Analysis of Drug Metabolites by Gas Chromatography-Mass Spectrometry Using Glass Capillary Columns

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Summary
The routine use of glass-capillary columns in a general applications laboratory for gas chromatography-mass spectrometry is discussed. The instrumentation is described with emphasis on the interface between glass capillary columns and the mass spectrometer. Two examples of the analysis of metabolites demonstrate the successful use of this system.

Introduction
Since many laboratories still hesitate to introduce glass-capillary columns for their routine work, this paper is intended to demonstrate the usefulness of glass capillary columns in combined gas chromatography-mass spectrometry (gc-ms) in a general applications laboratory. Even with the wide variety of samples, coming into a laboratory like this, about 75 % of the problems could be better solved on glass capillary columns, than on packed columns. The necessary hardware to achieve this will be described below and two examples are given.

Instrumentation
The instrumentation used in this work was the Finnigan 9500 gas chromatograph combined with the Finnigan 3200 quadrupole mass spectrometer. For data acquisition and reduction as well as mass spectrometer control the gc-ms system was on-line with the Finnigan Data System 6100.

The gas chromatograph was equipped with a Grob-type [1] capillary injector for splitless sample introduction and continuous septum flush. Both items are necessary, since they extend the sample range of the capillary column into that suitable for the analysis of trace components.

All our capillary columns were purchased at H. and G. Jaeggi, Trogen, Switzerland. We use a variety of stationary phases such as OV-1, OV-101, OV-61, SF-96, UCON-HB and FFAP. In all cases we use 20 m × 0.35 mm capillaries. Such columns show the desired high resolution and still require only slightly longer than a packed column would do for an analysis.

Two types of interfaces between the gc and the ms have been used, depending upon the application.

The first one is used in combination with the electron impact ion-source (Fig. 1). It consists of a parallel dual interface, which permits the use of both capillary and packed columns, without changing the interface.

Fig. 1

- (1) normal injector
- (2) Grob type injector
- (3) capillary column
- (4) packed column
- (5) teflon shrinking tube connection
- (6) 1/4" swagelok connection
- (7) glass jet separator
- (8) Pt-IR capillary
- (9) ion source
- (10) rotary pump

Packed columns are coupled via a one stage glass jet separator and glass lined stainless steel tubing.

The capillary column is coupled via a 30 cm × 0.1 mm Pt capillary directly to the ionizing chamber [2]. This Pt capillary provides sufficient restriction to be able to change the column while the system is running. No shut off valve is necessary or provided. Prior to the installation the Pt capillary is heated to a red glow with oxygen flowing through it. After installation a polar column eg. UCON-HB is attached and stationary phase is bled into the Pt capillary [3]. This “extends” the capillary column practically into the ion-source.

While working with the electron impact/chemical ionization combination source, a coaxial dual gas interface is used [4] (Fig. 2). It consists of the same Pt capillary as described above, but coaxially around it runs a glass coated 0.5 mm i. d. stainless steel tubing, through which the reactant gas is directed. With this arrangement He can be retained as the carrier gas with its desired qualities of inertness and low viscosity. At the same time even aggressive reactant gases such as ammonia or
water can be used without detrimental effects on the inner coating of the Pt capillary.

Only two requirements for the mass spectrometer should be mentioned. In order to couple capillary columns directly to the ion-source of the mass spectrometer its pumping speed has to be sufficient to handle up to 4 cm$^3$ atm min$^{-1}$ of He, without degrading resolution and sensitivity.

Secondly, the scan speed of the mass spectrometer has to be so high that the mass range of interest can be scanned in about one second. Only this will produce enough scans across a fast eluting gc peak, to decide whether the peak is a mixture or a pure compound. It is also important, that the system does not have a “long” back scan time while scanning in a cyclic mode, because this “blind” time of the mass spectrometer might lead to complete loss of a fast eluting gc peak. With this scan speed in mind, it is almost unnecessary to stress the importance of a data system.

**Application Examples**

1) The sample for this analysis was kindly supplied by Prof. J. Portig, Institute for Pharmacology and Toxicology, University of Marburg, Germany. His group is concerned with the metabolism of chlorinated hydrocarbons in mammals. In this experiment rats were intoxicated with hexachlorocyclohexane (1), a pesticide widely distributed throughout the world. Work-up of the urine of these rats furnished inter alia a fraction, which contains chlorophenylsulphonates (2) and chlorophenylmercaptates (3) which varying chlorine contents.

Hydrolysis and methylation with diazomethane furnishes chloroanisols (4) and chlorothioanisols (5). The fraction containing chiefly 5 was subjected to gc-ms analysis using a 20 m OV-1 capillary column. A temperature programme from 120 °C to 220 °C with 8 °C min$^{-1}$ was used, and the spectrometer scanned from mass 34 to mass 400 with a cycle time of 1.4 sec (Fig. 3).

Mass chromatograms, Figs. 4–6, generated by the data system, provide rapid correlation of gc peaks with expected compounds, as shown for:

- monochlorothioanisols m/e 158
- dichlorothioanisols m/e 192
- trichlorothioanisols m/e 226

(and other metabolites with M$^+$ 222)

Chromatography of this extract on a packed column – 6 ft X 2 mm, 3 % OV-1 on GasChrom Q 100−120 mesh – proved unsuccessful, since too many peaks could not be resolved (Fig. 7).

2) The second application example comes from Prof. A. Kalbhen’s group, Institute of Pharmacology, University of Bonn, Germany. This group tries to