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DIRECT SAMPLING METHOD FOR GAS CHROMATOGRAPHIC HEADSPACE ANALYSIS ON GLASS CAPILLARY COLUMNS

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SUMMARY

A method is described which allows a rapid, direct, gas chromatographic headspace analysis to be made on capillary columns. The procedure incorporates a simple concentration stage, on a conventional packed pre-column, such that minor components present in headspace vapours can be detected. The method is illustrated by reference to tobacco and other samples of plant origin.

INTRODUCTION

Gas chromatographic (GC) headspace analysis is now frequently used to examine the volatiles associated with a wide range of samples including foods[1], beverages[1], tobaccos[2,3], biological fluids[4,5] and polymers[6–8]. Such analyses may be used, in conjunction with other techniques such as mass spectroscopy, as a means of chemical identification or for obtaining characteristic "fingerprints" of samples in a procedure commonly known as "profile analysis". Whilst the use of chemical identification in flavour related studies is self evident, profile analysis[9] is used to correlate the "fingerprint" GC traces with the properties of the sample concerned. Such methods have been used in disease diagnosis[10,11], investigation of medical disorders[12,13], food quality evaluation[14,15] and air pollution studies[16,17].

Generally headspace vapours are complex mixtures containing chemicals over a wide range of concentrations. Satisfactory GC analysis requires good resolution to separate the components of the mixture and high sensitivity in order to detect those components that occur only at a low concentration. These requirements are generally met by using capillary column chromatography and employing a concentration stage prior to analysis. Several different methods of effecting a concentration of the headspace vapours have been reported[18] including the use of a transevaporator[19] and more commonly, carbonaceous[20,21] or porous polymeric adsorbents[22,23]. These methods may be called "indirect" as they involve a primary concentration of headspace vapours, usually carried out remote from the gas chromatograph prior to a desorption stage and finally chromatographic analysis. The tedious and sometimes lengthy procedures, often using large quantities of sample, involved in the concen-

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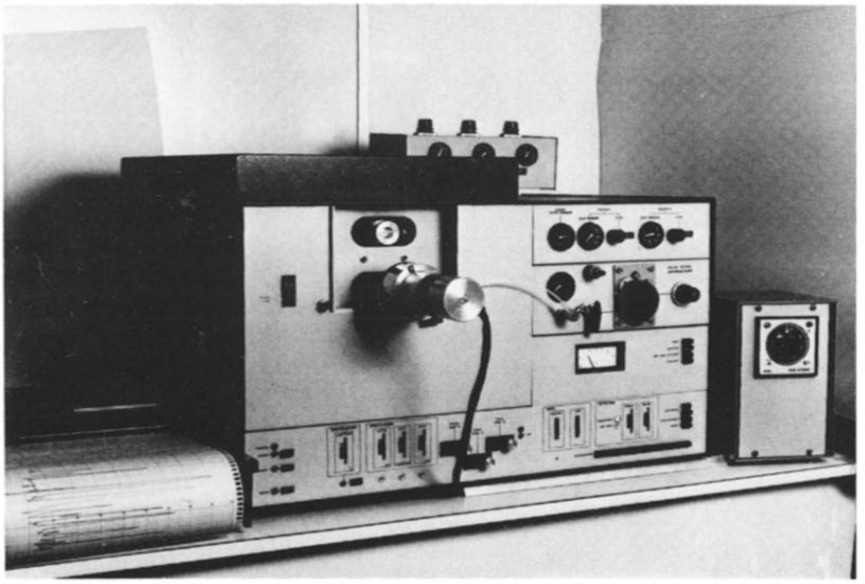

Fig. 1. Headspace sampling accessory assembled on a gas chromatograph.

tration stage led to the development of an essentially direct method. In this direct method, which uses the specially designed GC accessory[24] illustrated in Figs. 1 and 2, the headspace vapours are swept from the sample onto the head of a cooled chromatography column where the volatiles are trapped and therefore concentrated. Once sufficient sample has been collected on the column, GC analysis is initiated. This procedure however, in its originally reported form, was applicable only to packed columns; the analyses obtained therefore lacked the necessary resolution required for either successful mass spectroscopic identification of individual components or completely satisfactory profile analysis.

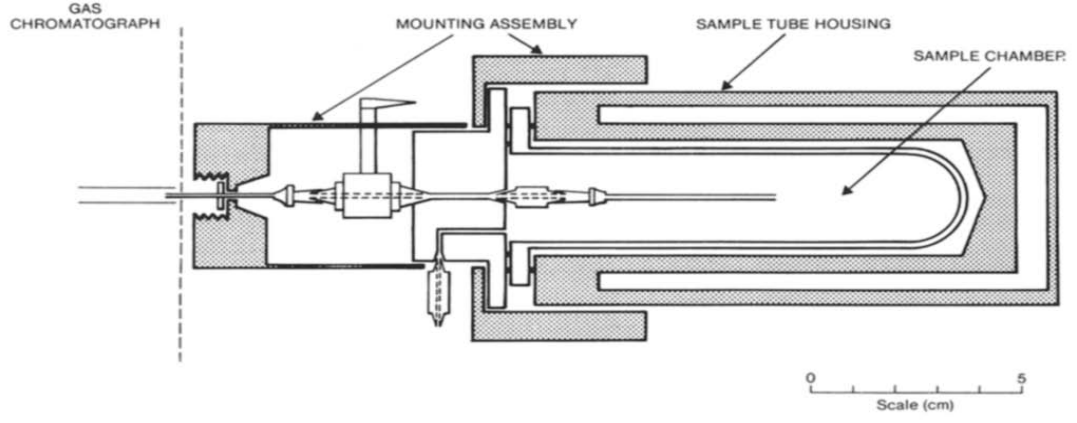

Fig. 2. Sectional diagram of sampling accessory.

Methods of obtaining direct headspace analyses using capillary column chromatography have been reported and suitable apparatus is commercially available. However, concentration stages, essential if minor components are to be detected, are not involved nor are they easily incorporated in these methods and, as a result, only the principal components of the headspace vapours figure in the results obtained.

The method described here allows a rapid, direct, GC headspace analysis to be made on capillary columns. The principle of this method involves using a short conventional packed column immediately before the capillary column. The sample capacity of the packed pre-column is used for collecting and concentrating the headspace vapours prior to the capillary column, with its superior resolving and separating power being used for the analysis.

EXPERIMENTAL

Apparatus

A Perkin Elmer gas chromatograph with a sub-ambient facility and a flame ionisation detector was used throughout this investigation. The specially designed, GC headspace sampling accessory, described elsewhere[24], was used in conjunction with the chromatograph and therefore no special capillary injection system is required.

A support-coated open tubular (SCOT) glass capillary column (61 m × 0.5 mm I.D.) coated with SP2250 and supplied by Scientific Glass Engineering (London,

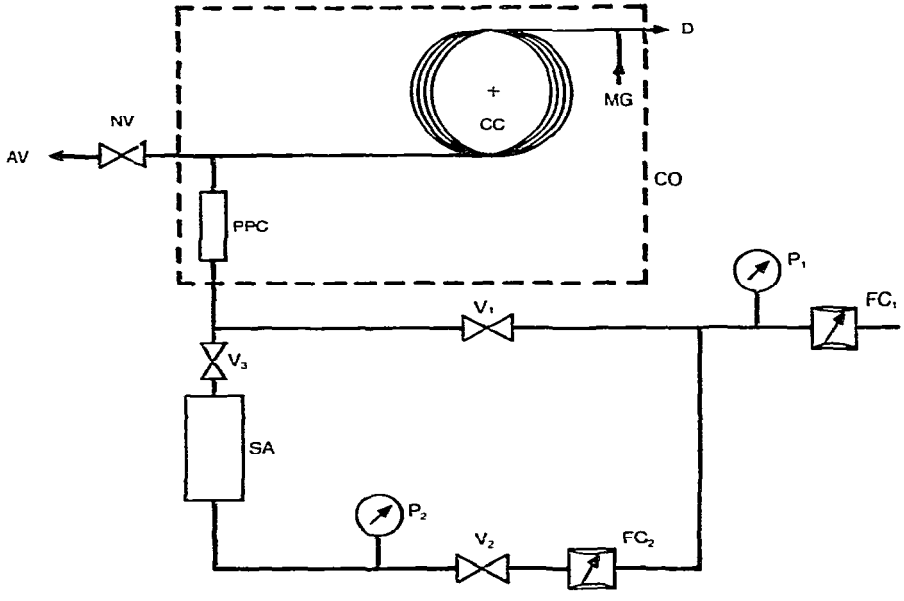

Fig. 3. Schematic representation of gas chromatographic apparatus to allow direct sampling of headspace vapours using capillary columns. AV = Atmospheric vent, CC = capillary column, CO = chromatograph oven, D = detector, FC_1/FC_2 = flow controllers, MG = make-up gas, NV = needle valve, P_1/P_2 = pressure gauges, PPC = packed pre-column, SA = sampling accessory (ref. 24) and $V_1/V_2/V_3$ = on/off valves.

Great Britain), was used and wherever possible glass lined stainless-steel tubing to connect pre-column to capillary and capillary to detector.

The pre-column (4 cm × 3 mm I.D.) was packed with 4% SP2250 on Gas-Chrom Q (80–100 mesh) and assembled between the injector block and the capillary column. A gas stream splitter, adjustable by means of a needle valve, is incorporated between the pre-column and the capillary column. This, together with the usual GC flow controls allows a suitable proportion of the sample to be presented to the capillary column (see *Transfer of sample from pre-column to capillary column*) whilst maintaining the optimum flow of carrier gas through the capillary column during the analysis.

High purity nitrogen was used as the carrier gas throughout these studies.

The apparatus is illustrated schematically in Fig. 3, the labelled components of which will be referred to in following sections.

Sampling procedure

The sample, typically between 0.5 and 3.0 g for solid samples, is placed in the sample chamber of the gas chromatographic headspace sampling accessory (SA) where it is equilibrated to the temperature required. With all the gas flows off (valves V_1, V_2 and V_3 closed) the pre-column (PPC) is cooled to −60°C with solid carbon dioxide or liquid nitrogen. If more convenient, the whole oven may be cooled to −60°C. A gas flow, controlled by using a flow controller (FC_2), is then initiated (valves V_2 and V_3 open) so as to sweep the headspace from the sample and onto the pre-column (PPC). A flow-rate of 25 cm^3 min^{-1} for 10 min usually results in sufficient volatiles from the headspace of the sample being condensed, as a discrete plug, on the initial millimetres of the pre-column to obtain a satisfactory GC trace. Alternative sampling times and flow-rates may be used if required for particular samples. The needle valve (NV) may be adjusted to allow practically all the sampling gas to pass out through the atmospheric vent (AV).

Transfer of sample from pre-column to capillary column

After the flow of carrier gas used to sweep the headspace vapours from the sample has been stopped (by closing valves V_2 and V_3) the oven is heated to the initial temperature of the chromatographic programme so as to vapourize the collected sample and prepare for the analysis. If only the pre-column has been cooled in order to trap the sample, with the oven kept at the initial temperature of the chromatographic programme, then clearly it is only the pre-column which needs to be heated. Once thermal equilibrium has been obtained in the oven the carrier gas is redirected along its usual course (valve V_1 open) using the inlet gas pressure (~650 kPa) to flush part of the collected sample rapidly through the pre-column and onto the capillary column. The fraction of sample presented to the capillary column can be controlled by operation of the needle valve (NV) which adjusts the split that allows the vapourized sample to be divided between the capillary column (CC) and the atmospheric vent (AV). This pressurized "injection", which occurs rapidly, (therefore introducing the sample to the capillary column over a very short time span) is followed by initiating the analytical run and adjusting, if necessary, the needle valve to a previously determined position in order to obtain the optimised flow through the capillary column for efficient separation.

RESULTS AND DISCUSSION

The method described above is direct in as much as there is no handling of the collected headspace volatiles between the concentration and analytical stages. This minimises the possibility of artefact formation.

The headspace vapours are collected on essentially the same material with which they come into contact during the gas chromatographic analysis, again this results in a minimisation of artefact formation.

The method is rapid requiring only a minimum of sample preparation and short sampling times.

The selectivity of some adsorbents commonly used in this type of analysis is well-known; the cooled pre-column used for concentrating the headspace vapours in this work results in complete retention of the volatiles. This has been shown by leading the atmospheric vent gas flow during the sampling time through a non-specific detector which resulted in no signal being recorded. Furthermore there was no evidence to suggest that the pre-column retained any of the trapped volatiles under the conditions of the pressurized "injections".

The apparatus described above has been used to examine a number of samples of plant origin where enrichment of the headspace vapours is essential if satisfactory chromatograms are to be obtained. The chromatographic details are given in Table I.

Three different tobaccos, each with its own post-harvest curing treatment (air curing, flue curing or fire curing[25]) were sampled in shredded form (3 g of each) and analysed according to the described procedure to obtain the fingerprint chromatograms given in Fig. 4. Visual inspection of the chromatograms shows qualitative and quantitative differences especially pronounced in the case of the Latakia tobacco which is cured in an atmosphere of smoke from which it adsorbs the volatiles.

The second application of the technique, illustrated here in Fig. 5, involves the

TABLE I

CHROMATOGRAPHIC DETAILS

Sampling details	
Amount of sample	$\geq$3 g depending on sample
Sampling temperature	75°C
Sampling time	10 min
Flow of sampling gas	25 cm^3 min^{-1}
Temperature of pre-column	−60°C
Analytical details	
Instrument	Perkin Elmer F17 fitted with sub-ambient temperature facility
Pre-column	4 cm × 3 mm I.D. glass
Pre-column packing	4% SP2250 on Gas-Chrom Q (80–100 mesh)
Capillary column	SCOT, 61 m × 0.5 mm I.D. glass supplied by Scientific Glass Engineering
Capillary column coating	SP2250
Temperature programme	+60°C for 4 min then 3°C/min to 200°C
Carrier gas flow	2.5 cm^3 min^{-1}
Injection block temperature	250°C
Detector	Flame ionisation

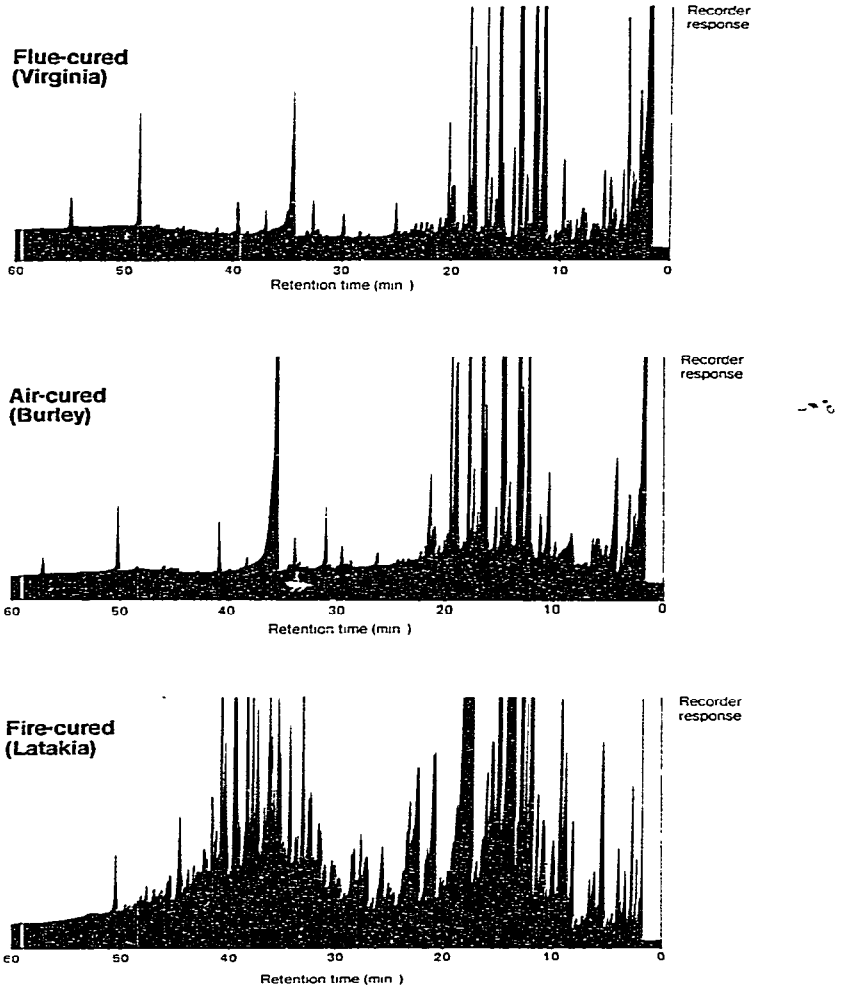

Fig. 4. Headspace chromatograms of cured tobacco samples.

headspace of three freshly-picked herbs: rosemary, sage and mint. In contrast to the tobacco samples, which contain only small amounts of moisture partly as a result of the drying that occurs during curing, the herbs contained substantial amounts of moisture. Nevertheless satisfactory chromatograms were obtained from samples of approximately 100 mg, being a single leaf in the case of sage and mint and a small sprig in the case of rosemary.

CONCLUSIONS

The method and apparatus described above provide a convenient route to obtaining headspace chromatograms of samples where an enrichment stage prior to

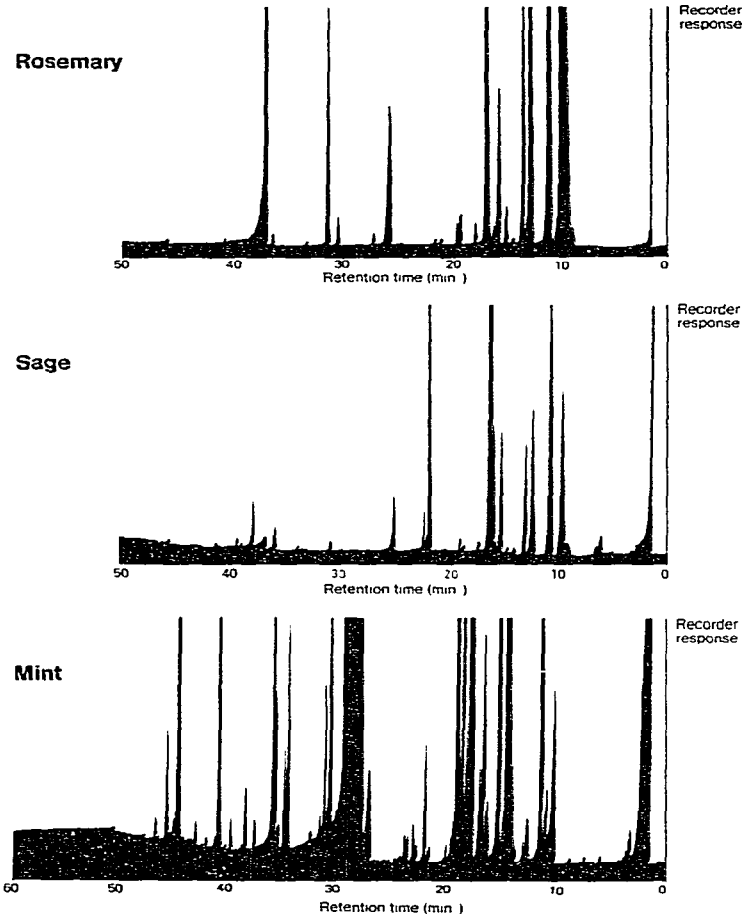

Fig. 5. Headspace chromatograms of freshly-picked herbs.

analysis is required. The examples cited show that the technique may be used successfully with both fresh and processed material of plant origin, although this is by no means the limit of its applicability. With sample handling kept to a minimum the method is convenient and the possibility of artefact formation is reduced.

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