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BINARY STATIONARY-PHASE COLUMNS FOR GAS CHROMATOGRAPHY OF BARBITURATES

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SUMMARY

A general procedure is presented which allows the prediction of gas–liquid chromatographic behaviour and the separation of complex barbiturate mixtures. Using E 301 and FFAP, binary stationary-phase columns were obtained that separated a complex mixture of barbiturates selected on the basis of the their frequency. The barbiturates were analysed in the free form using nitrogen saturated with formic acid vapour as the carrier gas at room temperature.

INTRODUCTION

Barbiturates have been determined by ultraviolet spectrophotometry at different pH values[1,2] but this method, although sensitive and specific, is limited because is does not allow differentiation between individual barbituric acids. As has been reported by several workers[3–8], gas chromatography offers many advantages, *viz.*, sensitivity, speed of analysis and the separation and determination of constituents of mixtures.

It has been noted that the column caracteristics for the gas chromatography of barbiturates are critical. A high liquid loading gives long analysis times, whereas with a low liquid loading high adsorption of barbiturates is observed, which tend to tail badly and highly inert support materials must be used for their analysis. Several workers have used phosphoric acid or organic acids of high molecular weight to reduce tailing[9,10]. This technique yields gas chromatographic columns that are sufficiently inert to barbiturates but of limited stability because of acid decomposition or elution.

Also, not all possible barbiturates can be separated on a single column, although several papers have been published on the use of stationary phases of varying polarity[6], or combinations of polar and non-polar stationary phases[5].

This paper describes the gas chromatography of barbiturates by column dynamic de-activation obtained using a carrier gas saturated with formic acid at room temperature. It also reports the separation, using binary stationary phases, of interesting pairs of barbiturates in a complex mixture that traditionally is separated on two columns of different polarity.

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BINARY STATIONARY-PHASE COLUMNS

It has been shown by Purnell and co-workers[11–13] that the simple equation

$$K_R = \varphi_A K^0_{R(A)} + \varphi_B K^0_{R(B)} \quad (1)$$

describes the gas–liquid chromatographic retention behaviour of solutes with a varied number of binary stationary phases, where K_R is the liquid–gas solute partition coefficient for a stationary phase composed of A of volume fraction φ_A and B of volume fraction φ_B, and $K^0_{R(A)}$ and $K^0_{R(B)}$ are the corresponding solute partition coefficients with pure stationary phases A and B. Eqn. 1 is very important in gas–liquid chromatography, as it can be used to predict the optimal composition of A and B that will separate any given mixture.

Eqn. 1 can be written in the form

$$K_R = K^0_{R(B)} + [K^0_{R(A)} - K^0_{R(B)}]\,\varphi_A \quad (2)$$

If no blending occurs between stationary phases A and B, then K_R is a linear function of φ_A. Given that $K_R = V_g\varrho$, where V_g and ϱ are the specific retention volume and the stationary phase density, respectively, and

$$\varphi_A = \frac{m_A \varrho^0_B}{\varrho^0_A + m_A\,(\varrho^0_B - \varrho^0_A)}$$

where m_A, ϱ^0_A and ϱ^0_B are the weight fraction and the densities of pure A and B, respectively, then $\varphi_A \approx m_A$ if $\varrho^0_A \approx \varrho^0_B \approx \varrho$. This approximation broadly applies to high-molecular-weight polymeric silicone oils or gums and so the weight fraction, rather than the volume fraction, can be used. After substitution in eqn. 2, the following equation is obtained:

$$V_g = V^0_g\,(B) + [V^0_g\,(A) - V^0_g\,(B)]\,m_A \quad (3)$$

The value V_{g_i} of the ith mixture component for any m_A fraction can be obtained from eqn. 3 or, better, from a graph of V_g *versus* m_A knowing only $V^0_{g_i}$ for the pure stationary phases. Thus, if we require to separate a mixture, a stationary-phase pair A–B is selected such that using stationary phase B it is possible to separate those components not separated by A and *vice versa*, and the optimal fraction m_A is obtained from a window diagram. This diagram is obtained from graphs of $\alpha_{i/j}$ *versus* m_A ($\alpha_{i/j} = V_{g_i}/V_{g_j}$). The largest minimal α value for any m_A fraction is obtained. A window diagram can be approximated to an open polygonal of which the absolute maximum coincides with optimal m_A.

The minimal number of theoretical plates required for baseline separate at the best m_A value can be calculated from the resolution equation. For mixtures of unknown composition and complexity, the above-described procedure can be also computerized.

EXPERIMENTAL

Dynamic deactivation of the gas chromatographic system was obtained using a trap with formic acid placed in the gas stream immediately before the injector and used at room temperature. The carrier gas was not bubbled through the formic acid.

The barbiturates were obtained from pharmaceutical products. The formic acid used as tailing reducer was 98% pure, containing 2% of water.

The binary stationary-phase columns were prepared with mechanical mixtures of the appropriate amounts of support + selected stationary phase A (FFAP) and support + selected stationary phase B (E 301). Table I lists the barbiturates of the selected model mixture examined. The components were selected on the basis of their frequency.

RESULTS AND DISCUSSION

Fig. 1 shows the shape of the amobarbital peak obtained without (peak A) and with (peak B) formic acid in the carrier gas as tailing reducer. Formic acid greatly reduces the adsorption of barbiturates on silanized or unsilanized Chromosorb. Indeed, from measurements carried out with the barbiturates in the mixture there is a linear response from 10^{-6} to 10^{-9} g injected. Further, with carrier gas saturated with formic acid it is possible to analyse directly barbiturates as their alkaline salts in water or methanol solutions.

A large number of stationary phases were examined but without obtaining the complete separation of all of the compounds of the mixture. With stationary phases of low polarity such as Apiezon L or silicone gum, having methyl or phenyl groups, it is possible to separate allobarbital–butethal and amobarbital–pentobarbital but not

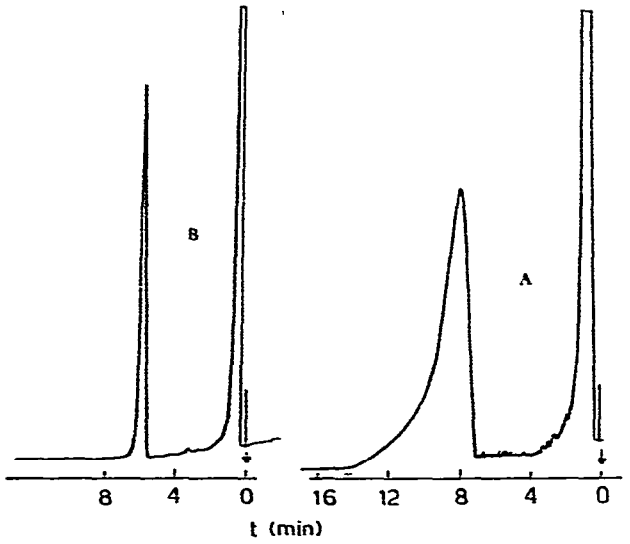
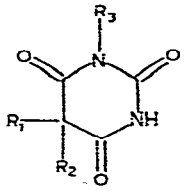


Fig. 1. Shape of amobarbital peak obtained (A) without and (B) with formic acid in the carrier gas. Column temperature, 200°C; carrier gas, nitrogen; detector, flame ionization.

TABLE I

BARBITURATES EXAMINED



No.	*Compound*	R_1	R_2	R_3
1	Barbital	Ethyl	Ethyl	Hydrogen
2	Allobarbital	Allyl	Allyl	Hydrogen
3	Butethal	Ethyl	Butyl	Hydrogen
4	Amobarbital	Ethyl	3-Methylbutyl	Hydrogen
5	Pentobarbital	Ethyl	1-Methylbutyl	Hydrogen
6	Secobarbital	Allyl	1-Methylbutyl	Hydrogen
7	Hexobarbital	Methyl	1-Cyclohexenyl	Methyl
8	Phenobarbital	Ethyl	Phenyl	Hydrogen
9	Heptabarbital	Ethyl	1-Cycloheptenyl	Hydrogen

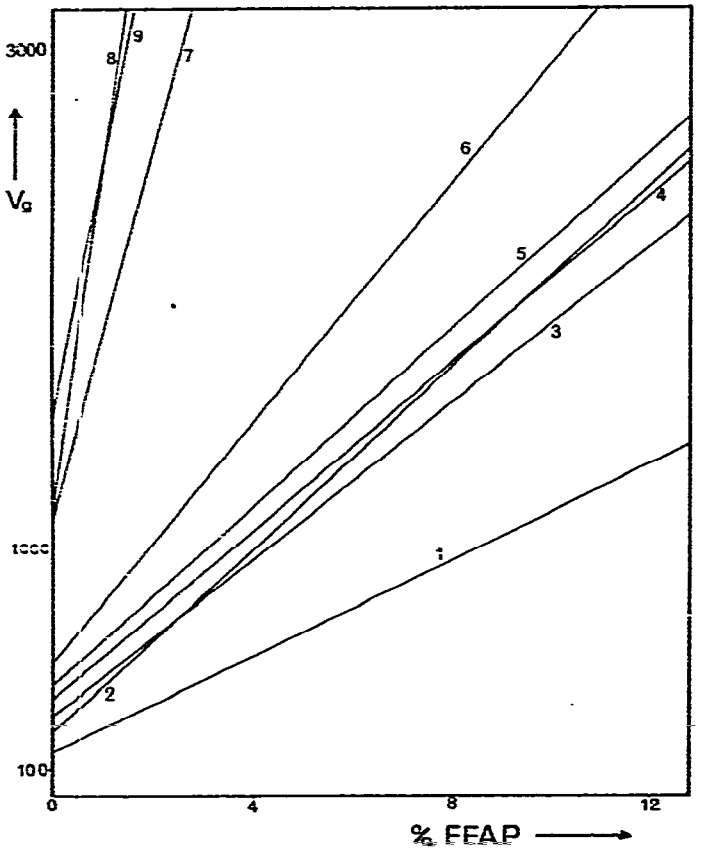


Fig. 2. V_g *versus* concentration of FFAP in E 301 for the barbiturates listed in Table I. Column temperature, 200°C; carrier gas, nitrogen saturated with formic acid. Apolar column: 3% (w/w) E 301 on Chromosorb W (80–100 mesh). Polar column: 1% (w/w) FFAP on the same support.

hexobarbital–phenobarbital. With polar stationary phases containing groups such as RCN, ROH, RCOOR′, ROR′ and RCOOH it is possible to separate the last pair but not the others. To separate all of the barbiturates in the mixture a binary stationary phase column was prepared containing E 301 (silicone grease) and FFAP (prepared from Carbowax 20M and a derivative of terephthalic acid) (Carlo Erba, Milan, Italy).

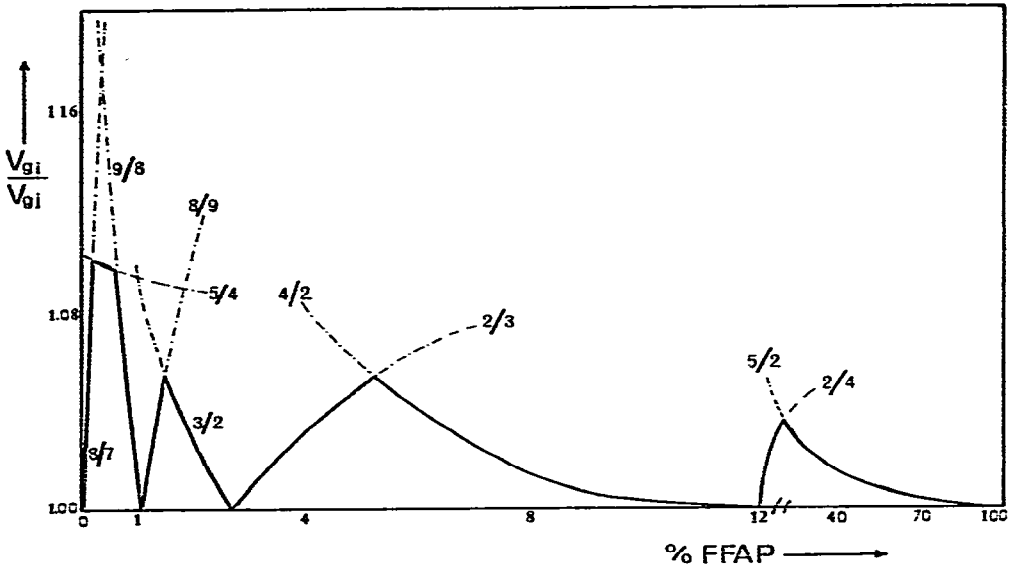


Fig. 3. Window diagram for the barbiturates listed in Table I. The best α value is predicted to lie at 0.2% FFAP in E 301.

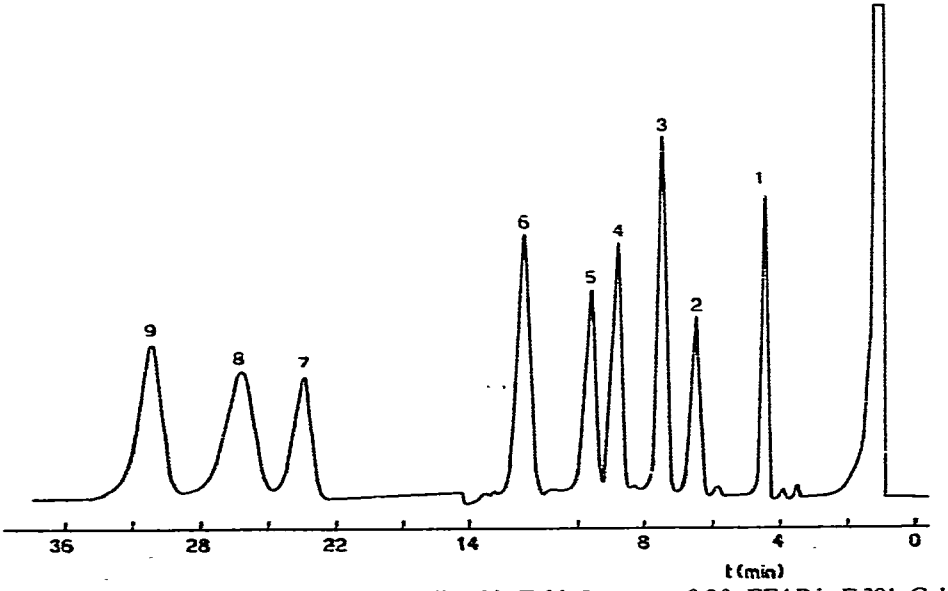


Fig. 4. Chromatogram of the barbiturates listed in Table I at $m_A = 0.2\%$ FFAP in E 301. Column, 3 m × 0.3 cm I.D.; packing, E 301 (3%, w/w) and FFAP (1%, w/w) on Chromosorb W (80–100 mesh), mechanically mixed; column temperature, 200°C; carrier gas, nitrogen saturated with formic acid vapour.

The apolar column was prepared from 3% (w/w) E 301 on chromosorb W (80–100 mesh). The polar column was prepared from 1% (w/w) FFAP with the same support.

Fig. 2 shows graphs of V_g *versus* FFAP concentration for each of the barbiturates in the mixture. Only the range of FFAP concentrations of interest, 0–12%, is reported. The points where the lines cross each other corresponds to $\alpha_{i/j} = 1$.

Fig. 3 shows the window diagram for the selected mixture. The largest minimal α value is obtained for the pairs phenobarbital–hexobarbital and amobarbital–pentobarbital and corresponds to 0.2% FFAP in E 301. With this α value, for a baseline separation of the reported barbiturates 4500 theoretical plates are required, which was obtained with a column 3 m long.

The gas chromatogram in Fig. 4 shows that a complete separation of all of the barbiturates in the mixture is achieved with such a column.

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