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J.J.C. Scheffer (editors)

Essential Oils and Aromatic Plants

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Proceedings of the 15th International Symposium on Essential Oils, held in Noordwijkerhout, The Netherlands, July 19–21, 1984

edited by

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INTRODUCTION

The 15th International Symposium on Essential Oils was organized in Noordwijkerhout in the neighbourhood of Leiden, The Netherlands, also to celebrate that the very first beginning of these symposia took place in Leiden in the fall 1969. Then the pharmacognosists Dr. F.W. Hefendehl (Freiburg, FRG), Dr. K.-H. Kubeczka (Karlsruhe, FRG), Dr. J. Karlsen and Prof.Dr. A. Baerheim Svendsen (both Leiden, The Netherlands) came together to find out if it could be possible to organize quite informal meetings annually in order to discuss common problems concerning essential oil research. The meetings started fairly modest and the group was small, but it increased little by little, and later on more pharmacognosists and other people — from universities and from the industry — interested in essential oil research joined the group.

The 15th symposium was attended by about 80 participants from the following countries: Belgium, Egypt, England, Federal Republic of Germany, Finland, France, Hungary, Israel, Italy, The Netherlands, Portugal, Rwanda, Switzerland, Turkey and Yugoslavia.

So far, new methods and techniques in essential oil research have always been the main topics of the symposia; in addition reports on current research on essential oils and essential oil-bearing plants have been delivered.

The 15th symposium had as main topic 'headspace analysis of essential oils and aromatic plants', and the method as well as some applications of it were dealt with in a series of main and short lectures. Also other methods and techniques were discussed in the lectures. In a number of short lectures and in posters reports were given on current research on essential oils and their constituents as well as on essential oil-bearing plants, their propagation, cultivation, etc. A review was given on the biological effects and side-effects of essential oils and their constituents. 'The medicinal plants in the mirror of Dutch painting' was the title of the opening lecture.

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CONTENTS

Difficile est scribere,
praesertim edenda

QUANTITATIVE ASPECTS OF FLAVOUR ANALYSIS BY EQUILIBRIUM AND DYNAMIC HEADSPACE GAS CHROMATOGRAPHY WITH CAPILLARY COLUMNS

B. KOLB

ABSTRACT

Equilibrium (E-HSGC) and dynamic (D-HSGC) headspace sampling procedures with capillary columns are compared. Split injection is widely used in headspace gas chromatography (HSGC) since a homogeneous gas mixture is injected and non linear split behaviour is unknown here. Splitless injection is recommended if headspace sampling is combined with cold trapping in order not to disturb the equilibrium, since a large sample is withdrawn over a longer period. In this case the well established methods for quantitave analyses with E-HSGC can be applied. These methods, including sample identification by pattern recognition, calibration by the technique of standard addition and the procedure of multiple headspace extraction (MHE) are discussed. Problems with D-HSGC techniques, particularly concerning an exhaustive stripping of all the volatile constituents from the sample without loss of the highly volatile ones by breakthrough in the adsorption tube are discussed also. In cases of mixtures with a wide range of volatilities, where the sample transfer can hardly be quantitative, the application of the MHE principle is suggested as a possible solution.

INTRODUCTION

Headspace gas chromatography (HSGC) is straightforward when volatiles have to be separated from a solid or liquid prior to the GC analysis, e.g. in cases where the sample itself cannot be injected. The analysis of the headspace vapours above foodstuffs by GC has been widely applied in aroma research, but various techniques have been used which are all called headspace techniques. The different meaning of what is called headspace may cause some confusion, particularly if the quantitative aspects are concerned.

In agreement with Wyllie et al. (1) there are good reasons to define headspace as the gaseous mixture surrounding a sample within a closed system, which according to all thermodynamic rules is in equilibrium. Contrary to this equilibrium headspace method there are a variety of methods which are used to isolate the volatiles from a sample by stripping with an inert gas. Such continuous stripping procedures need an intermediate storage zone, either a cold trap or an adsorption tube, in

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which the volatiles are first separated from the stripping gas and further concentrated by band focusing, particularly necessary for the rigorous demands of capillary GC. Both the equilibrium and the dynamic headspace procedures have their particular advantages and typical applications, which should be discussed with special emphasis on the quantitative aspects. One of the most attractive properties of the equilibrium headspace procedure is its inherent simplicity, which lends itself to automation, while the expenditure to automate a dynamic headspace procedure is much more costly. The need for automation is not so well recognized, particularly not in research institutes where most of the work is related mainly to qualitative investigations. Most papers therefore deal with identification of flavour compounds preferably with GC-MS combination, and for such application neither the quantitative aspects nor a high sample throughput seem to be important. The situation, however, might be quite different when quality control in the production, e.g. food processing, is concerned. Differences in the flavour, which might be quite obvious to the human nose, are often only related to small differences in chromatographic peak relations. The significance of small differences in an analytical result, however, needs to be confirmed by statistical evaluation, and even only two samples require a series of chromatograms to be processed, and this immediately calls for automation. Automation in general improves the precision of the measurements and is thus a necessary prerequisite for statistical data processing and for the confirmation of the final result.

INSTRUMENTATION OF EQUILIBRIUM HEADSPACE GAS CHROMATOGRAPHY (E-HSGC)

HSGC is in fact a gas analysis and the same sampling techniques are often used as common in gas analysis. Gas-tight syringes or gas sampling valves with sample loops are thus widely employed. A gas syringe is the most popular device for transferring a gas sample from the headspace vial into a gas chromatograph. Apart from the contamination and adsorption problems with such a device, there is a more serious drawback, which is caused by the fact that a syringe is an open system during the sample transfer. Any pressure which has been generated by the increase of the partial vapour pressures of all the volatiles in the sample by heating the vial, also extends into the syringe. During sample transfer, however, the syringe needle is open to atmosphere and an undefined amount of the gas sample will be lost by expansion of the headspace gas through the needle. Even small differences in the humidity of two otherwise identical

samples can simulate a big quantitative difference in the concentration of a volatile compound, although the same sample volume is withdrawn from the vial. Obviously not only the volume but also the pressure must be held constant to get the same sample amount onto the GC column, and independent of the sample composition. Such constant pressure sampling is most easily achieved if the vials are first pressurized with inert gas up to a constant pressure. By this procedure the sample amount becomes independent of the sample composition and good reproducibility of sampling is obtained (2). Several such systems are commercially available, from which the sampling principle as used in the HS-100 Automatic Headspace Sampler from Perkin-Elmer (Fig. 1) will be discussed. The whole instrument with all its possibilities is described elsewhere (2).

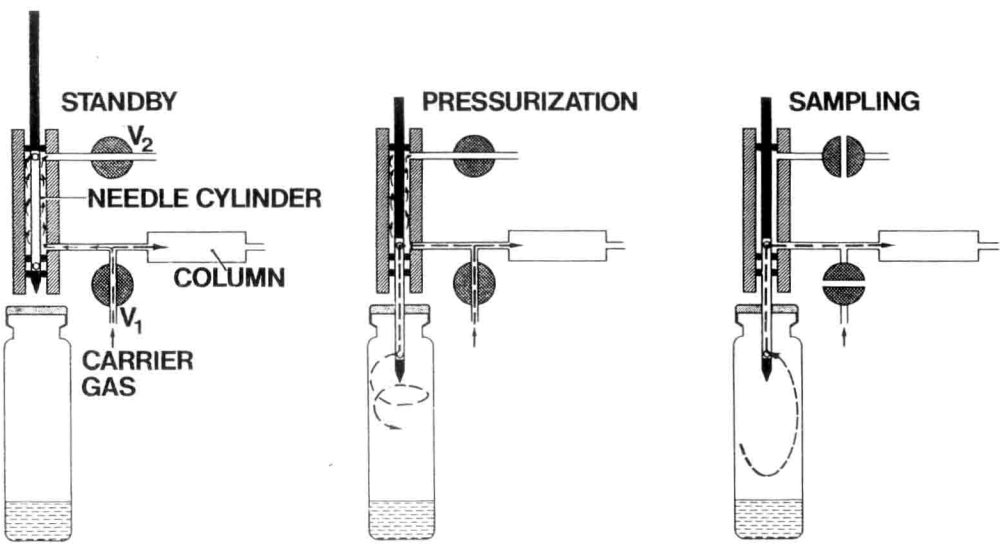

Fig. 1. Balanced pressure sampling with the Perkin-Elmer HS-100 Automatic Headspace Sampler.

The sampling device comprises a heated movable sampling needle with two vents and a solenoid valve, V1, in the carrier gas supply line. This needle moves up and down in a heated cylinder and is sealed by three O-rings. In the standby position the lower needle vent is placed between the two lower O-rings and thus sealed against atmosphere, while the carrier gas flows through valve V1 to the column. A small cross-flow purges the cylinder and is vented via valve V2. At the end of the selected thermostatting period, the sampling needle descends, pierces the septum