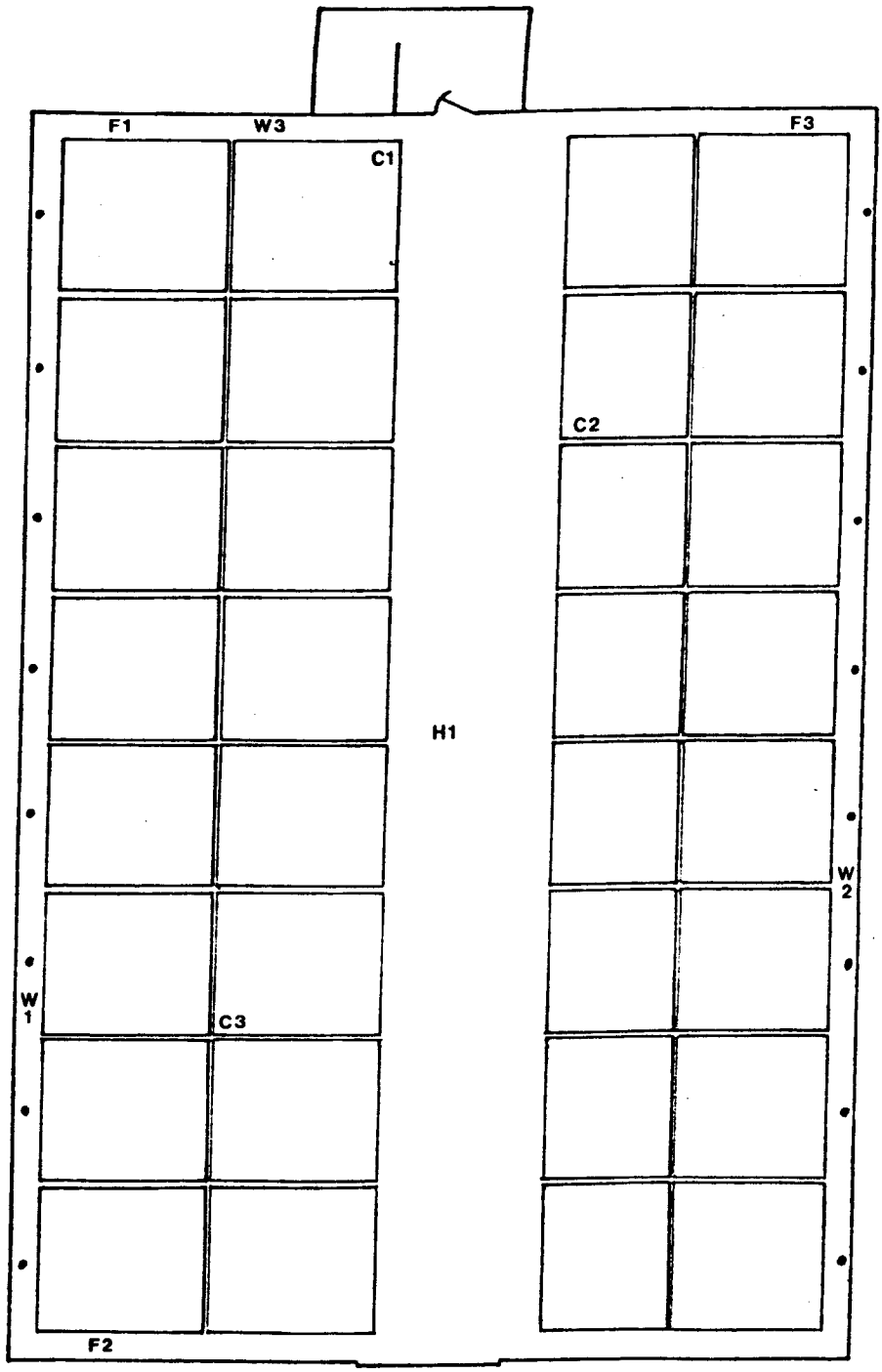
1. October 1981: Stores A and G loaded with main crop potatoes.
2. November 3rd 1981: Stores A and G sampled for headspace and residue CIPC.
3. November 14th 1981: Stores A and G treated with a thermal fog of CIPC, A at a rate of 14 mg CIPC $kg^{-1}$ potatoes, G at a rate of 20 mg CIPC $kg^{-1}$ potatoes.
4. November 25th 1981: Stores A and G sampled for headspace CIPC, headspace samples were then taken at weekly intervals from both stores until June 31st 1982.
5. December 14th 1981: Stores A and G treated with a thermal fog of CIPC, A at a rate of 14 mg $kg^{-1}$ potatoes, G at a rate of 20 mg $kg^{-1}$ potatoes.
6. January 23rd 1982: Stores A and G sampled for residue CIPC.
7. March 26th 1982: Stores A and G treated with a thermal fog of CIPC, A at a rate of 14 mg $kg^{-1}$ potatoes, G at a rate of 20 mg $kg^{-1}$ potatoes.
8. July 1982: Stores A and G emptied of the remaining stored potatoes.

FIGURE 14.1

GRIMSBY STORE A AND G DIAGRAM

H1 - H6 HEADSPACE SAMPLING SITES
C1 - C3 CRATE SAMPLING SITES
W1 - W3 WALL SAMPLING SITES
F1 - F3 FLOOR SAMPLING SITES

SCALE: 1 cm = 2 METRES



Headspace Samples

The first set of headspace samples taken from A and G stores on November 3rd 1981 were obtained using the headspace sampling apparatus described in Chapter 11. The samples were taken in the middle of the central corridor, 0.5m off the floor, H1 as shown in Figure 14.1. Triplicate headspace samples were taken at flow rates of 200 cc $min^{-1}$ - 400 cc $min^{-1}$ and for sample volumes of 7 $dm^3$ - 10 $dm^3$. The precolumns were sealed with PTFE caps, stored in glass screw top jars before and after sampling and were analysed within four days of being sampled.

The subsequent series of headspace samples from both stores were obtained with a small peristaltic air sampling pump ((Polymeter) Draeger Safety, Chesham, England), these samplings commenced on November 25th 1981 and ceased on June 31st 1982. Between these periods both A and G stores were sampled once every week. The precolumns were attached to the pump with a 300 mm length of silicone rubber tubing ((Esco Rubber Ltd), Teddington, England), the store air was drawn through the precolumn at a flow rate of 10 cc $min^{-1}$ - 14 cc $min^{-1}$ for sample volumes of 3 $dm^3$ - 7 $dm^3$ over a period of 5 hr - 8 hr. This, therefore, gave a time weighted average samples of the headspace CIPC over the 5hr - 8 hr sampling period. The samples were taken in the middle of the central corridor, 0.5 m off the floor, H1, Figure 14.1. These samples were taken by the store manager, who sampled the store headspace, as described, at any particular time during the week that was convenient. Freshly conditioned precolumns sealed with PTFE caps were posted to the store

manager, and the sampled precolumns were sealed with PTFE caps and posted back to the laboratory for analysis. This proved to be a convenient system, the sampled precolumns were analysed within four days of being sampled.

Residue Samples

Both A and G stores were sampled for residue CIPC from the walls, floor and potato crates. The stores were sampled on two occasions, November 3rd 1981, before the initial seasons application of CIPC, and on January 23rd 1982, after two successive applications of CIPC had been made. Before the samples were taken the surfaces were brushed and the top 1mm - 2mm removed and discarded thereafter, the samples were taken to a depth of 5mm - 10mm.

The floor was sampled at three sites within each store, F1 - F3, as shown in Figure 14.1, the samples weights were 100 g - 200 g containing a mixture of stones and cement. The wall samples were taken at three sites within each store, W1 - W3, as shown in Figure 14.1, the samples weights sere 40 g - 60 g. The crate samples were taken at three sites within each store, C1 - C3, as shown in Figure 14.1, the samples weights were 20 g - 40 g.

Analysis

The headspace samples of CIPC were analysed according to the method described in Chapter 11. The residue samples were analysed according to the method described in Chapter 13, for the previous residue analysis.

## 14.3 RESULTS

The headspace levels of CIPC in A and G stores are given in Table 14.1, together with the store air temperature and humidity at the time of sampling. The headspace levels of CIPC plotted against time are shown in Figures 14.2 and 14.3 for stores A and G respectively. The headspace levels of CIPC plotted against store atmosphere temperature are shown in Figures 14.4 and 14.5 for stores A and G respectively. The headspace levels of CIPC plotted against store atmosphere humidity are shown in Figures 14.6 and 14.7 for stores A and G respectively. The residue levels of CIPC in store A are given in Table 14.2, the residue levels of CIPC in store G are given in Table 14.3.

TABLE 14.1

Headspace Levels of CIPC in A and G Stores

| Week | STORE A | | | STORE G | | |
|---|---|---|---|---|---|---|
| | CIPC (ug 10 $dm^3$) | TEMP. (°C) | HUMID. (%) | CIPC (ug 10 $dm^3$) | TEMP. (°C) | HUMID. (%) |
| 1 | 0.17 | 10 | - | <0.01 | 10 | - |
| 2 | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - |
| *4 | 0.15 | 10 | 72 | <0.01 | 12 | 82 |
| 5 | 0.05 | 9 | 75 | <0.01 | 11 | 77 |
| 6 | 0.05 | 11 | 74 | <0.01 | 11 | 68 |
| *7 | 0.84 | 11 | 85 | 0.30 | 12 | 70 |
| 8 | 0.28 | 11 | 75 | 0.52 | 12 | 58 |
| 9 | 0.29 | 10 | 85 | 0.62 | 12 | 60 |
| 10 | - | - | - | - | - | - |
| 11 | 0.13 | - | - | 0.13 | - | - |
| 12 | 0.44 | - | - | 0.36 | - | - |
| 13 | 0.48 | 11 | 84 | 0.46 | 10 | 70 |
| 14 | 0.27 | 11 | 90 | 0.44 | 11 | 76 |
| 15 | 0.20 | 11 | 85 | 0.16 | 12 | 80 |
| 16 | - | - | - | - | - | - |
| 17 | 0.19 | 11 | 82 | 0.24 | 10 | 78 |
| *18 | 0.42 | 11 | 80 | 0.16 | 10 | 70 |
| 19 | 0.12 | 10 | 82 | 0.28 | 10 | 76 |
| 20 | 0.30 | 10 | 76 | 0.13 | 10 | 82 |
| 21 | 0.23 | 10 | 78 | 0.10 | 11 | 80 |
| 22 | - | - | - | 0.34 | 8 | 78 |
| 23 | 0.21 | 13 | 80 | 0.18 | 10 | 80 |
| 24 | - | - | - | - | - | - |
| 25 | 0.33 | 12 | 82 | 0.17 | 11 | 98 |
| 26 | 0.23 | 14 | 75 | 0.35 | 12 | 98 |
| 27 | - | - | - | - | - | - |
| 28 | 0.24 | 12 | 80 | 0.20 | 11 | 90 |
| 29 | 0.18 | 15 | 84 | 0.27 | 13 | 100 |
| 30 | 0.42 | 18 | 88 | 0.52 | 14 | 85 |
| 31 | 0.73 | 19 | 82 | - | - | - |
| 32 | 0.13 | 14 | 75 | 0.41 | 12 | 85 |

TABLE 14.1 (continued)

| Week | STORE A | | | STORE G | | |
|---|---|---|---|---|---|---|
| | CIPC (ug 10 $dm^3$) | TEMP. (°C) | HUMID. (%) | CIPC (ug 10 $dm^3$) | TEMP. (°C) | HUMID. (%) |
| 33 | 0.17 | 14 | 78 | 0.33 | 14 | 87 |
| 34 | 0.22 | 13 | 78 | 0.34 | 13 | 82 |
| 35 | 0.32 | 13 | 80 | 0.15 | 13 | 82 |
| $\bar{x}$ | 0.28 | 12.1 | 80 | 0.26 | 11.3 | 79 |
| S.D. | 0.18 | 2.4 | 9 | 0.17 | 1.4 | 10 |

Notes on Table 14.1

Week : The number of weeks after the first headspace sampling on November 3rd 1980, week 1.

* : CIPC applied to both stores during this week.

All the headspace samples were taken at site H1, Figure 14.1.

The headspace CIPC levels for week 1 are the arithmetic mean of three samples. All subsequent headspace CIPC levels are from one sample.

$\bar{x}$ arithmetic mean of all results.
S.D. standard deviation of all results.

TABLE 14.2

Residue Levels of CIPC in A Store

| Material | Site | CIPC CONCENTRATION (mg CIPC/g material) | | | | | |
|---|---|---|---|---|---|---|---|
| | | First Sampling | | | Second Sampling | | |
| | | 1 | 2 | $\bar{x}$ | 1 | 2 | $\bar{x}$ |
| Floor | F1 | 6.52 | 6.41 | 6.46 | 7.01 | 7.11 | 7.06 |
| Floor | F2 | 5.76 | 5.92 | 5.84 | 5.64 | 5.78 | 5.71 |
| Floor | F3 | 0.60 | 0.61 | 0.60 | 0.42 | 0.57 | 0.49 |
| Wall | W1 | 0.17 | 0.14 | 0.15 | 0.18 | 0.22 | 0.20 |
| Wall | W2 | 2.93 | 2.84 | 2.88 | 3.47 | 3.22 | 3.34 |
| Wall | W3 | 0.55 | 0.61 | 0.58 | 0.94 | 0.83 | 0.88 |
| Crate | C1 | 0.38 | 0.33 | 0.35 | 0.33 | 0.34 | 0.33 |
| Crate | C2 | 0.21 | 0.21 | 0.21 | 0.33 | 0.40 | 0.36 |
| Crate | C3 | 0.31 | 0.33 | 0.32 | 0.05 | 0.07 | 0.06 |

TABLE 14.3

Residue Levels of CIPC in G Store

| Material | Site | CIPC CONCENTRATION | | | | | |
|---|---|---|---|---|---|---|---|
| | | (ug CIPC/g material) | | | (mg CIPC/g material) | | |
| | | First Sampling | | | Second Sampling | | |
| | | 1 | 2 | $\bar{x}$ | 1 | 2 | $\bar{x}$ |
| Floor | F1 | <0.1 | <0.1 | <0.1 | 0.52 | 0.47 | 0.49 |
| Floor | F2 | <0.1 | <0.1 | <0.1 | 0.16 | 0.15 | 0.15 |
| Floor | F3 | <0.1 | <0.1 | <0.1 | 0.33 | 0.29 | 0.31 |
| Wall | W1 | <0.1 | <0.1 | <0.1 | 0.07 | 0.10 | 0.80 |
| Wall | W2 | <0.1 | <0.1 | <0.1 | 0.40 | 0.44 | 0.42 |
| Wall | W3 | <0.1 | <0.1 | <0.1 | 0.22 | 0.21 | 0.21 |
| Crate | C1 | <0.1 | <0.1 | <0.1 | 0.02 | 0.07 | 0.04 |
| Crate | C2 | <0.1 | <0.1 | <0.1 | 0.04 | 0.04 | 0.04 |
| Crate | C3 | <0.1 | <0.1 | <0.1 | 0.12 | 0.15 | 0.13 |

Notes on Tables 14.2 and 14.3

| | | |
|---|---|---|
| Site | : | Refers to the sites marked on Figure 14.1. |
| First Sampling | : | Residue samples taken on November 3rd 1981. |
| Second Sampling | : | Residue samples taken on January 23rd 1982. |
| 1, 2 | : | Duplicate analyses of the sample. |

FIGURE 14.2

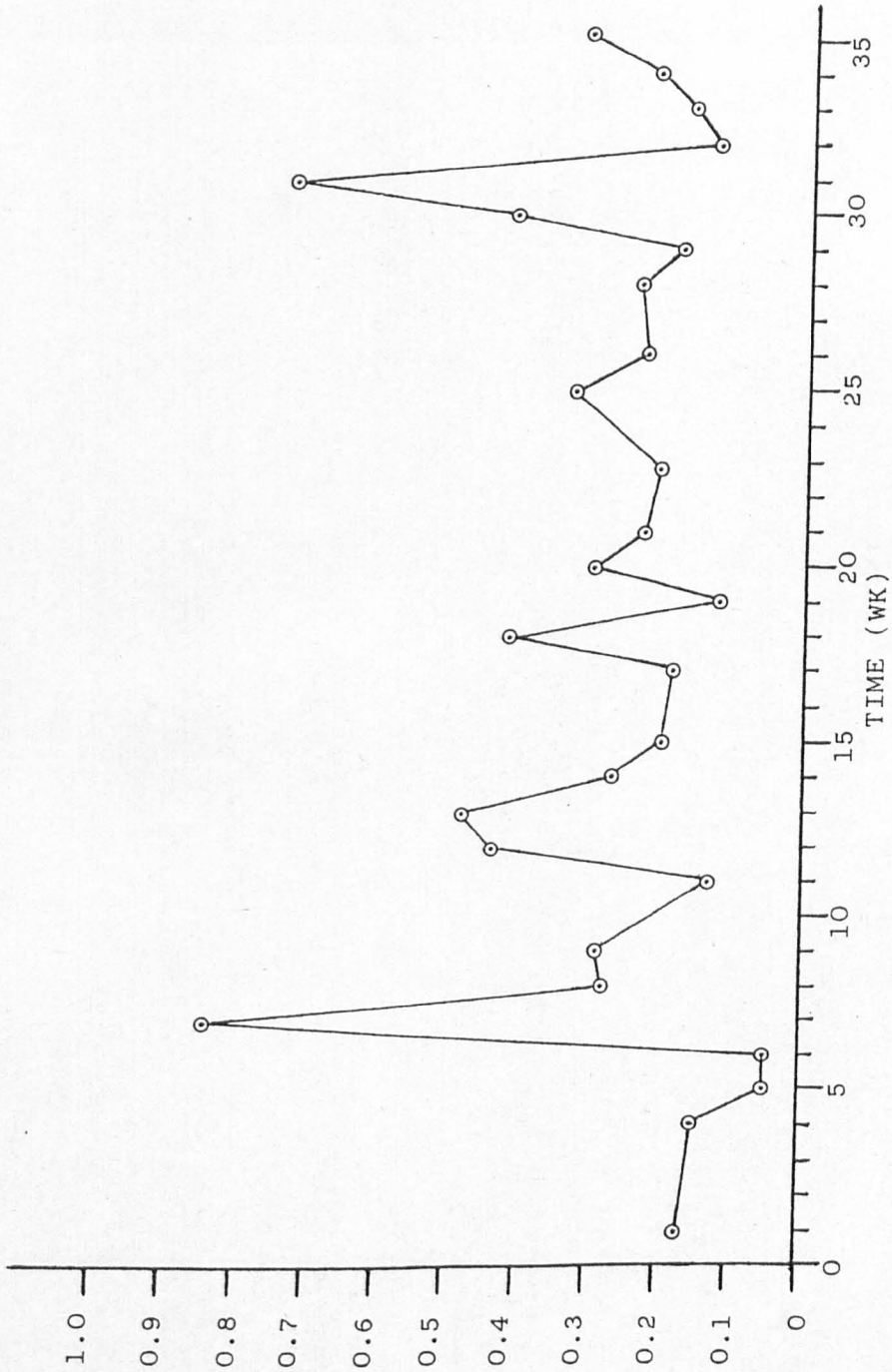


FIGURE 14.2
A STORE HEADSPACE CIPC VS TIME Site H1
HEADSPACE CIPC
(ug 10 dm-3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
TIME (WK)

FIGURE 14.3

G STORE HEADSPACE CIPC VS TIME Site H1

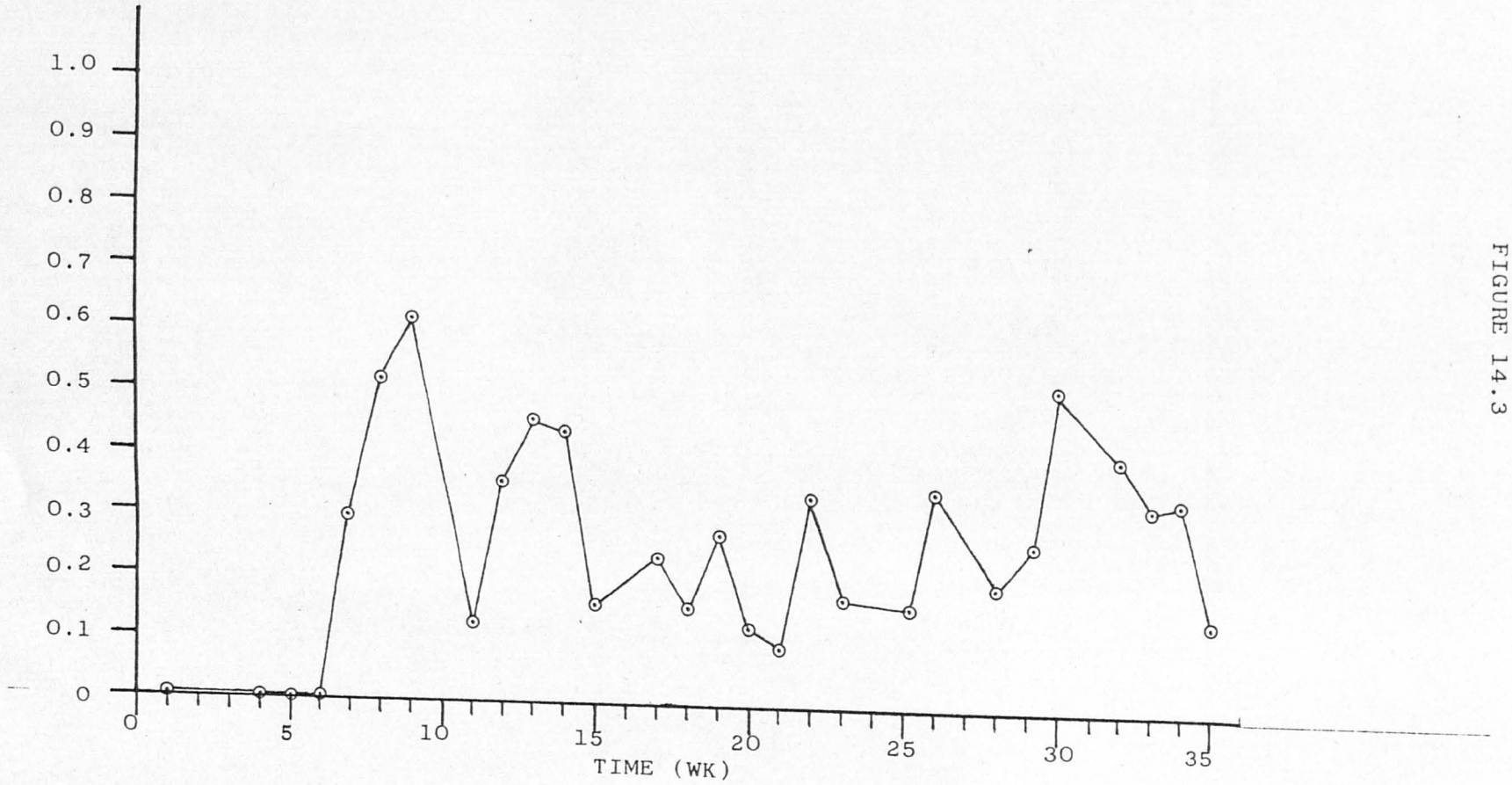



FIGURE 14.3

FIGURE 14.4

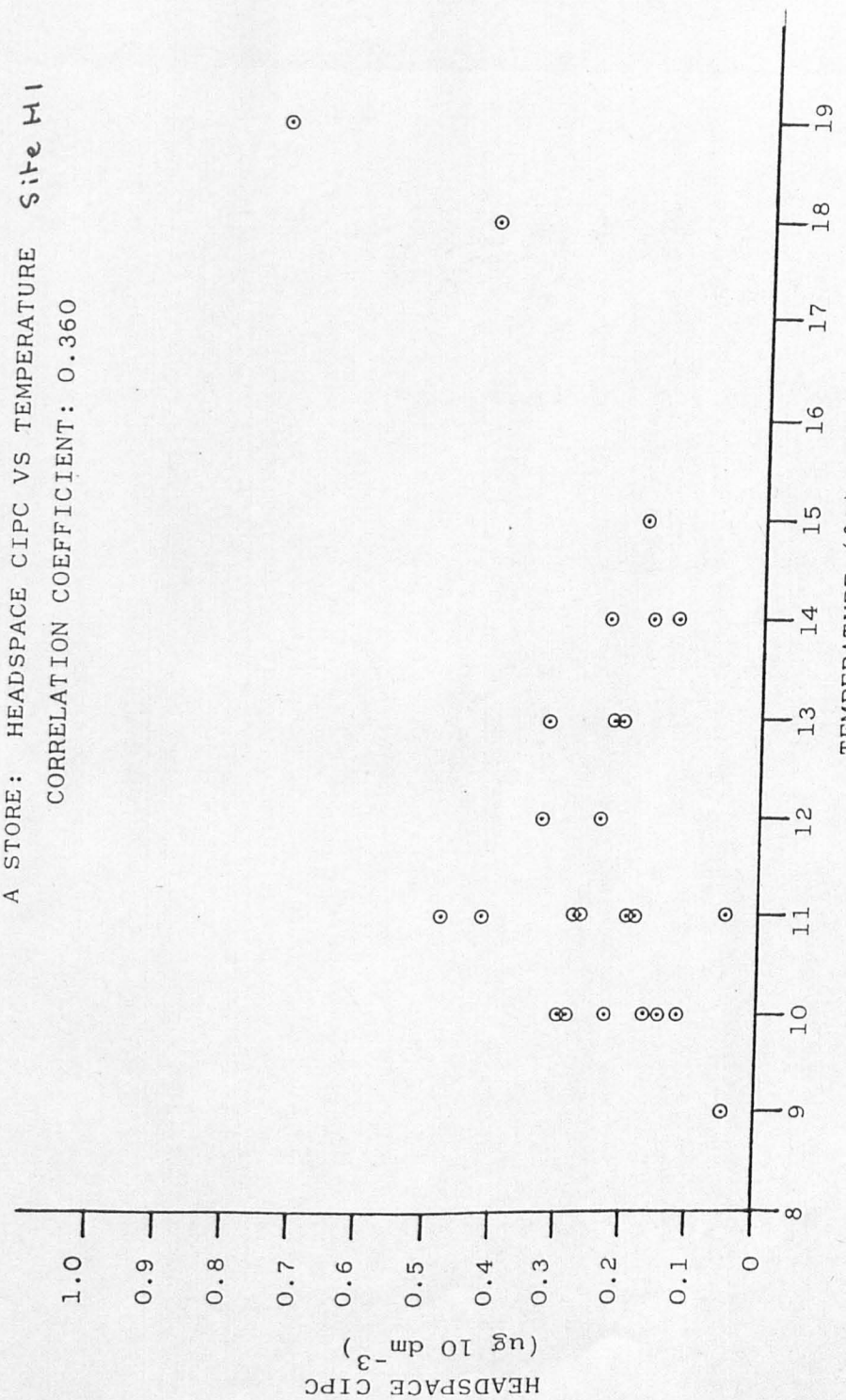


FIGURE 14.4
A STORE: HEADSPACE CIPC VS TEMPERATURE Site H1
CORRELATION COEFFICIENT: 0.360
HEADSPACE CIPC
(ug 10 dm-3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
8
9
10
11
12
13
14
15
16
17
18
19
TEMPERATURE (°C)

FIGURE 14.5

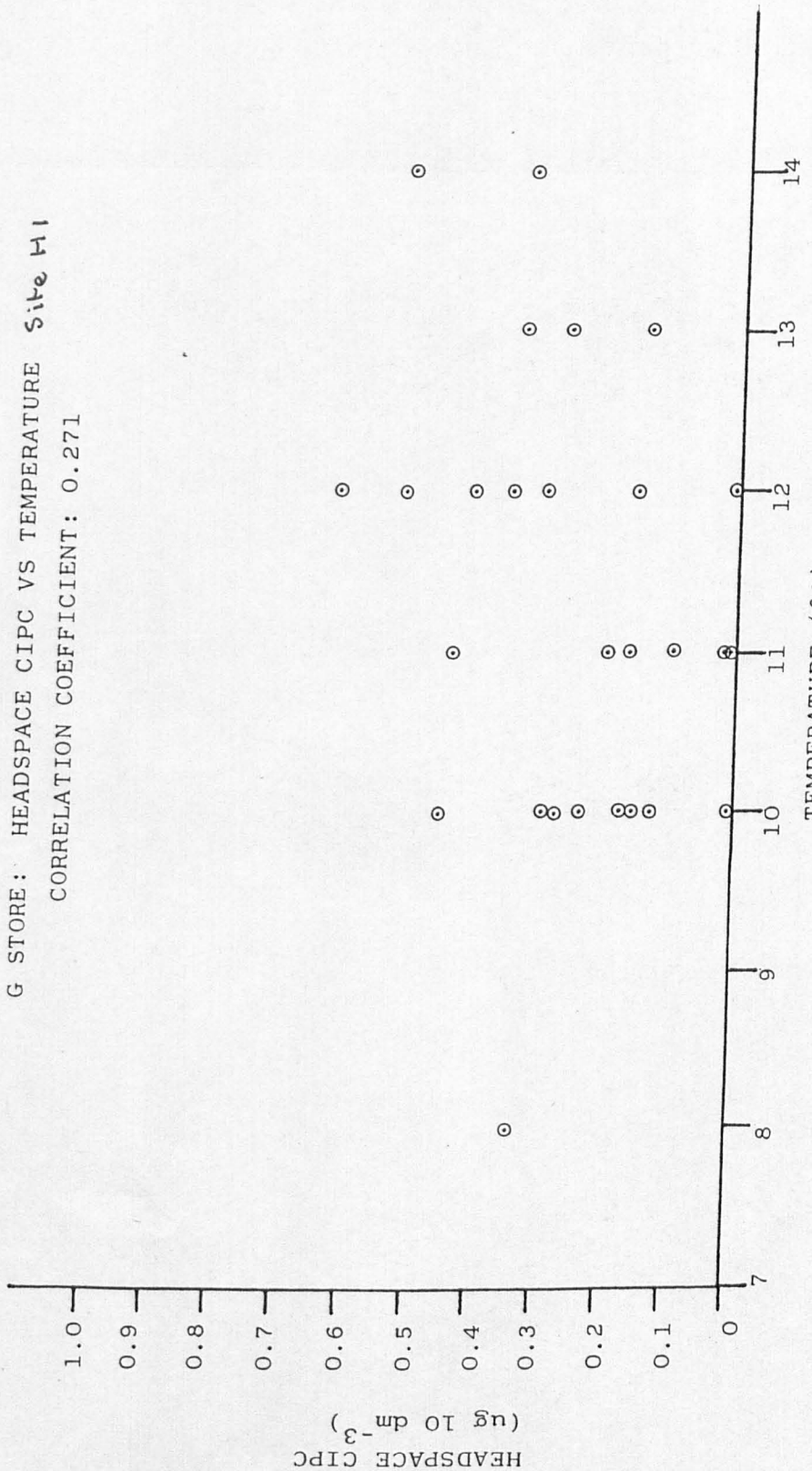


FIGURE 14.5
G STORE: HEADSPACE CIPC VS TEMPERATURE Site H1
CORRELATION COEFFICIENT: 0.271
HEADSPACE CIPC
(ug 10 dm-3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7
8
9
10
11
12
13
14
TEMPERATURE (°C)

FIGURE 14.6

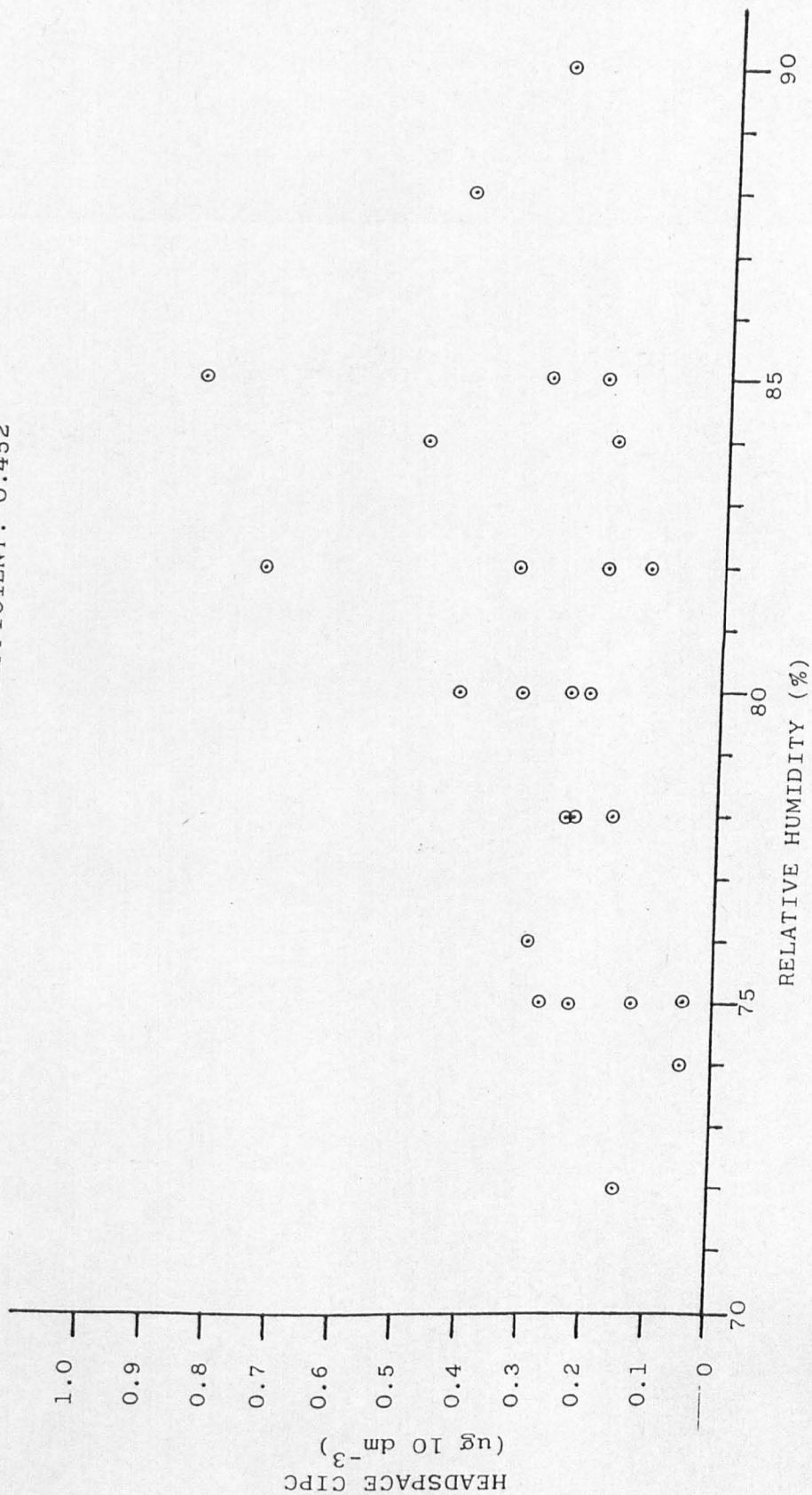


FIGURE 14.6
A STORE: HEADSPACE CIPC VS HUMIDITY Site H1
CORRELATION COEFFICIENT: 0.452
HEADSPACE CIPC
(ug 10 dm-3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
70
75
80
85
90
RELATIVE HUMIDITY (%)

FIGURE 14.7

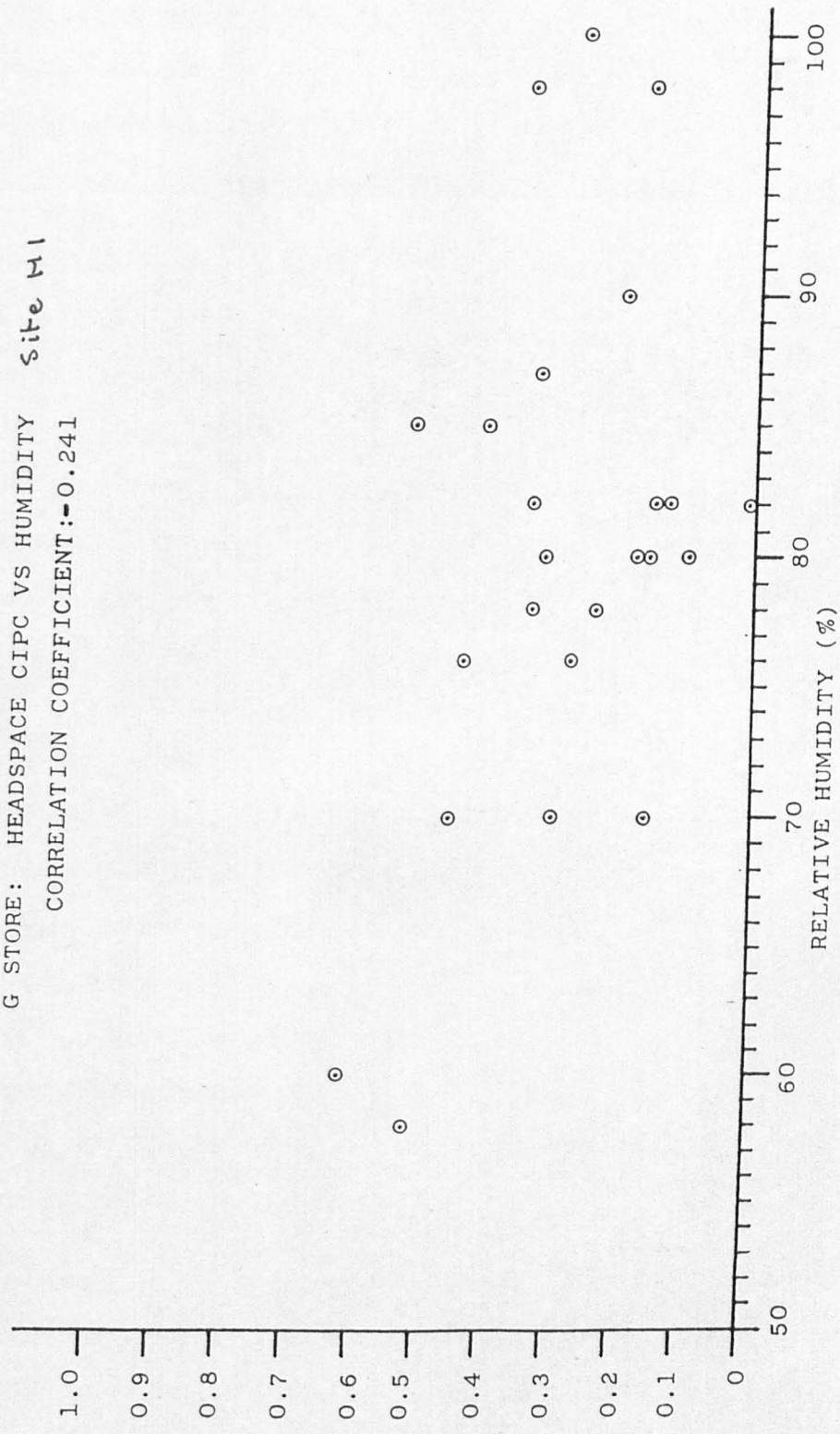


FIGURE 14.7
G STORE: HEADSPACE CIPC VS HUMIDITY site H1
CORRELATION COEFFICIENT:-0.241
HEADSPACE CIPC
(ug 10 dm-3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50
60
70
80
90
100
RELATIVE HUMIDITY (%)

## 14.4 DISCUSSION

Figures 14.2 and 14.3 show that the headspace levels of CIPC in A and G stores were variable over the 35 week sampling period. However, the following points were noted about the headspace levels of CIPC in both A and G stores.

The first application of CIPC on week 4 did not increase the levels of headspace CIPC, in A store the headspace CIPC levels decreased between weeks 4 and 6, in G store the headspace CIPC was undetectable. The second application of CIPC on week 7 dramatically increased the levels of headspace CIPC to the highest concentrations that were measured over the sampling period. The third application of CIPC on week 18 again increased the levels of headspace CIPC but not to as great an extent as the previous application. There were two other increases in headspace CIPC common to both A and G stores, these were between weeks 11 and 15 and weeks 29 and 32, the reason for the increase between weeks 11 and 15 is not known, as it could not be attributed to a varation in the store atmosphere temperature or humidity. However, the increase between weeks 29 and 32 was probably due to the higher store atmosphere temperatures, which would have promoted increased volatilisation of the residue CIPC in the fabric of the store, thereby increasing the levels of headspace CIPC.

Throughout the storage period the sprout control of the potatoes was good, therefore the headspace concentrations of CIPC in A and G store were sufficiently high to arrest sprout growth. The average concentration of headspace

CIPC was 0.28 ug 10 $dm^{-3}$ in A store and 0.26 ug 10 $dm^{-3}$ in G store, the minimum concentrations of headspace CIPC for active sprout suppression was estimated to be 0.3 ug 10 $dm^{-3}$ for the two stores investigated.

Figures 14.4 and 14.5 are a plot of the levels of headspace CIPC against store atmosphere temperature. As noted previously at the highest store atmosphere temperatures the levels of headspace CIFC were also high, e.g: the high headspace CIPC levels at 18°C and 19°C for store A at 14°C for store G. At the lower store atmosphere temperatures between 9°C and 13°C, which are more consistent with normal storage regimes, the levels of headspace CIPC are highest at 11°C for store A and 12° for store G, the reason for the apparent headspace CIPC maxima at these temperatures is not known.

Figures 14.6 and 14.7 are a plot of the levels of headspace CIPC against the relative humidity of the store atmosphere, no distinct correlation was observed between these two factors.

The levels of residue CIPC increased in both A and G stores after two applications of CIPC had been made, Tables 14.2 and 14.3. A store at the initial sampling was found to contain high levels of CIPC from the previous storage seasons CIPC applications. The highest levels of CIPC were present in the floor of the store A, this was expected as the CIPC "thermal fog" when applied falls out onto the floor along with the carrier solvent Dichloromethane, the Dichloromethane dissolves the CIPC which can then readily penetrate into the cement of the floor. Intermediate levels of CIPC were found

in the walls of the store, and the lowest levels of CIPC were found in the crates. Both the floor and the walls of the store contain alumina, and it is therefore not surprising that high levels of CIPC are present in these materials as aluminium oxide is a strong adsorbent of CIPC. The crates were thought to have the lowest levels of CIPC as during the summer when the store is not in use it is normal practice for the crates to be stored outside the store in the open, where they are exposed to environmental factors such as wind, rain and direct sunlight which would remove the residue CIPC by washing and evaporation.

The residue levels of CIPC in G store were undetectable before the initial application of CIPC, this was expected as the store had not been previously treated with CIPC and the crates were new and had not been exposed to CIPC. After two applications of CIPC the residue levels of CIPC in G store increased, with the same relative distribution of CIPC between the floor, walls and crates as found for A store.

The conclusions drawn were that A store was heavily contaminated with residue CIPC, and was still able to assimilate higher levels of residue CIPC, G store after two treatments quickly built up a high residue level of CIPC and it was thought that the residue levels after a few storage seasons would match those found in A store. The headspace levels of CIPC in A and G stores were variable, these variations were in part attributed to the application of CIPC to the stores and the store atmosphere temperature. However,

with hindsight, a more frequent sampling of the store headspace would have been desirable, preferably with the samples being duplicates, in order to establish the exact nature of the variations in the store headspace CIPC. It was thought that the residue levels of CIPC on the potatoes themselves were of minor importance to the levels of headspace CIPC, as during the latter part of the samplings of store G, weeks 28 - 35, most of the stored tubers had been removed from the store. During that period the levels of headspace CIPC in G store remained high, this headspace CIPC being derived from the floors, walls and crates of the store.

The facts that the active headspace concentration of CIPC for stores A and G is approximately 0.3 ug 10 $dm^{-3}$, and that the stores themselves became quickly, heavily and probably irreversably contaminated with CIPC, means that both stores could generate an active headspace level of CIPC capable of affecting sprout growth over a considerable period of time.

In conclusion to this section of the thesis, some points can be made about the results obtained and the commercial implications of the work.

This study has developed a simple technique for the headspace analysis of CIPC and TCNB in box potato stores, the levels and distribution of CIPC and TCNB within a potato store have been established and an estimation of the level of headspace CIPC necessary for sprout suppression has been deduced. In addition it has been shown that a considerable and significant build up of residue CIPC occurs in potato

stores that have previously been treated with CIPC, with the result that the stores themselves are capable of generating a CIPC headspace which is physiologically active. It is recommended that any future work should extend the knowledge of how the distribution of CIPC and TCNB in the store headspace is affected by the levels of residue CIPC/TCNB and the storage conditions and practices. This would entail more extensive sampling of the residue and headspace CIPC/TCNB during the storage period. However, the results would enable a more precise and economical use of both chemicals through a knowledge of the optimum conditions and timing for their application to achieve an efficient sprout suppression. The latter work on the headspace analysis of the potato stores at Grimsby, Chapter 14, showed that the logistics of such an operation were feasible, the precolumns were sampled on site and then sent by post for analysis at this laboratory. As an extension of this it would be possible to have a number of large scale potato stores either box or bulk sampled for headspace CIPC/TCNB and then the sampled precolumns sent to a central laboratory for analysis. The only development needed being in the degree of sophistication and automation in the apparatus used.

Finally, these headspace analysis techniques having been tested in the field for the analysis of two potato sprout suppressants could also be applied to the headspace analysis of other commercially and environmentally important volatiles where their levels are of interest either from the point of view of their efficiency or their toxicology.

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