Food and Environmental Analysis by Capillary Gas Chromatography

Hints for Practical Use

Edited by Lothar Matter

Translated by Anthony J. Rackstraw

With 104 Figures and 12 Tables

Hüthig Verlag Heidelberg

Lothar Matter (Ed.)
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Chromatographic Methods

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Foreword

Advances in important areas of environmental and food analysis would be unthinkable without gas chromatography in capillary columns. Many years elapsed between the introduction of capillary columns (open-tubular columns, Golay 1958) into gas chromatographic analysis and the widespread adoption of this technique in practice. The reasons for this delay lay in the difficulties of column technology, instrumentation, and methodology encountered in the use of very narrow capillaries with their very small sample capacity. The process of acceptance received a considerable boost through the introduction of fused silica capillaries (Dandeneau, 1978). Thanks to the column geometry, capillary GC is a miniaturized separating analytical process. In general, GC can only be used for volatile compounds or those which can be vaporized without decomposition at high temperatures. Because of the low sample capacity of systems with capillary columns, environmental analysis with high resolution chromatographic methods places stringent demands on the separation, detection, and identification of analytes in matrices of complex composition. This is particularly true of samples containing very small concentrations of the toxic substances to be detected and determined. Such analysis is possible only with special sample introduction techniques. Detection with the necessary very low detection limits for toxic compounds can be accomplished with comparative ease on use of the ionization detectors available in GC.

Even after sophisticated sample preparation, precise and accurate analyses of trace components in difficult matrices are possible only on optimal use of the best instruments and modern analytical methods of capillary GC.

Particular difficulties are encountered, as in all analytical chromatographic techniques, in forensically reliable identification of target compounds. These can only be overcome by use of hyphenated techniques, preferentially GS/MS.

This book, containing a collection of important applications, will be of great value to those engaged in the practice of environmental analysis. It demonstrates, for a number of typical examples of food and environmental analyses, how modern GC analysis with capillary columns can be successfully applied to this area.

Mülheim an der Ruhr *Prof. Dr. Dr. Gerhard Schomburg*

Preface

The objective of this book differs from that of most other publications on capillary gas chromatography. Instead of presenting all known methods of determination, it focuses on those which are important to the user. The book does not aim to transform the reader into a capillary GC expert, but instead addresses those readers who use capillary gas chromatography because of its various applications, i.e. practitioners. This is therefore *a book by practitioners for practitioners.*

The content is based on a number of meetings held by the German Chemical Society on the same topic and comprises selected presentations delivered on those occasions.

Well known and experienced authors have contributed their knowledge and experience to the individual chapters. All the applications described have been tried and tested and are reproducible on adherence to the basis rules of chromatography.

I wish to express my gratitude to the authors for their readiness to contribute to this work.

Special thanks goes to my family, without whose understanding this book would never have been published.

Dinslaken, May 1997 *Lothar Matter*

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1 Application of the Rules of Chromatography to Capillary Gas Chromatography

Lothar Matter

1.1 Introduction

High resolution capillary gas chromatography is the analytical method of choice for organic trace analysis in foods and in the environment. Thanks to the wide range of detection methods (flame ionization, electron capture, nitrogen/phosphorus, flame photometric, mass selective, and ion trap detection, to name but a few) it is possible too determine a variety of "contaminants/impurities", both qualitatively and quantitatively.

Capillary gas chromatography is fast, highly sensitive, and accurate. It is one of the most powerful of all separation methods. However, its use calls for knowledge and skill; in other words, the dream of a "black box" or a single push-button instrument into which a sample is placed and which promptly yields the correct results with nothing more ado, will remain a dream for ever.

Finding the appropriate solution for the analytical task at hand requires some degree of experience and a critical approach to the material. The analyst should/must be aware of the properties, composition, and pitfalls of the sample to be analyzed in order to choose and optimize the right analytical method. The generally valid rules of chromatography must be strictly applied in the field of capillary gas chromatography if useful results are to be obtained. If newly published work of interest is to be repeated then all the gas chromatographic parameters given in the publication must also be repeated. Details such as the temperature program, the carrier gas used, column length, column diameter, film thickness, phase, etc., play an important role in the description

of the method. This information is essential not only for evaluation of the method but also for the analyst interested in using the method [1-1]. However, most publications, including many recent ones, fall far short of this mark [1-2, 1-3, 1-4].

The common remark or statement "just inject" should be consigned to the past.

1.2 Rules of Chromatography

In my opinion the most important rules of chromatography are the following (no claim is made to completeness of this list):

- Sample introduction
- Selection of the "right" stationary phase
- Column length
- Column internal diameter
- Film thickness
- Carrier gas
- Clean separation of the components to be analyzed from the matrix

1.2.1 Sample Introduction

Correct sample introduction is a prerequisite for successful chromatographic analysis. Even today the manner is which this is performed is a matter of sometimes heated discussion. Back in 1983 Pretorius and Bertsch wrote: "If the column is described as the heart of chromatography, then sample introduction must be its Achilles tendon" [1-5]. One can only concur with this statement.

The requirements to be fulfilled by a sample introduction system can be described as follows [see 1-1]:

The injection method should not have a discriminatory effect on individual substances. Direct or indirect sample transfer (via an intermediate step) must occur "linearly" into the column, i.e. the original mutual ratios of the components of the sample must remain constant, at least for the substances to be determined.

- *Accuracy:* This important demand is often misunderstood and replaced by good reproducibility. A small standard deviation does not rule out systematic errors/incorrect results.
- *Thermal and/or catalytic decomposition:* The risk of decomposition and /or rearrangement on active surfaces should be reduced to a minimum, or, whenever possible, completely eliminated. If a septum is used, it is subject to constant thermal and mechanical stress and can also influence the chromatographic result by releasing volatile components (plasticizers ...).
- *No band broadening:* The separation performance of the capillary column should not be impaired to any significant extent by the injection method employed.
- *Reproducible retention times:* Unequivocal identification of retention times and exact reproducibility of sample components in a complex matrix go hand in hand.
- *Contamination:* Entry of non-volatile or only slightly volatile sample components into the actual separation system leads to a drop in column performance through peak broadening, shortens the lifetime of the capillary column, or exerts other detrimental effects.

In the area of trace analysis it is necessary to transfer the substances with *minimal possible losses* into the system and to generate peaks that are as high as possible and have steep flanks. We shall not consider the various sample introduction techniques, such as split/splitless, PTV, and direct (or on-column) injection because they are described at length in the literature. However, mention should be made of cold on-column injection according to G. Schomburg and later modified by K. Grob which, in my opinion, represents the most reliable and most accurate sample introduction technique for high resolution capillary gas chromatography [1-6, 1-7].

All other sample introduction systems in which the sample is first heated up in the injector and then transferred in vaporized form to the capillary column are subject to the danger of pyrolysis of sample components [1-8]. They constitute sources of constant misinterpretation in organic trace analysis. The popular determination of hydrocarbons can be cited as an example. Figure 1-1 (see p. 4) shows the result of a quantitative determination of hydrocarbons in a solution with a known content of eicosane, which was part of a round robin test for the recognition of irradiated chicken, pork, and beef by gas chromatographic identification of volatile hydrocarbons [1-9].

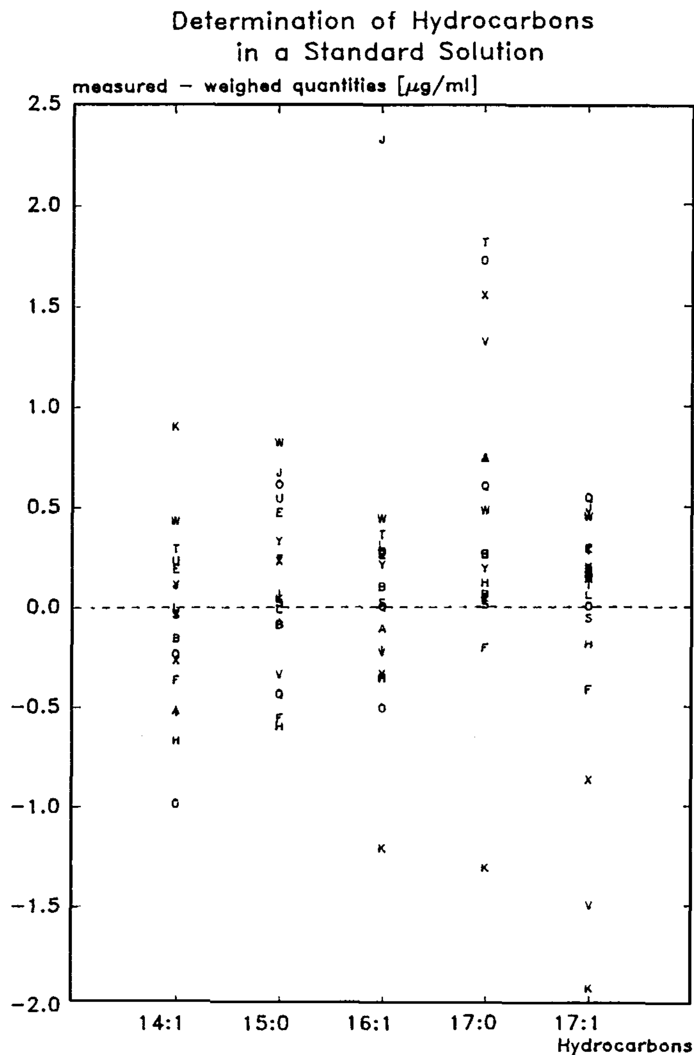

Fig. 1-1: Determination of hydrocarbons in a standard solution.

The figure shows the differences between the values found (mean of several determinations) and the actual amounts present. Of the 18 participating laboratories, only 4 reported values for all hydrocarbons which were very close to the actual values. It can also be said that the rules of chromatography were observed; the GC analysts knew what they were doing.

1.2.2 Choice of the "Right" Stationary Phase

No generally valid rules exist for the selection of the right stationary phase for a given analytical problem. However, experience shows the following guidelines to apply: like dissolves in like. This means that the stationary phase should have a polarity similar to that of the substances to be separated (apolar phases for apolar substances, high boiling samples require high temperature stable phases). Apolar phases are more stable towards oxidative processes than polar ones. Inappropriate storage or leaks in operation of a capillary column (and hence uncontrolled entry of air) are "forgiven" more readily by apolar phases than by polar ones.

Wherever possible, stationary phases which contain a functional group giving a detector response should be avoided. Thus cyanopropyl phases should not be used with a nitrogen/phosphorus detector, or trifluoropropyl phases with the electron capture detector. The signals of both these detectors would be swamped by the normal level of bleeding of the column [1-10].

1.2.3 Column Length

The column length is dependent upon the degree of difficulty of the separation problem. The only general recommendation that can be made is that the column should not be longer than necessary for the problem at hand. The shortest column permitting a good separation should be selected. It is inefficient to use excessively long columns for samples containing only few components requiring determination. The use of capillaries of lengths exceeding 60 or more meters lies in the separation of complex mixtures such as polychlorinated biphenyls.

1.2.4 Column Diameter

The internal diameter of columns used in capillary gas chromatography ranges between 0.53 mm (megabore columns) via standard columns with 0.32 mm to columns with 0.10 mm i.d. (microbore columns). Microbore columns have a high separation performance per unit time but require state-of-the-art instrumentation and are not easy to handle.

The normal standard columns (0.32 mm i.d.) or so-called narrow-bore capillaries (0.25 mm i.d.) are used in most laboratories.

So-called megabore columns (0.53 mm i.d.) have recently come into use as alternatives to packed columns. After examining all the facts supposedly favoring megabore capillaries, K. Grob and P. Frech came to the conclusion