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Comparison of several methods for the determination of trace amounts of polar aliphatic monocarboxylic acids by high-performance liquid chromatography

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ABSTRACT

Reversed-phase high-performance liquid chromatography followed by direct detection by UV absorbance at 200 nm is a convenient method for the determination of polar aliphatic carboxylic acids. It can only be used for "clean" samples, except for formic and acetic acid, owing to a lack of retention. Up to butyric acid, ion-exclusion chromatography with conductimetric detection is generally preferred, allowing the detection of less than 120 μg/l. Each ion-exclusion column has its own selectivity and efficiency; interfering compounds are another problem. The precision at the 1 ppm level is about 2% ($n = 10$). Pre-column derivatization of carboxylic acids with 4-bromomethyl-6,7-dimethoxycoumarine has been fully automated but is not recommended; limitation of the water content, internal standardization and carry-over correction are necessary. Pre-column derivatization with 2-nitrophenylhydrazine is a better way to obtain detection limits of one nanogram (or 50 μg/l) of C_4–C_9 carboxylic acid. The analyses are fully automated, can be performed in an aqueous environment and give a precision better than 4% at levels of 1 mg/l of analyte.

INTRODUCTION

In the chemical and polymer industries there is an increasing demand for the determination of trace amounts of polar aliphatic carboxylic acids in aqueous extracts of process fluids.

In this work, four high-performance liquid chromatography (HPLC) methods were compared with respect to the concentration and mass detection limit, precision and ease of use. Selectivity was only considered in general terms as the targeted analyte matrices were of diverse origins.

The four methods studied were: (1) ion-suppression reversed-phase HPLC (RP-HPLC) with UV absorbance detection; (2) a high performance ion-exclusion chromatography (HPICE) method with conductimetric detection; (3) the BMMC method, pre-column derivatization with 4-bromomethyl-6,7-dimethoxycoumarine (BMMC) and RP-HPLC with fluorescence detection; and (4) the NPH method, pre-column derivatization with 2-nitrophenylhydrazine (NPH) and RP-HPLC with absorbance detection at 400 nm.

EXPERIMENTAL

The four methods are summarized in Table I. The pre-column derivatization of the BMMC method was performed in a Promis (Spark, Emmen, Netherlands) unit, which is shown schematically in Fig. 1. All the tubing of the peristaltic pump was poly (vinyl chloride). During the load cycle the reagent flow-rate was 0.1 ml/min and the waste flow-rate was 0.32 ml/min. The sample was differentially aspirated at 0.12 ml/min. Reagent 1 (75 mg of potassium carbonate and 21 μl of water) was added to 7.5 ml of a 10 mM 18-crown-6 solution in acetonitrile. The mixture was sonicated for 15 min, diluted with 3.75 ml of acetonitrile, filtered through a 0.2-μm filter and diluted with 0.5 ml of acetonitrile. Reagent 2 was 1 mg/ml of BMMC (Kodak, Rochester, NY, USA) in acetonitrile.

The pre-column derivatization unit of an HP-1090 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) was used for the NPH reaction (see Fig. 2). The NPH reagent consisted of 0.04 M NPH hydrochloride (Janssen, Beerse, Belgium)

TABLE I

SUMMARY OF METHODS

|	Method			
	UV	HPICE	NPH	BMMC
Sample acids R–COOH	R = C2, C3, C5, C7, C9	R = C0, C1, C2, C3	R = C5, C6, C7, C8, C9	R = C4, C5, C6, C7, C8
Liquid chromatography	Gilson	Dionex 20001	HP-1090	HP-1050
Column	250 × 4 mm; Nucleosil 120-5 C_{18}	250 × 10 mm; HPICE-AS1	100 × 4 mm; Nucleosil 120-5 C_{18}	100 × 3 mm; Nucleosil 102-5 C_{18}
Eluent A	10 mM H_3PO_4, pH 2.5	1 mM octanesulphonic acid	Water, pH 4.5 with HCl	10 mM H_3PO_4, pH 6.5 with NaOH
Eluent B	Acetonitrile	—	Acetonitrile	Acetonitrile
Gradient	20 → 100% B in 10 min	—	30 → 75% B in 15 min	5 → 100% B in 30 min
Temperature (reaction)	Ambient	Ambient	60°C	Ambient
Temperature (column)	Ambient	Ambient	50°C	Ambient
Flow-rate	1 ml/min	1.2 ml/min	1 ml/min	2 ml/min
Pre-column derivatization unit	Gilson 231-401		HP-1090	Promis, Spark
Injection volume	1000 µl	500 µl	26 µl	20 µl
Detector	Linear UV 204 nm	Waters 431 conductivity	Diode array (HP-1090)	HP-1046 A fluorescence
Wavelength(s)	λ = 200 nm	—	λ = 400 nm	λ_{ex} = 340 nm; λ = λ_{em} = 417 nm
Pre-column derivatization reagents	—	—	2-nitrophenylhydrazine hydrochloride	4-bromomethyl-6,7-dimethoxy coumarine
Reaction time	—		20 min	15 min
Suppressor		AMMS-ICE (Dionex)		
Suppressor fluid	—	10 mM tetrabutylammo-niumhydroxide, 2 ml/min		
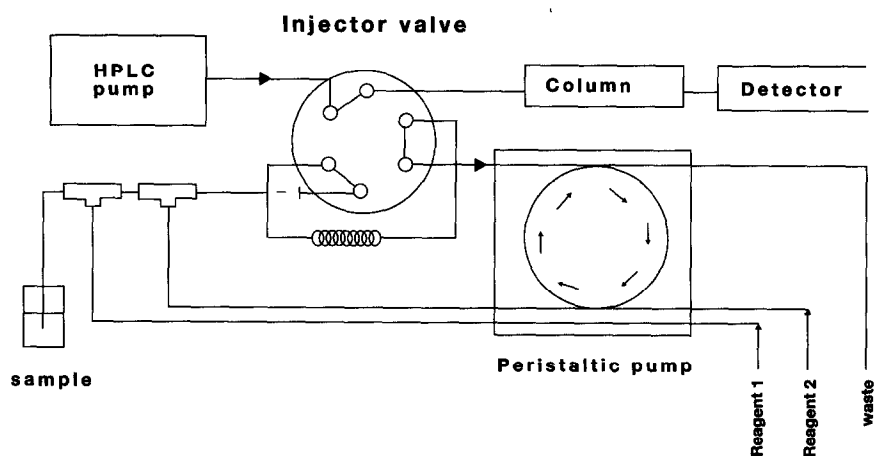
Fig. 1. Schematic diagram of the Promis pre-column derivatization unit.

in 50% aqueous ethanol; the EDC reagent solution (see Table II) contained 3% pyridine, 47% ethanol and 0.25 M 1-ethyl-3-(3-dimethylaminoropyl)carbodiimide hydrochloride (Polysciences, Warring-' ton, PA, USA) in water.

The liquid chromatograph for the UV detection method consisted of a Gilson 305/302 gradient pumping system with an 805 monometric module, an 811 gradient mixer and a Model 231-401 auto-

sampler. Detection at 200 nm was performed on a Linear UV-204 detector (Linear, Reno, NY, USA).

For the HPICE method a Dionex 2000i or 4000i ion chromatograph (Dionex, Sunnyvale, CA, USA) was used, equipped with an anion micromembrane suppressor and a Waters 431 conductivity detector (Waters, Milford, MA, USA).

An HP-1050 gradient solvent delivery system with an HP-1046A fluorescence detector was used
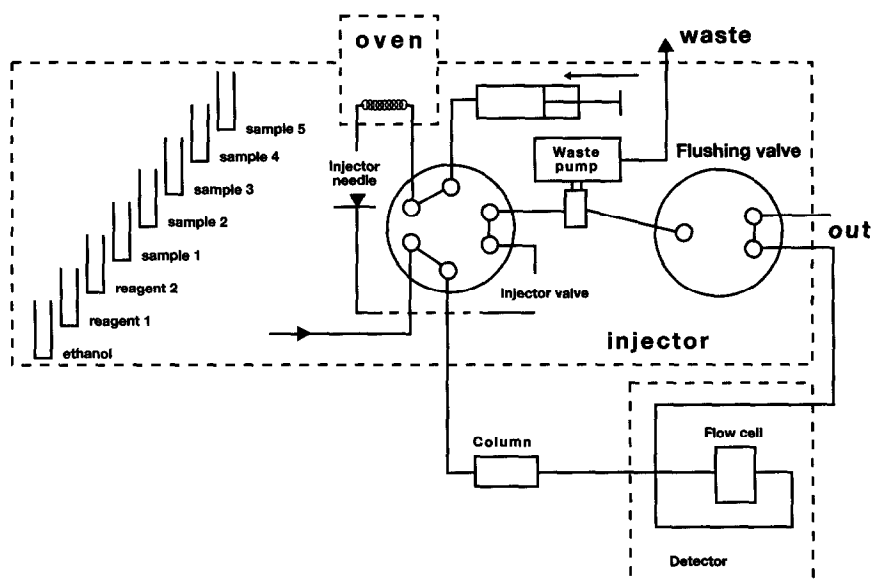

Fig. 2. Schematic diagram of the HP-1090 liquid chromatograph used for the NPH method.

for the BMMC method and an HP-1090 system with a built-in diode-array detector for the NPH method (all from Hewlett-Packard).

All reversed-phase columns were Nucleosil 120-5C_{18} (Macherey-Nagel, Düren, Germany); all solvents were of P.A. quality (Merck, Darmstadt, Germany).

RESULTS AND DISCUSSION

UV method

Many monocarboxylic acids show enough retention to be detected by HPLC at a mobile phase pH lower than the pK_a value of their carboxylic acid groups.

Direct UV absorbance detection at short wavelengths is often problematic becase of its lack of selectivity and sensitivity. Most organic compounds with a polar group have an absorbance in the region 180–205 nm, comparable with or larger than that of carboxylic acids [1].

The problem with the sensitivity can be partly overcome by using on-column concentration [2]. A signal-to-noise ratio of 3 is obtained at about 100 ng of analyte injected onto the column. By introducing the largest possible volume of aqueous sample into the column, the detection limit, expressed as the concentration in the original sample, is reduced.

This method is applicable to the determination of carboxylic acids with a higher retention than acetic acid (*cf.* Fig. 3).

Disturbances from the injection plug in the elution region of formic and acetic acids and interferences from impurities in the solvents used do not allow the extension of this method to more polar carboxylic acids or larger injection volumes.

HPICE method

Formic acid, acetic acid and the more polar carboxylic acids such as diglycolic acid and monochloroacetic acid can be determined by separation on a low-capacity ion exchanger, such as Dionex AS-4A, followed by suppressed conductivity detection. Monocarboxylic acids containing a longer aliphatic chain are not sufficiently retained for determination by this method. An alternative method uses a combination of adsorption and ion-exclusion chromatography on a sulphonated polystyrene–divinylbenzene resin [3].

In applying this method, striking differences in the selectivities among the HPICE AS-1 columns were observed (compare Fig. 4A and B). At concentrations compatible with UV detection (see under *UV method*) the ratio of the absorbance to the conductimetric signal of the method, the low selectivity and the variability of the columns stress the need for
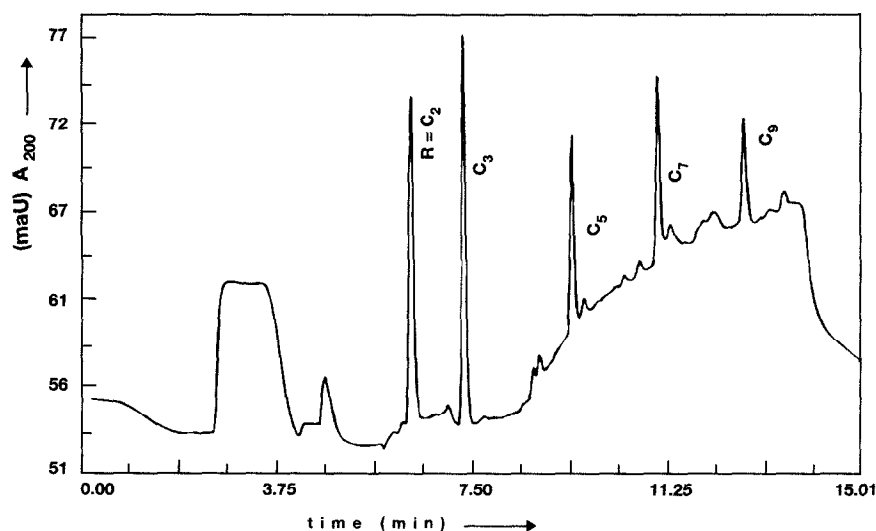

Fig. 3. Chromatogram of five aliphatic carboyxlic acids, obtained by RP-HPLC and direct UV detection at 200 nm. See under Experimental and Table I for details. Injection: 10 mg/l of each acid in water.
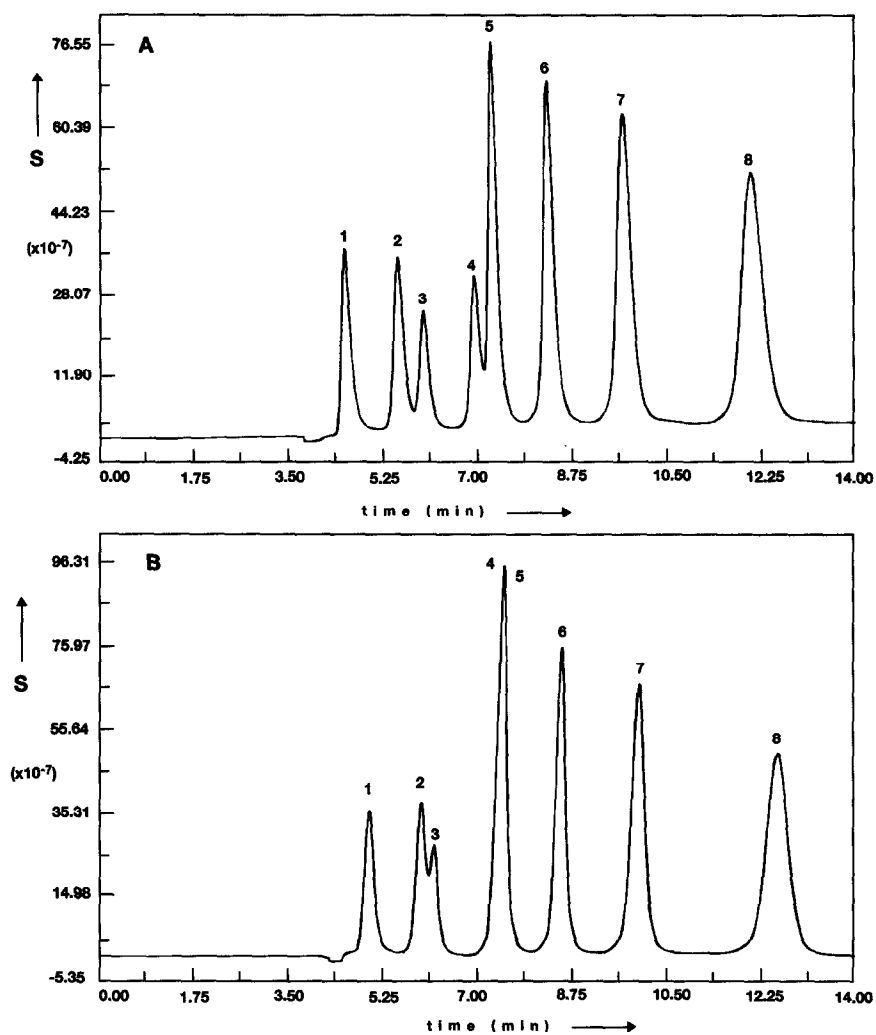
Fig. 4. Chromatograms of a test mixture of carboxylic acids on HPICE-AS1 columns. Signal-to-noise ratios: column A, 2612; B, 5098. For conditions see Table I. Injection: 40 μl of test mixture consisting of (1) 30 mg/l citric acid, (2) 34 mg/l diglycolic acid, (3) 38 mg/l monochloroacetic acid, (4) 37 mg/l hydroxyacetic acid, (5) 47 mg/l formic acid, (6) 81 mg/l acetic acid, (7) 127 mg/l propionic acid and (8) 198 mg/l butyric acid.

high efficiency to avoid the use of a corroborative analysis method at lower analyte concentrations. The Van Deemter curve (Fig. 5) indicates that the optimum flow-rate for resolution per unit time, at (H/u) min (where H = theoretical plate height and u = linear velocity) [4], is about 1.2 ml/min at 80 atm. The HPLC equipment allows operation at 275 atm, so four columns could be coupled. The result is given in Fig. 6. A much better resolution of the first

five components can only be obtained by changing the selectivity of the system, e.g. by modifying the octanesulphonic acid concentration.

The detection limits for formiate and butyrate in water with octanesulphonic acid added to give a 1 mM solution, using a 500-μl loop for injection, are 20 and 120 μg/l, respectively. The maximum loop volume is dependent on he analyte to be determined and its matrix.
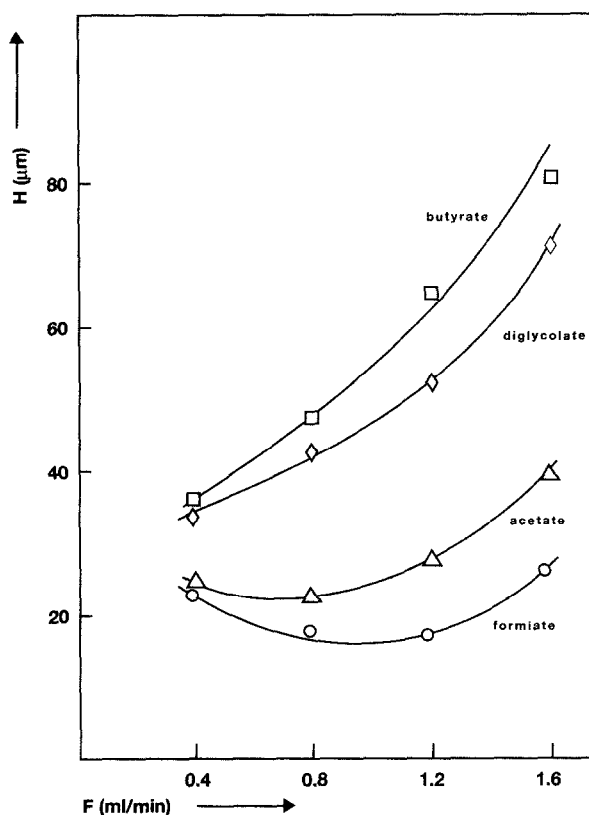
Fig. 5. Theoretical plate height (*H*) as a function of flow-rate (*F*) for different analytes on a Dionex system with two HPICE-AS1 columns in series. For conditions see Table I.

BMMC method

In the first two methods discussed the main drawbacks are the low selectivity and the large injection volumes necessary for acceptable limits of detection.

Pre-column derivatization with BMMC seems attractive as a result of its high selectivity, the improvement in sensitivity for carboxylic acids, the short reaction time and availability of reagents. The reaction is represented schematically in Fig. 7. The potassium counter-ion of the carboyxlic anion is selectively complexed by 18-crown-6, allowing the "naked" carboxylic anion to substitute for a bromine atom of the reagent.

The resulting ester can be detected fluorimetrically. It is resistant to light and stable in an aqueous environment, in contrast to the reagent. Automated on-line pre-column derivatization, even at room temperature, using BMMC was possible with the introduction of an 18-crown-6–potassium carbonate suspension [5]. BMMC is slightly more polar than 4-bromomethyl-methoxycoumarine and its derivative has a higher intrinsic fluorescent sensitivity [6].

Base-catalysed solvolysis to coumaric acid is a problem for all coumarine reagents [7]. This problem manifests itself as a ghost peak which competes with the analyte for the reagent. The prouct of the analyte may also be solvolysed, which will lead to
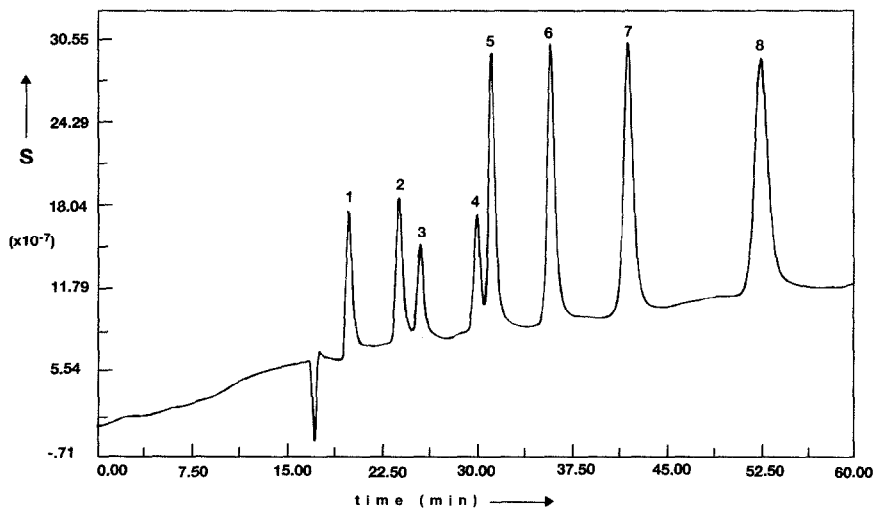

Fig. 6. Chromatogram at optimum conditions for resolution per unit time. Columns: four 250 × 10 mm HPICE-AS1 columns in series. Flow-rate: 1.2 ml/min 1 m*M* octanesulphonic acid, 220 atm. Injection as in Fig. 4.
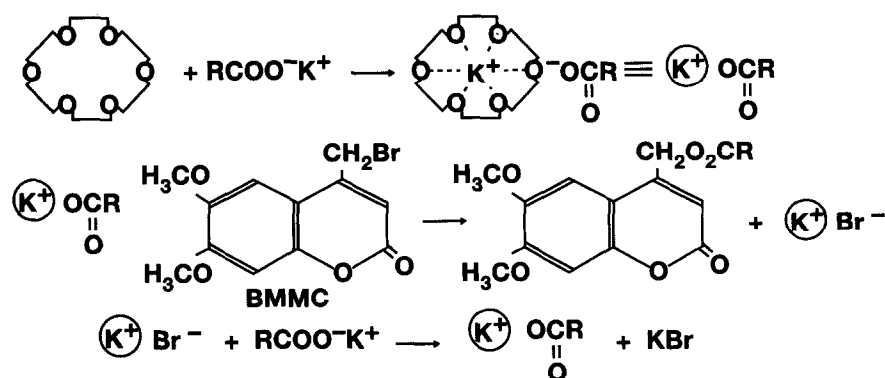
Fig. 7. Schematic representation of of the BMMC reaction.

multiple derivatives and irreproducible results. The exclusion of water and limiting the concentration of the base to a stoichiometric amount cannot be carried out in practice, so internal standardization has to be used.

The pre-column derivatization was carried out as in Wolf and Korf [5], but the dimethoxy reagent and a filtered suspension were used to prevent clogging of the derivatization unit. A chromatogram of an acetonitrile sample containing 1.4 mg/l of each the carboxylic acids indicated is shown in Fig. 8.

An injected blank sample of acetonitrile indicated the presence of carboxylic acids, which could not be traced to any of the reagents or solvents used and thus has to be attributed mainly to carry-over from the previous injection, in spite of extensive rinsing with ethanol, the most effective solvent except for formic acid (which will carry-over and compete for the reagent). This percentage of carry-over (PCO) is determined as a function of the analyte concentration in the sample, A, from the first and the fifth consecutive blank, B, respectively:
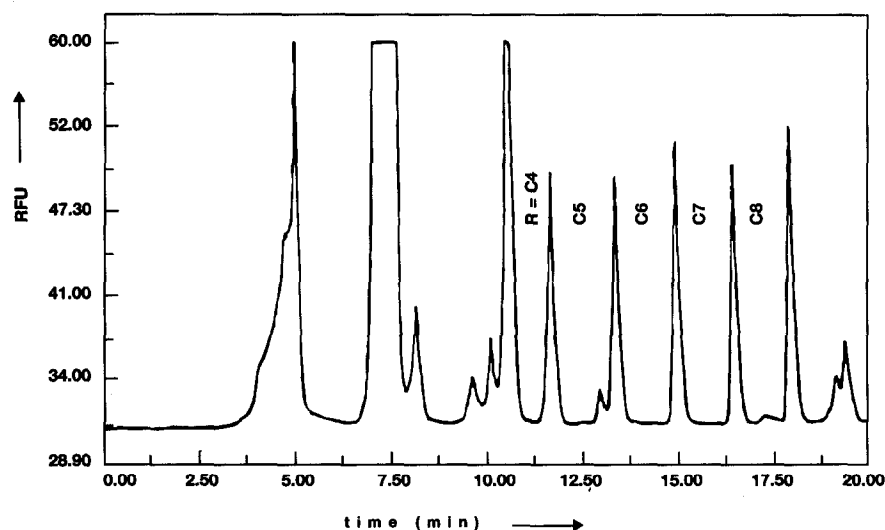
$$PCO(\%) = (B_1 - B_5/A - B_5) \times 100$$

Fig. 8. Separation of BMMC derivatives of pentanoic to nonanoic acid. For conditions see Table I. Injection: 1.4 mg/l of each carboxylic acid in acetonitrile. RFU are relative fluorescence units.
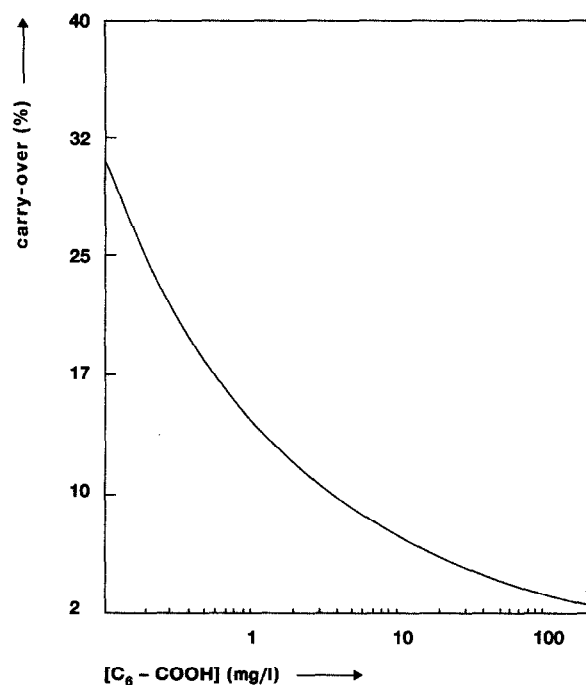
Fig. 9. Percentage of carry-over as a function of heptanoic acid.

The result is given in Fig. 9.

By subtracting the carry-over of the previous sample and adding that of the sample itself, the corrected concentrations can be reconstructed from the measured data.

Calibration graphs show a linear dynamic range of approximately 10^3. The system appeared to be contaminated with the analytes at about 100 ppb after three months of intermittent use and was abandoned in favour of the following method.

NPH method

Aliphatic as well as aromatic carboxylic acids react with NPH to form acid hydrazides when 1-eth- yl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride is added as a coupling agent (see Fig. 10). Derivatization with NPH has as an advantage over almost all published carboxylic acid derivatization methods in that the reaction can be carried out in an aqueous environment [8].

The reaction product can be detected by its absorbance at 230 or 400 nm (see Fig. 11). Reaction by-products which may interfere when detecting at 230 nm can be removed by a second reaction stage after the addition of potassium hydroxide. At 400 nm the acid hydrazides are detected with a four-fold less sensitivity ($\varepsilon_m \approx 5 \cdot 10^3$) than at 230 nm, but only one reaction step is necessary as the main interferences do not absorb at this wavelength. At mg/l levels the derivatives could not be determined by fluorescence detection.

Without a second reaction stage and extraction the derivatization was optimized for the concentration of HCl and NPH, reaction temperature and reaction time in a fully automated HP-1090 pre-

TABLE II

NPH DERIVATIZATION PROTOCOL

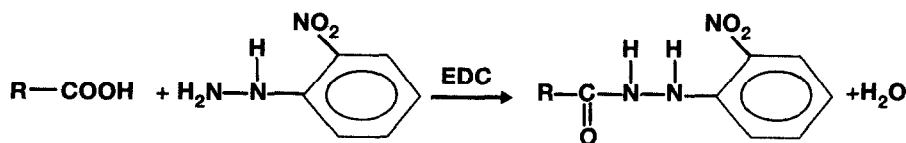| Line	Function	
1	Draw	0.0 μl from Vial 0 Ethanol
2	Draw	1.0 μl from Vial 1 2NPH · HCl
3	Draw	0.0 μl from Vial 0
4	Draw	2.0 μl from Vial 2 1EDC · HCl
5	Draw	0.0 μl from Vial 0
6	Draw	20.0 μl from Sample
7	Draw	0.0 μl from Vial 0
8	Draw	2.0 μl from Vial 2
9	Draw	0.0 μl from Vial 0
10	Draw	1.0 μl from Vial 1
11	Draw	0.0 μl from Vial 0
12	Mix	30.0 μl cycles 10
13	Wait	20.0 min
14	Inject	

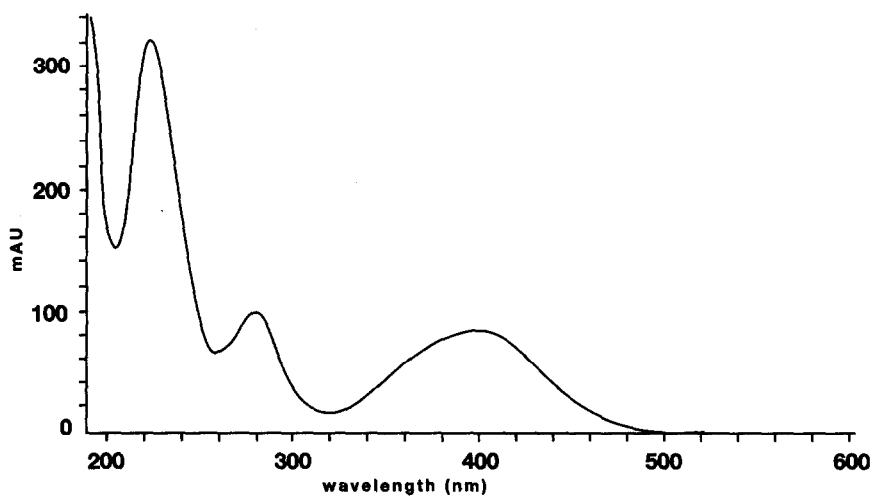Fig. 10. Schematic representation of the NPH reaction.

Fig. 11. Absorbance spectrum of the NPH derivative of hexanoic acid in the mobile phase used. For conditions see Table I.
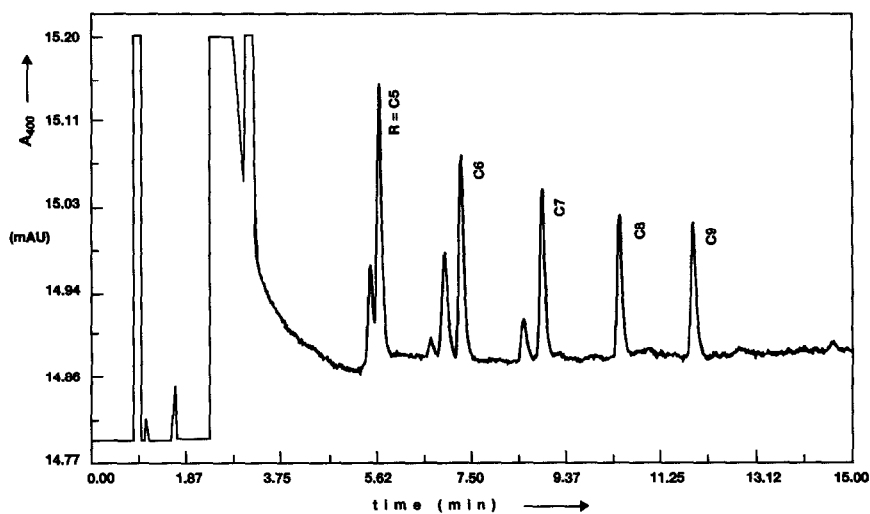

Fig. 12 Separation of NPH derivatives of hexanoic to decanoic acid. For conditions see Table I. Injection: 1.3 mg/l of each carboxylic acid in water.

TABLE III

COMPARISON OF THE FOUR METHODS

R.S.D. = Relative standard deviation; I.S. = internal standard

Method	Injection volume (μl)	R-COOH: R =	Detection limit		R.S.D. (%)	I.S.
			μg/l	ng		
UV	1000	C_6	100	100	–	–
HPICE	500	C_1	40	20	1.9	–
NPH	26	C_6	50	1.3	3.2	C_7
BMMC	20	C_6	60	1.2	7.6	C_7

column derivatization unit (see Fig. 2). The optimum protocol for determining analytes at a concentration of less than 10 mg/l is given in Table II. At higher concentrations the volume of sample should be decreased or the calibration graphs will not be linear as there will not be sufficient excess reagent. Sample volumes larger than 20 μl have not yet been used successfully. A different protocol may improve incomplete mixing for larger sample volumes. Some ethanol (1–10%) was added to the test sample to ensure the complete dissolution of the less polar carboxylic acids. A chromatogram of 1.3 mg/l of each of the five aliphatic carboxylic acids is shown in Fig. 12. The coefficient of variation of the peak areas is 7.0 (R = C_5), 5.6 (C_6), 8.2 (C_7), 9.2 (C_8) and 8.7% (C_9), respectively, and 3.2, 2.6, 2.6 and 3.9% when using the C_7–COOH peak as an internal standard ($n = 10$).

COMPARISON AND CONCLUSIONS

An overall comparison of the four methods studied here is given in Table III. At the 1 mg/l level HPICE can be recommended for carboxylic acids at least as polar as butyric acid, whereas the fully automated NPH method can be most effectively used for less polar carboxylic acids.

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