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SEPARATION AND DETERMINATION OF LOW-MOLECULAR-WEIGHT STRAIGHT-CHAIN (C<sub>1</sub>-C<sub>8</sub>) CARBOXYLIC ACIDS BY GAS CHROMATOGRAPHY OF THEIR ANILIDE DERIVATIVES

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## SUMMARY

The analysis for formic, acetic, propionic, *n*-butyric, *n*-valeric, *n*-hexoic, *n*-heptoic and *n*-octoic acids in aqueous and non-aqueous mixtures by the gas chromatography of their anilide derivatives on sodium dodecylbenzenesulphonate coated on Chromosorb G is described. The derivatives of C<sub>2</sub>-C<sub>8</sub> acids are formed in non-aqueous conditions via thionyl chloride treatment while the formic acid derivative is formed in aqueous conditions after pH adjustment. Optimum conditions for quantitative derivative formation are discussed. Variations of the p*K*<sub>a</sub> values of the acids with derivative retention times and retention times and response factors with the number of carbon atoms are illustrated.

## INTRODUCTION

Most of the methods described in the literature for the analysis of low-molecular-weight C<sub>1</sub>-C<sub>7</sub> carboxylic acids by gas chromatography involve the direct injection of the acids into the column. This often necessitates the use of special combinations of stationary phases to obtain the optimum conditions for separation, but even then formic acid decomposition or lack of separation of it has been reported on some of these columns under the experimental conditions<sup>1,2</sup>. JANÁK AND KAPLANOVA<sup>3</sup> separated acetic and formic acids and water on a 1:3 mixture of bis(2-ethylhexyl) sebacate and citric acid coated on silanised Celite. MCKINNEY AND JORDAN<sup>4</sup> resolved formic, acetic, acrylic, propionic, *n*-butyric and isopentanoic acids and low-molecular-weight alcohols and aldehydes on 4% ethofat/2% isophthalic acid on Chromosorb T. A packing that will resolve a mixture of volatile fatty acids, C<sub>1</sub>-C<sub>5</sub>, has been reported by JACKSON<sup>5</sup>, namely Ucon LB 550-X, sebacic acid and polypropylene glycol on 80/100 mesh AW Chromosorb W. Free formic and acetic acids in cigarette smoke have been determined by gas chromatography of their methyl esters on 20% Carbowax 20 M on 60/80 mesh Chromosorb P<sup>6</sup>.

In the present work the anilide derivatives of formic, acetic, propionic, *n*-

butyric, *n*-valeric, *n*-hexoic, *n*-heptoic and *n*-octoic acids are chromatographed for quantitative determination on a single stationary phase coated on Chromosorb G. The derivatives are stable, non-volatile and easy to form. The method described lacks the disadvantages of the use of mixtures of stationary phases. The column packing remains stable and efficient over a long period of time because no free acids are passed through it.

#### EXPERIMENTAL

A Perkin Elmer Model F11 gas chromatograph equipped with dual flame ionisation detector was used for the analysis, and nitrogen served as the carrier gas.

##### *Column preparation*

2.5 g of sodium dodecylbenzenesulphonate (DBS) were dissolved in water-methanol (1:1) and the solution was added to 97.5 g of NAW 60/80 mesh Chromosorb G. After mixing by stirring the solvent was evaporated in the oven at 100° with occasional stirring until the solid appeared dry. It was cooled and packed by suction into 6 ft. (A) and 12 ft. (B) by 3 mm I.D. glass columns.

*Column C.* 1 g of potassium hydroxide was dissolved in 50 ml of methanol and added with more methanol to 99 g of NAW Chromosorb G. After mixing by stirring the solvent was removed. Then 97.5 g of the dry solid were added to 2.5 g of DBS dissolved in water-methanol, mixed thoroughly and the solvent removed as above in the oven. The solid was cooled and packed in a 6 ft. × 3 mm I.D. glass column (C).

The three packed columns A, B and C were conditioned at 220° for 18 h.

##### *Instrument analytical conditions*

The instrumental conditions and the columns employed for the various separations and determinations are listed in Table I below.

TABLE I

#### INSTRUMENTAL CONDITIONS FOR THE ANALYTICAL SEPARATIONS

Columns: (A) 6 ft. × 3 mm I.D. glass column packed with 2.5% (w/w) sodium dodecylbenzenesulphonate (DBS) on NAW 60/80 mesh Chromosorb G; (B) the 12 ft. × 3 mm I.D. version of column A; (C) 6 ft. × 3 mm I.D. glass column packed with 2.5% (w/w) DBS on NAW 60/80 mesh 1% (w/w) base-loaded Chromosorb G.

The oxygen and hydrogen inlet pressures and the injection port temperature are the same for all the separations, *viz.* 26/18 lb./in.<sup>2</sup> and 250°, respectively.

<i>Chromatography of the anilide derivatives of</i>	<i>Instrumental conditions</i>
Formic and acetic or propionic or butyric acid using methyl stearate as internal standard	Columns A and C; oven temperature, 200°; nitrogen inlet pressure, 9 lb./in. <sup>2</sup> (Fig. 2)
C <sub>1</sub> -C <sub>8</sub> (formic, acetic, propionic, <i>n</i> -butyric, <i>n</i> -valeric, <i>n</i> -hexoic, <i>n</i> -heptoic and <i>n</i> -octoic) acids, using methyl myristate as internal standard	Column B; oven temperature, 200°; nitrogen inlet pressure, 15 lb./in. <sup>2</sup> (Fig. 3)
Acetic, propionic, <i>n</i> -butyric and <i>n</i> -valeric acids (for quantitative separation)	Columns A and C; oven temperature, 200°; nitrogen inlet pressure, 10 lb./in. <sup>2</sup> (Fig. 5)
	Column B; oven temperature, 200°; nitrogen inlet pressure, 10 lb./in. <sup>2</sup> (Fig. 4)

### Chemicals

The chemicals were used as obtained from BDH without further purification. The anilide derivatives of the carboxylic acids were prepared as described by OPEN-SHAW<sup>7</sup> for retention time determinations.

### PROCEDURE

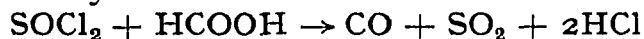
#### *Preparation of the anilide derivatives of C<sub>2</sub>-C<sub>8</sub> straight-chain carboxylic acids and chromatography*

0.5 ml of the ethyl acetate solution of acetic, propionic, *n*-butyric, *n*-valeric, *n*-hexoic, *n*-heptoic, and *n*-octoic acids (0.5 *M* in each acid) were pipetted into a dry 50-ml stoppered tube containing 0.15 ml thionyl chloride. 0.5 ml aniline were then gently added. (The aniline was allowed to fall in drops directly onto the carboxylic acid-thionyl chloride mixture from a pipette without touching the walls of the tube.) The tube was placed unstoppered on a sand bath at 60–80° for 5 min and then cooled for 2 min in an ice bath. 10 ml of 0.1% (w/v) methyl myristate solution in ethyl acetate was added and the tube swirled round gently to mix. 10 ml of 1 *M* aqueous solution of sodium bicarbonate were added and the tube shaken unstoppered until the effervescence died down. It was then stoppered and shaken vigorously for 30 sec and left to stand for the layers to separate. 2  $\mu$ l of the ethyl acetate layer were taken in a hypodermic syringe and injected into the instrument (columns B and C).

When the injection was not being done immediately after the derivative preparation the clear ethyl acetate layer was transferred with a clean dropping pipette to a 10–20 ml dry stoppered tube containing 2–5 g anhydrous sodium sulphate. The derivatives could stand in the solution for up to a week without deterioration.

### RESULTS AND DISCUSSION

The anilide derivatives of a mixture of acetic to *n*-octoic acids may be prepared quantitatively by the method described only in non-aqueous conditions via thionyl chloride treatment. In aqueous medium and at the working concentrations (0.1–1.0 *M*) the anilides were hardly formed due to the ease of hydrolysis of the acid chlorides. On the other hand formanilide was hardly formed in non-aqueous medium especially after thionyl chloride treatment due to the reaction:



In aqueous medium, however, formanilide was obtained quantitatively in all working concentrations (0.05–1.0 *M*) even with thionyl chloride treatment.

When these acids are in an aqueous mixture they may be determined as described but must first be extracted into an organic (ethyl acetate or diethyl ether) layer. Though formic, acetic, propionic and *n*-butyric acids are miscible with water, they were extracted into the ethyl acetate layer in measurable amounts. However, the amount extracted individually into the organic layer, using equal volumes of distilled water and ethyl acetate and shaking vigorously for 1 min in a separatory funnel, varied with the amount of acid present, *e.g.* formic, acetic, propionic and *n*-butyric acids over a concentration range of 0.5 *M*–4.0 *M* in water were extracted 39.3–41.4%, 45.0–50.0%, 71.8–75.0% and 89.4–90.6%, respectively, into the organic layer. The rest of the acids gave 98–100% extraction when extracted individually.

Duplicate extractions of an equimolar (0.5 M) mixture of the C<sub>2</sub>-C<sub>8</sub> acids gave a different extraction picture for each acid, *viz.* acetic acid, 15.5%; propionic acid, 30.3%; *n*-butyric acid, 36.8%; *n*-valeric acid, 39.2%; *n*-hexoic acid, 39.5%; *n*-heptoic acid, 39.5%; and *n*-octoic acid, 40.0%. The presence of mineral acids and salts also affected the carboxylic acid recoveries in the organic layer, so did the variation of the volume ratio of the organic and aqueous portions and the total acid concentration in the mixture. The investigation of these effects was not carried to the final conclusions as it might detract from the main object of this work. It must be pointed out that the recovery of each acid in a mixture would vary from situation to situation. It is therefore necessary to calibrate the extraction procedure for the acids in any given aqueous condition before proceeding with the derivative formation and gas chromatography.

The presence of water in the ethyl acetate solution of the acids reduces the amount of derivatives formed (hence lowers the sensitivity of the method) and distorts the chromatograms obtained from column B. Therefore after extraction of the acids from an aqueous medium the ethyl acetate layer must be dried with anhydrous sodium sulphate before being sampled for derivative formation.

Unknown acid mixtures may be determined by plotting calibration curves with standard mixtures, or by the use of the relative response factors (*i.e.*, weight of acid taken in mg/peak area *vs.* methyl myristate, Table III), using methyl myristate as the internal standard in both cases. The relative amount of each anilide formed (measured as the peak area ratio) in the mixture with any fixed amount of thionyl chloride present varies with the amount of aniline added (Fig. 1). Acetic, propionic and *n*-butyric acids on the one hand, and *n*-valeric, *n*-hexoic, *n*-heptoic and *n*-octoic acids on the other yield maximum amounts of derivatives at different concentrations of aniline. It was found reproducible and accurate enough in practice, however, to estimate first the number of mmoles of acid in an aliquot of the unknown mixture, and then to add thionyl chloride 1-2 mmoles in excess, followed by an amount of

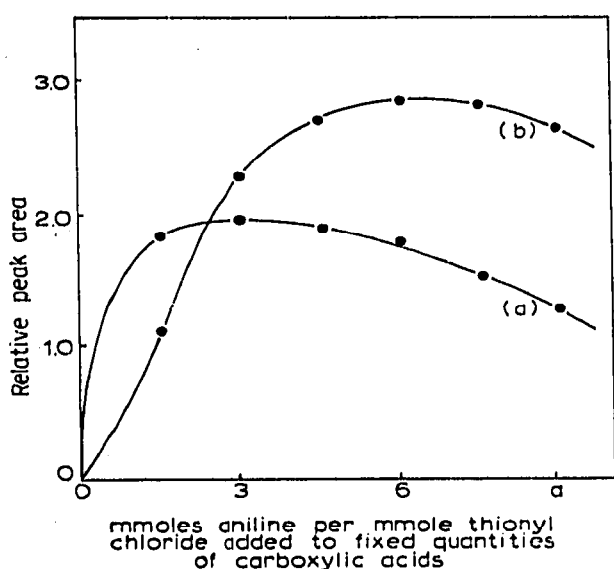


Fig. 1. Variation of quantity of derivative formed with increasing amounts of aniline. (a) *n*-Valeric-*n*-octoic acids; (b) acetic, propionic and *n*-butyric acids.

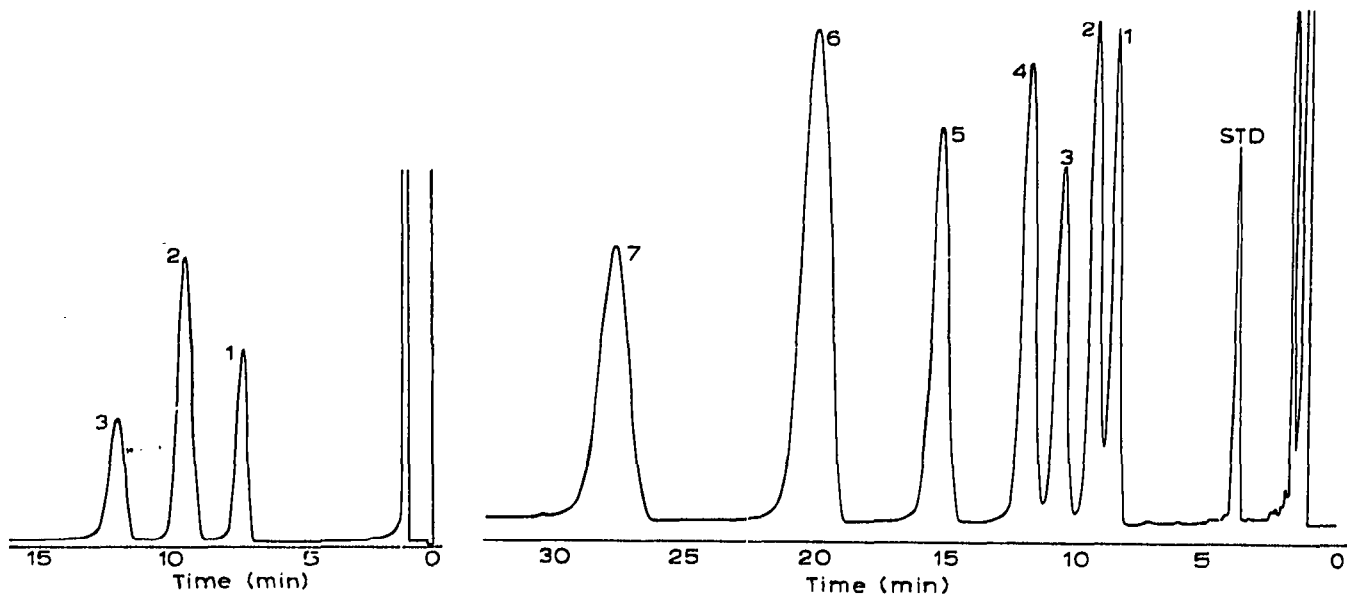


Fig. 2. Separation of acetanilide (1) and formanilide (3) with methyl stearate (2) as internal standard on a 6-ft. 2.5% DBS on Chromosorb G column.

Fig. 3. Separation of anilide derivatives of  $C_1$ - $C_8$  straight-chain carboxylic acids on a 12-ft. 2.5% DBS on Chromosorb G column. 1 = Propionic acid, 2 = *n*-butyric acid, 3 = acetic acid, 4 = *n*-valeric acid, 5 = *n*-hexoic acid, 6 = formic acid and *n*-heptoic acid, and 7 = *n*-octoic acid; STD = methyl myristate.

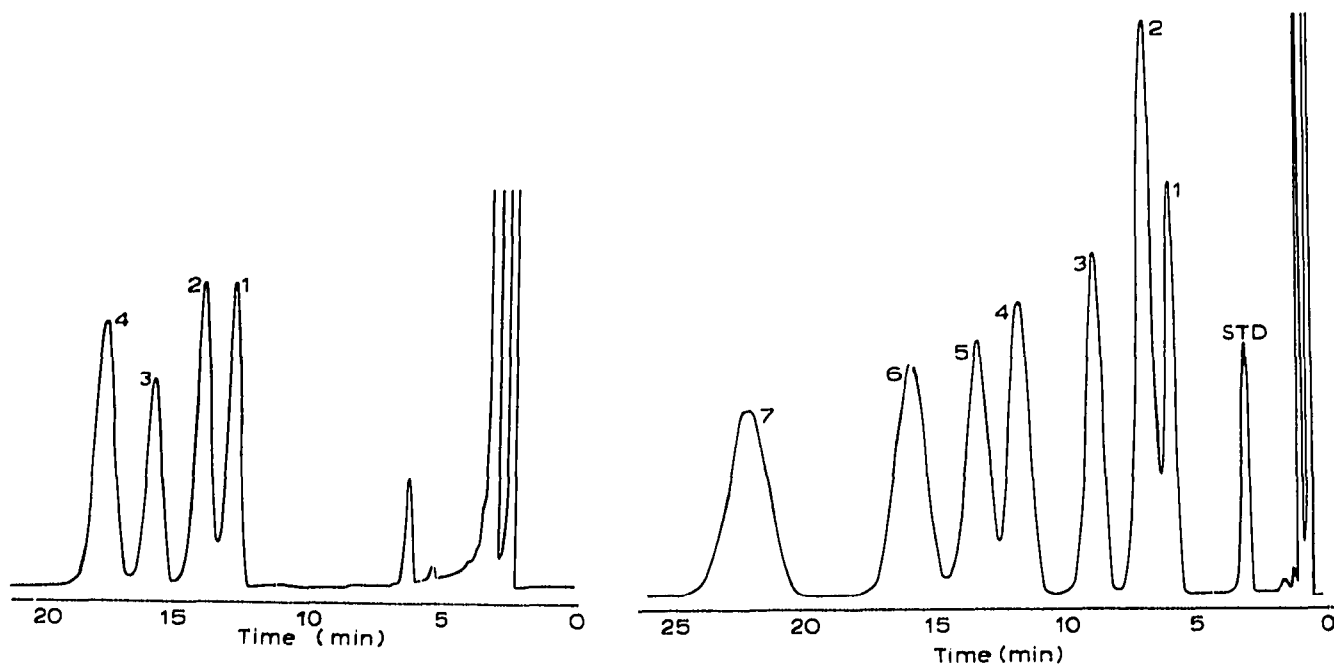


Fig. 4. Quantitative separation of anilide derivatives of (1) propionic acid, (2) *n*-butyric acid and (3) acetic acid on a 12-ft. 2.5% DBS on Chromosorb G column. 4 = Valeric acid.

Fig. 5. Separation of anilide derivatives of  $C_1$ - $C_8$  straight-chain carboxylic acids on a 6-ft. 2.5% DBS on Chromosorb G column coated 1% w/w with potassium hydroxide. 1 = Propionic acid, 2 = acetic and *n*-butyric acid, 3 = *n*-valeric acid, 4 = *n*-hexoic acid, 5 = formic acid, 6 = *n*-heptoic acid, 7 = *n*-octoic acid; STD = methyl myristate.

TABLE II

RELATIVE RETENTION TIMES OF THE ANILIDES OF THE C<sub>1</sub>-C<sub>8</sub> CARBOXYLIC ACIDS

Acid	$pK_a$ (25°) <sup>a</sup>	Relative retention time	
		Column B	Column C
Formic (C <sub>1</sub> )	3.77	6.34	5.0
Acetic (C <sub>2</sub> )	4.76	3.21	2.41
Propionic (C <sub>3</sub> )	4.88	2.57	2.0
<i>n</i> -Butyric (C <sub>4</sub> )	4.82	2.86	2.41
<i>n</i> -Valeric (C <sub>5</sub> )	4.81	3.79	3.16
<i>n</i> -Hexoic (C <sub>6</sub> )	4.85	4.92	4.33
<i>n</i> -Heptoic (C <sub>7</sub> )	4.89	6.64	6.08
<i>n</i> -Octoic (C <sub>8</sub> )	4.85	9.42	8.66

aniline three to six times the number of mmoles of thionyl chloride added. The relative amount of aniline added to sample and standard aliquots must be the same.

The anilides of the C<sub>1</sub>-C<sub>8</sub> carboxylic acids were separated on columns A, B and C. Heptoic and formic acid anilides were not separated on column B but they were

TABLE III

Acid	Relative response factor	
	Weight (in mg)	Mmoles
	Peak area	Peak area
Acetic	8.4	0.14
Propionic	8.14	0.11
<i>n</i> -Butyric	7.75	0.09
<i>n</i> -Valeric	7.14	0.07
<i>n</i> -Hexoic	6.96	0.06
<i>n</i> -Heptoic	7.02	0.05
<i>n</i> -Octoic	7.64	0.05

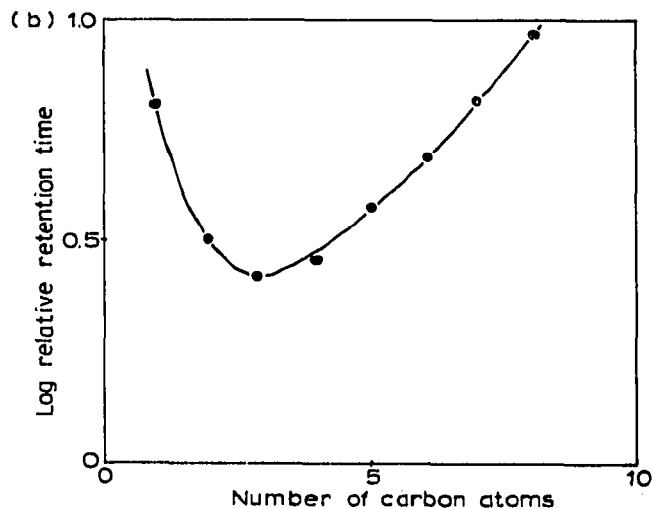
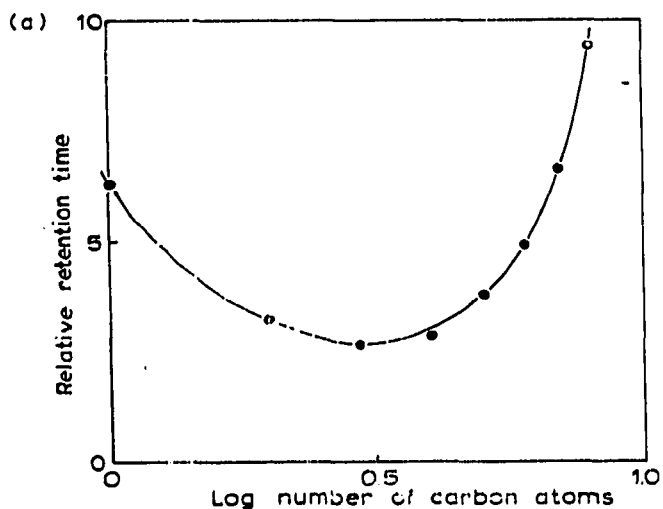


Fig. 6. (a and b) Variation of relative retention time with the number of carbon atoms on the carboxylic acids.

on columns A and C. Similarly, while *n*-butyric, acetic and propionic acid anilides were not resolved on columns A and C they were on column B. In the absence of *n*-butyric or acetic acid in the mixture the rest of the acids were quantitatively separated on columns A and C (Figs. 2-5).

The relative retention times (on columns B and C) and the response factors (on column B) of the acids are shown in Tables II and III, respectively. Their variations with the number of carbon atoms are illustrated in Figs. 6 and 7. The relative response factor decreases (in other words, the relative peak area per mmole of acid present increases) with increasing number of carbon atoms.

The effect of the  $pK_a$  of the acids on their relative retention times is not very clear. A plot of the  $pK_a$  ( $25^\circ$ ) vs. relative retention time produces the curves in Fig. 8. The  $pK_a$  values of formic, acetic, propionic and *n*-butyric acids lie on a straight line in relation to their retention times on column B.

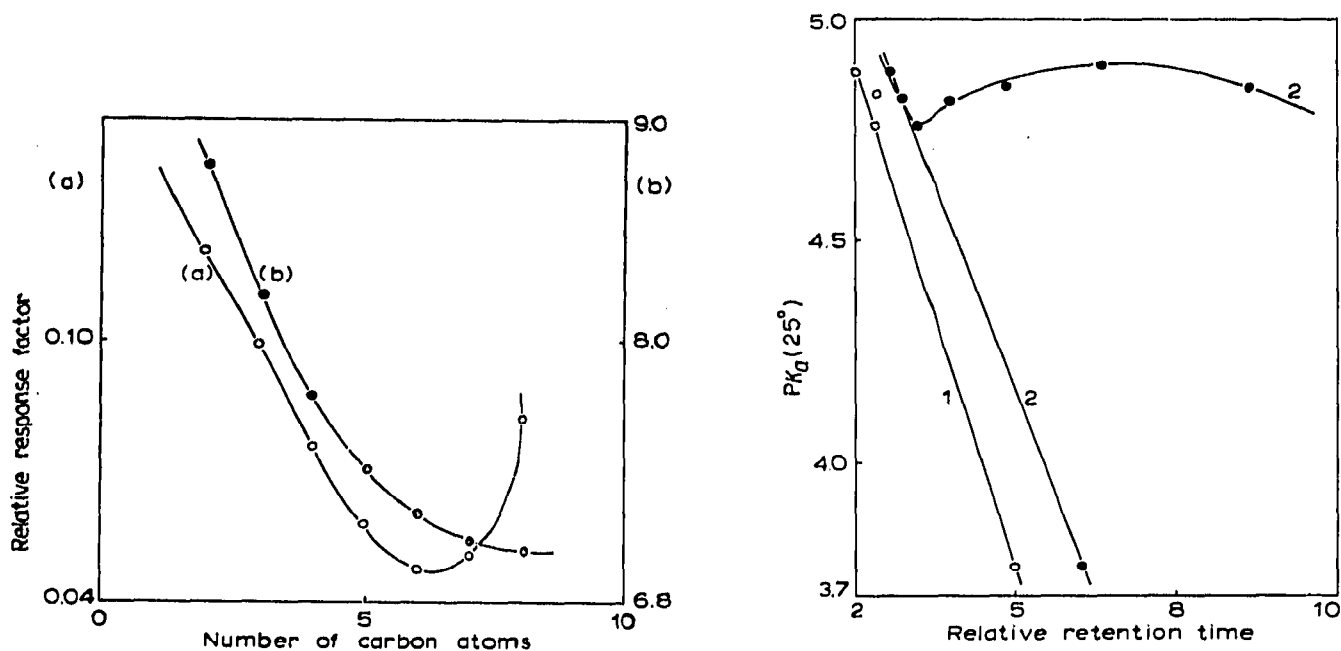


Fig. 7. Variation of relative response factors with the number of carbon atoms.

(a) (Weight of acid in mg/peak area) vs. number of carbon atoms

(b) (Mmoles acid taken/peak area) vs. number of carbon atoms

Fig. 8.  $pK_a$  ( $25^\circ$ ) vs. relative retention times of the anilides of the straight-chain  $C_1-C_9$  carboxylic acids on columns B and C. (1) On column C; (2) on column B.

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