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TECHNIQUES FOR THE DETECTION OF LIPIDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The use of an infrared detector for the detection of lipids in both normal and reversed-phase chromatography is demonstrated. A system is also described for the derivatisation of lipid molecules containing a $C=O$ group to form the hydroxamic acids and their separation and detection as the iron(III) hydroxamates.

INTRODUCTION

As early as 1972, Parry *et al.*[1] described the necessary properties of a high-performance liquid chromatographic (HPLC) detector system, requirements that still serve as a basis for detector evaluation. The detector characteristics should be such that it should (i) respond to all compounds (except those comprising the mobile phase), or possess a predictable specificity; (ii) give a highly sensitive and predictable response; (iii) give a linear response; (iv) be insensitive to changes in the mobile phase composition, flow-rate and temperature; (v) not contribute to band spreading; (vi) be non-destructive; (vii) give some information to assist compound identification; (viii) be easy to use and capable of continuous separation; (ix) have a low initial cost as well as low running costs.

Most present HPLC detectors are a compromise of these criteria and, despite recent developments, many compounds pose difficulties in detection, particularly if the molecules do not possess a UV chromophore or a fluorophore, are not electrochemically active or do not give a good response with other commercial detectors.

Lipids are a group of important compounds for which HPLC could provide valuable information if the problems encountered in their analysis could be overcome. The separation of simple lipid mixtures into component classes by thin-layer chromatography (TLC), column chromatography, gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) is well established[2–6]. For complex lipid mixtures, no one technique is completely satisfactory, and a combination of two or more of the above techniques is usually used.

HPLC has a number of advantages over other chromatographic techniques

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for lipids *viz.* (i) it can be quantitated more easily than TLC; (ii) lipids which cannot be separated by GLC, because they would be decomposed by the high temperatures required or by the catalytic activity of the column, may be analysed using HPLC; (iii) the separated components can be collected and subjected to further analysis, *e.g.* by spectroscopic techniques.

Lipids in food include glycerides, free fatty acids, fatty acid esters, phospholipids, sterols, terpenes, carotenoids and hydrocarbons. Few of these classes of compounds have a strong UV chromophore, and the wide-ranging polarity of the compounds requires gradient elution for their successful resolution. These two facts rule out UV detection of the underivatised lipids and the use of the refractive-index detector.

We have used two approaches to overcome these problems: (i) to utilise a specific functional group present in the molecule (*e.g.* the carbonyl group) for infrared detection; and (ii) to develop a system that would enable the UV–visible detector to be used for lipid analysis.

Infrared detection

A detection system based on spectroscopy in the infra-red region of the spectrum has the advantage that it can be used as a universal detector, monitoring the C–H frequency at 3.40 μm, or by choosing a wavelength corresponding to a particular atomic grouping, *e.g.*, the C=O frequency at 5.7 μm, the detector may be used in a specific mode.

The two major problems associated with the infrared detector are: (i) the lack of "spectral windows" in the spectrum of the mobile phase, *i.e.* regions in the spectrum where the transmission is greater than 30%. This not only limits the choice of mobile phase but also the path-length of the detector cell, *e.g.* acetonitrile in a 1-mm cell has a spectral window from 6–8 μm and from 8.5–11 μm, but with a 3-mm cell, both of these regions are spectrally opaque. Thus, the choice of path-length is a compromise between spectral transmission and sensitivity; (ii) the change in absorbance of the mobile phase during gradient elution. This leads to base-line shifts, the size of such shifts depending on the extent of compositional changes and on the absorbance characteristics of the mobile phase, the optical path-length and the detector sensitivity. This problem can be overcome by absorbance matching, *i.e.* the blending of solvents to give mobile-phase pairs which have a similar absorbance but different solvent strengths for use in gradient elution. This can be a long and tedious process unless a microprocessor-controlled mobile-phase system is used for optimisation. The use of the infrared detector for the analysis of various oils and waxes has already been demonstrated[7].

Derivatisation of lipids

Iron(III) hydroxamates have been used successfully for the determination of esters, anhydrides, etc.[8]. The reaction is a two-stage process:

(i) The reaction of a carboxyl function with hydroxylamine under alkaline conditions to form a hydroxamic acid.

$$\underset{\text{R–C–OR}'}{\overset{\text{O}}{\overset{\|}{}}} + NH_2OH \rightarrow \underset{\text{R–C–NH–OH}}{\overset{\text{O}}{\overset{\|}{}}} + R'OH$$
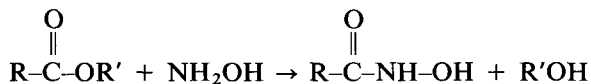
(ii) The formation of a coloured complex with a strong absorption between 460 and 530 nm, by reaction of the hydroxamic acid with an iron(III) salt.
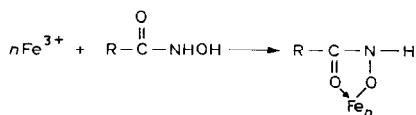

where $n = 1$–3.

The reaction, whilst being useful for some lipids, does not, for instance, work for fatty acids.

It has been shown[9–11] that carboxylic acids, esters, amides, lactones, imides, lactams, anhydrides, thiol esters, glycerides, aldehydes and penicillin type compounds undergo hydroxamic acid formation when in the presence of Ni^{2+}. The reaction was shown by Jencks[12,13] to be a two-step process, the first reaction giving an O-acyl hydroxylamine which then reacts with hydroxylamine to yield the hydroxamic acid:

$$RCOOR' + NH_2OH \rightarrow RCOONH_2 + R'OH$$

$$RCOONH_2 \xrightarrow{\ NH_2OH\ } RCONHOH \ \rightarrow$$
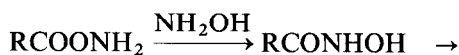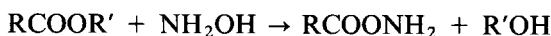
It has been proposed[11] that the Ni^{2+} catalyses the conversion of O-acyl hydroxylamine to acyl hydroxamic acid through a five-membered chelate as below.
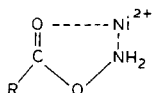
This chelation will polarise the carboxyl function, rendering attack by hydroxylamine at the acyl carbon. The hypothesis that chelation by Ni^{2+} is responsible for catalysis of hydroxamic acid formation leads to the view that other carboxylic acid derivatives of suitable structure, as II, where Y is a group capable of coordination to a metal ion, might be liable to nucleophilic attack upon chelation with Ni^{2+}.
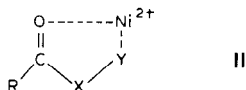
EXPERIMENTAL

Infrared

An SP8000 liquid chromatograph (Spectra-Physics, St. Albans, U.K.) with an infrared detector (DuPont, Stevenage, U.K.) and a Model R400 refractive-index detector (Millipore, Harrow, U.K.) were used. The infrared detector was operated at 5.75 μm, range 10, attenuation 0.1 A, slit width 1.0 cm and cell length 1.5 mm. The mobile phases were mixtures of acetonitrile (HPLC grade, BDH, Poole, U.K.), tet-

rahydrofuran (HPLC grade, Fisons, Loughborough, U.K.), n-hexane, n-heptane, dichloromethane and chloroform (all analytical reagent grade, BDH).

For the synthetic lipid mixtures, two columns (20 cm × 4.6 mm I.D.) packed with 5 μm Spherisorb S5W (Phase Separations, Deeside, U.K.) were connected in series. Reversed-phase separations were carried out on 5-μm Spherisorb ODS columns (Phase Separations) (25 cm × 4.6 mm I.D.) or on 5-μm Zorbax ODS columns (25 cm × 4.6 mm I.D.) (DuPont). The neutral lipids were extracted from Babassu seed kernels using light petroleum (b.p. 40–60°C). Standard lipid mixtures were prepared, containing components representative of those commonly found in waxes. Mixture 1 contained n-eicosane, stearyl decanoate, methyl caprate, methyl undecyl ketone and trimyristin. Mixture 2 contained n-eicosane, octadecyl decanoate, methyl stearate, methyl undecyl ketone, tripalmitin, octanol, 1,2-dipalmitoyl glycerol and glycerol monostearate (all from Sigma, Poole, U.K.).

Derivatisaton

The nickel on silica catalyst was prepared[14] by adding an aqueous solution of $NiNO_3 \cdot 6H_2O$ (700 g/l) and an aqueous solution of urea (500 g/l) acidified to pH 3 with nitric acid, successively to a stirred suspension of silica (100 g/l) (Chromosorb W, Phase Separations) also at pH 3. After stirring and refluxing the mixture for 4 h it was filtered, washed and dried. The nickel hydroxide was reduced to metallic nickel by heating at 450°C in hydrogen for 24 h. This produced a catalyst containing approximately 40% nickel. The actual percentage of nickel was determined volumetrically by complexation of the nickel with a known amount of potassium cyanide in ammoniacal solution followed by back-titration of the excess cyanide with standard silver nitrate solution.

Fig. 1 shows the arrangement of the reactor system used to evaluate the derivatisation reaction. The catalyst was dry packed into a 2 m × 1/16 in. I.D. stainless-steel tube with 2 μm fritted disks at either end. A Swagelock T-piece was drilled-out to form a low dead-volume mixer, and 4 m × 1/16 in. O.D. × 0.02 in. I.D. PTFE tubing formed the reaction coil. The catalyst bed, mixer and reaction coil were thermostatted in a gas chromatograph oven. The hydroxylamine hydrochloride solution (0.075 *M*) and the aqueous iron(III) chloride (0.01 *M*) were both pumped with LC-XP pumps (Pye-Unicam, Cambridge, U.K.). The lipid samples were injected
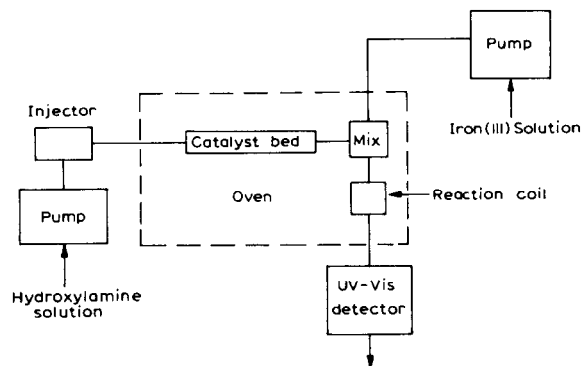

Fig. 1. Nickel catalyst reactor system.

into the flowing hydroxylamine hydrochloride solution using a Rheodyne Model 7125 injector (Rheodyne, Berkeley, U.S.A.) with a 20-μl sample loop. After passing through the catalyst bed the hydroxamic acid formed was mixed with the iron(III) chloride solution and the iron(III) hydroxamates produced in the reaction coil were detected with a Spectra-Physics Model 770 UV–visible detector at 460 or 530 nm.

RESULTS AND DISCUSSION

Infrared detection

Fig. 2 shows the separation of a synthetic mixture of common wax components on a Spherisorb S5W column with a binary gradient of *n*-hexane and tetrahydrofuran (THF). The infrared absorption of the mobile phase increases as the percentage THF increases (gradient profile given in Table I) giving rise to a large shift in base-line. Using the technique of absorbance matching and a ternary gradient with acetonitrile replacing much of the THF (gradient profile given in Table II) the base-line shift can be limited to 0.03 absorbance units (Fig. 3). The detection limit for tripalmitin was 1.2 μg injected on-column ($5 \cdot 10^{-5}$ g ml^{-1}) and the response was linear over a concentration range of 10^2 (a longer range was not tested). Other lipid molecules gave a similar response. This detection limit is slightly higher than the usual quoted value of 10^{-6} g ml^{-1} for the infrared detector. For quantitative analysis the molar response factors are required. These are given in Table III for mixture 1. Also given in Table III are the maximum wavelengths for the $C=O$ absorption and the absorp-
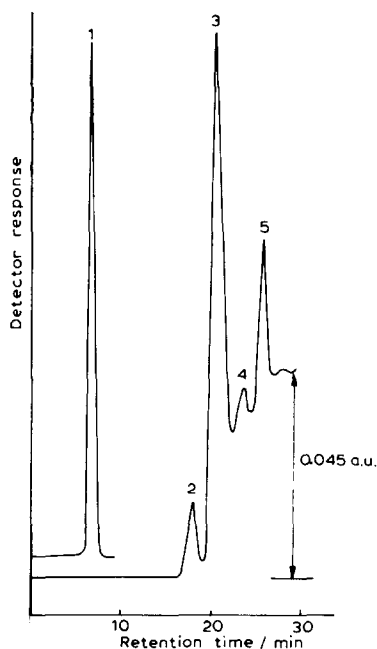

Fig. 2. Lipid mixture 1 eluted from silica with a binary gradient. Column, 5 μm Spherisorb S5W; detector, infrared; gradient, see Table I; flow-rate, 1.0 ml min^{-1}; temperature, 40°C. Peaks: 1 = *n*-eicosane (refractometer); 2 = octadecyl decanoate; 3 = methyl caproate; 4 = methyl undecyl ketone; 5 = trimyristin.

TABLE I

GRADIENT PROFILE FOR SEPARATION OF MIXTURE 1

Time	Hexane (%)	THF (%)
0	100	0
5	100	0
24	70	30
40	70	30

TABLE II

TERNARY GRADIENT PROFILE USED TO CHROMATOGRAPH MIXTURE 2

Time (min)	Hexane + 0.05% 2-propanol (%)	THF (%)	Acetonitrile (%)
0	100	0	0
5	100	0	0
20	80	10	10
30	65	10	25
45	45	15	40
55	15	45	40
56	0	100	0
65	100	0	0

tion coefficients at that wavelength for each component. It is obvious that the choice of 5.75 μm gives a good approximation to maximum absorption for most of the components, but if maximum sensitivity for the ketone is required the monitoring wavelength would have to be changed.

Although adsorption chromatography is satisfactory for the separation of a lipid mixture into its constituent chemical types, a reversed-phase system is necessary

TABLE III

COMPONENT IDENTIFICATION AND DATA

See Fig. 2 for chromatographic conditions.

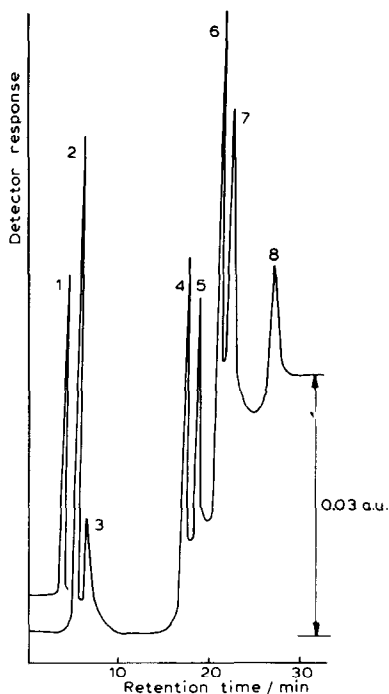| Peak No. and identification	Retention time (min)	Molar response factor	Carbonyl λ_{max} (μm)	Absorption coefficient at λ_{max}
1 n-Eicosane	7.0	—	—	—
2 Stearyl decanoate	18.0	3.27	5.76	500
3 Methyl caprate	21.0	1.00	5.75	500
4 Methyl undecyl ketone	24.0	12.80	5.82	300–500
5 Trimyristin	26.5	1.39	5.73	500

Fig. 3. Lipid mixture 2 eluted from silica with a ternary gradient. Column, 5 μm Spherisorb S5W; detector, infrared; gradient, see Table II; flow-rate, 1.5 ml min^{-1}; temperature, 40°C. Peaks: 1 = n-eicosane (refractometer); 2 = octadecyl decanoate; 3 = methyl stearate; 4 = methyl undecyl ketone; 5 = tripalmitin; 6 = octaldehyde; 7 = 1,2-dipalmitoyl glycerol; 8 = glycerol monostearate.

for the resolution of compounds within a given class. The mobile phases normally used in reversed-phase chromatography lack the necessary spectroscopic windows however[15].

HPLC with an infrared detector has been used by Hamilton and Sewell for the separation of individual chemical classes in seed fats and oils and lipolysis mixtures[9].

Fig. 4 shows the separation of the neutral lipid fraction from Babassu oil into the mono, 1,2- and 1,3-diglycerides and triglyceride fractions by stepwise gradient elution (Table IV) with an n-heptane–chloroform–acetonitrile mobile phase on a 5-μm Spherisorb S5W column (25 cm × 4.6 mm I.D.) with infrared detection at 5.75 μm. No attempt was made to use absorbance matching as can be seen by the large base-line shift when the mobile phase was changed for the elution of the 1,2-diglycerides. The triglyceride fraction was collected and analysed in a reversed-phase system on a Zorbax ODS column with an acetonitrile–dichloromethane (3:2) mobile phase at 1.0 ml min^{-1} and infrared detection at 5.75 μm (Fig. 5). The retention time of the C_{54} triglyceride was 27.5 min. For comparison, the same triglyceride fraction was analysed by GLC on a 3% OV-17 column (0.5 m × 4 mm I.D.), temperature programmed from 240 to 360°C at 6°C min^{-1}. The sensitivity and resolution was better using GLC but the analysis time was similar. The analysis time in the HPLC method could be reduced by using gradient elution and Fig. 6 shows the separation of a
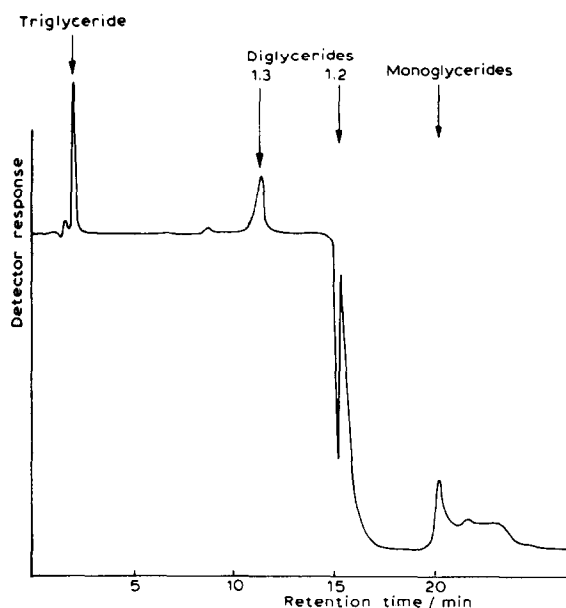
Fig. 4. Separation of neutral lipid fraction from babassu oil. Column, 5 μm Spherisorb S5W; detector, infrared; gradient, stepwise (see Table IV).

triglyceride standard mixture on a Zorbax ODS column with the gradient profile given in Table V. The C_6 and C_{12} triglycerides overlapped under these conditions but the retention time of the C_{54} triglyceride was reduced to 13 min.

Thus, the infrared detector can be used with HPLC for the analysis of lipids but it lacks sensitivity compared to other detection systems (e.g. UV or fluorescence spectroscopy) and for this reason a novel system was developed which would allow the use of the UV–visible detector for the analysis of lipid mixtures.

Derivatisation of lipids

The reaction in solution between a range of carboxylic acids, ranging from acetic to stearic acid, hydroxylamine hydrochloride and nickel chloride at pH 6.5

TABLE IV

STEPWISE GRADIENT PROFILE FOR SEPARATION OF NEUTRAL LIPIDS FROM BABASSU OIL

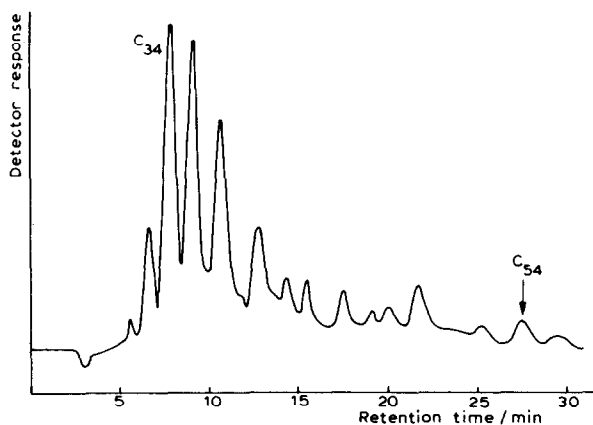| Time (min)	Hexane (%)	Chloroform (%)	Acetonitrile (%)
0.0	70	30	0
14.0	70	30	0
14.1	0	90	10
32.0	0	90	10
32.1	70	30	0

Fig. 5. Triglyceride fraction from babassu oil. Column, 5 μm Zorbax ODS; detector, infrared; mobile phase, acetonitrile–dichloromethane (3:2); flow-rate, 1.0 ml min^{-1}.
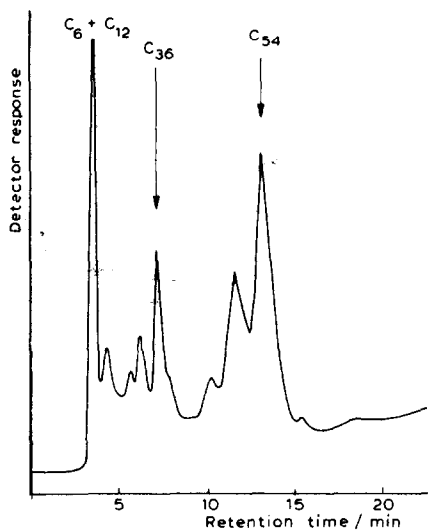

Fig. 6. Separation of a triglyceride standard mixture with a binary gradient. Column, 5 μm Zorbax ODS; detector, infrared; gradient, see Table V, flow-rate, 1.0 ml min^{-1}.

TABLE V

GRADIENT PROFILE FOR TRIGLYCERIDE STANDARD MIXTURE.

Time	Acetonitrile (%)	THF–dichloromethane (52:48) (%)
0.0	60	40
5.0	60	40
20.0	40	60

was studied. To increase the solubility of the analyte, it was found necessary to use methanol–water (1:1) with the addition of a surfactant as the reacting medium. Under these conditions, hydroxamic acid was formed but at very low levels.

Using the reactor system described earlier the reaction was studied in the presence of the silica-supported nickel catalyst. Those parameters normally associated with catalytic reactors were investigated; reactant pH, reaction time, temperature and particle size of the catalyst support. The reaction time was investigated by changing the flow-rate of the hydroxylamine solution and also by changing the reactor length. Changing the reactor length by a factor of 2 yielded an increase by a factor of 24 in the amount of hydroxamate produced. That this increase was not solely due to a change in the reaction time is shown by a comparison of the magnitude of change produced by altering the flow-rate (Fig. 7). An increase in the available catalyst surface could account for the difference, a change in mesh size from 100–120 to 30–60 gave an increase of conversion by a factor of 1.6. The effect of pH and temperature on the reaction was very marked (Fig. 8), a temperature of 55°C and a pH of 6.5 giving maximum conversion.

Thus, the optimum conditions were: temperature, 55°C; reagent, 0.075 M hydroxylamine hydrochloride at pH 6.0; and flow-rate, 0.8 ml min^{-1}.

In order to confirm the identity of the hydroxamic acid produced, samples were collected and the UV–visible spectrum obtained. The visible spectrum showed two absorption maxima at 530 nm and at 460 nm. This spectrum was compared with those, obtained from a pure sample of acetohydroxamic acid in iron(III) chloride (0.1 M) when two peaks were again obtained at 530 and 460 nm, and with that obtained with pure acetohydroxamic acid in iron(III) chloride (0.1 M) + 1% acetic acid when only one peak was observed at 530 nm. It has been shown[17] that different ligand/metal ratios give different absorption maxima, the ratio being controlled by the pH of the reaction media. When a 1:1 or 2:1 complex (iron:acid) is present, only one maximum occurs, but when a 3:1 complex is present (at neutral pH), two absorption maxima occur at approximately 480 and 530 nm.

The reaction system was characterised with acetic acid in an aqueous reaction medium. With longer-chain acids the analyte solubility becomes a problem and an organic solvent must be added to increase solubility. Bearing in mind that it was hoped to chromatograph the iron(III) hydroxamates using reversed-phase chro-
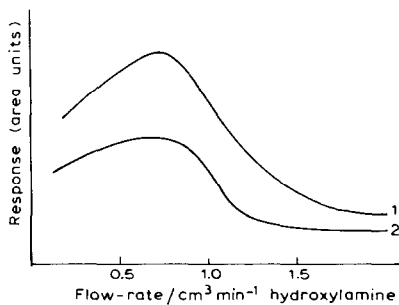

Fig. 7. Variation of detector response with hydroxylamine flow-rate. Reactor: 2 m nickel on silica catalyst at 70°C. Reactant: hydroxylamine (0.1 M) at pH 6. 1 = 460 nm; 2 = 530 nm.
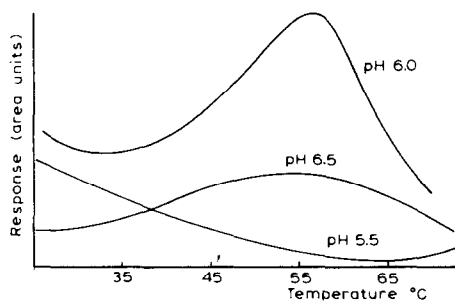
Fig. 8. Variation of detector response at 460 nm for different reactor temperatures and pH values.

TABLE VI

EFFECT OF ORGANIC SOLVENT ON FORMATION OF IRON(III) HYDROXAMATES

Reactant media: 0.1 M hydroxylamine hydrochloride.

Methanol		THF	
Amount (%)	Normalised* detector response	Amount (%)	Normalised* detector response
0	1.00	0	1.00
25	1.04	25	1.10
50	0.76	50	0.80
75	0.67	75	0.80
100	0.39	100	0.70

* Detector response relative to that from the reaction in the reaction media alone.

matography in a continuous-flow system the effect of methanol and THF on the reaction efficiency was studied. Table VI shows the normalised detector response for various percentages of methanol or THF added to the reaction media (0.1 M hydroxylamine hydrochloride). Whilst an increase in organic content reduces the overall efficiency of the reaction, particularly with methanol, THF can still be used at the 75% level and give an 80% reaction efficiency (compared to using 100% aqueous reaction medium). Addition of a surfactant to the analyte (methylmyristate) in THF–water (1:1) reaction medium was found to increase the reaction yield by 57%. However, it is not clear whether this is attributable to the increased solubility of the analyte or a "wetting" of the catalyst surface allowing better reaction.

The long-term stability of the catalytic reactor was studied and a slow conversion back to the nickel oxide was observed after three months of operation (representing some 400–500 injections), a change which would not create a serious problem. For replicate injections the relative standard deviation was 4.5% and on a day-to-day basis it was 4.6%. The response (for acetic acid) was linear over a concentration range of 10^3 (a larger range was not studied).

Thus, it was shown that the catalytic conversion of molecules containing a carbonyl function to form the hydroxamic acid and the formation by reaction with iron(III) chloride of the iron(III) hydroxamate and its visualisation at 460 nm or 530 nm is suitable for the detection of these compounds.

In order to get more information about the distribution of compounds within a given class of lipids a separation by carbon number in a reversed-phase system would be required. Therefore, the chromatography of the hydroxamic acids was fully investigated. A series of aliphatic hydroxamic acids with carbon chainlengths from C_2 to C_{22} was prepared and subjected to reversed-phase HPLC on a 5-μm Spherisorb ODS I column at 40°C with methanol–water mixtures as mobile phase and UV detection at 210 nm. Whilst some separation was achieved, the peaks tailed badly (asymmetry factor, A_s, ca. 0.3) and reproducibility was poor. The use of a fully "capped" stationary phase, of ion-pairing agents, of buffer solutions and combinations of all three failed to produce reproducible, symmetrical peaks. It was suspected
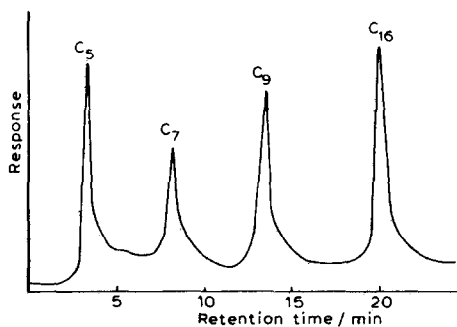
Fig. 9. Separation of C_5, C_7, C_9 and C_{16} hydroxamic acids (as chelates). Column, 5 μm Spherisorb ODS I; detector, UV–Visible at 530 nm. Mobile phases, (A) iron(III) chloride (0.01 M)/1% acetic acid in water (B) iron(III) chloride (0.01 M)/1% acetic acid in methanol. Gradient, A to B in 40 min at 1.0 ml min^{-1}; temperature, 35°C.

that the poor results were due to chemisorption. Similar problems have been reported by Lochmüller and Hanagac[18] in ligand-exchange chromatography when an immobilised metal was used in the stationary phase. It was attributed to slow desorption kinetics. The interaction between the hydroxamic acids and residual Fe^{3+} in the silica could lead to the formation of a stable chelate with poor chromatographic properties.
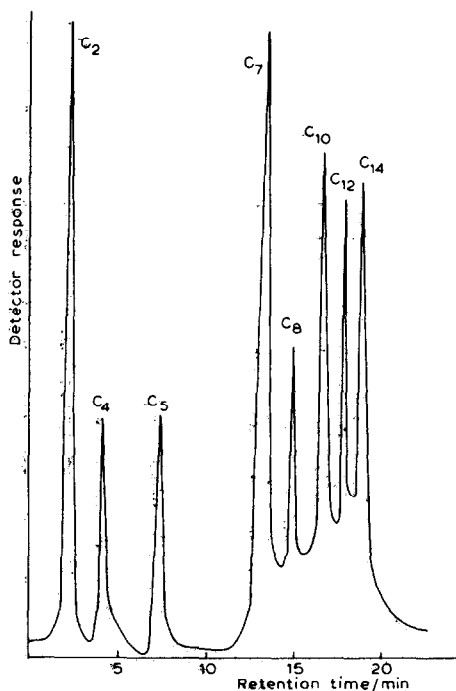

Fig. 10. Separation of C_2, C_4, C_5, C_7, C_8, C_{10}, C_{12} and C_{14} hydroxamic acids (as chelates). Column, 5 μm Spherisorb ODS I; detector, UV–Visible at 530 nm. Mobile phases, (A) iron(III) chloride (0.01 M)/1% acetic acid in water (B) iron(III) chloride (0.01 M)/1% acetic acid in methanol. Gradient, A to B in 40 min at 1.0 ml min^{-1}; temperature, 40°C.
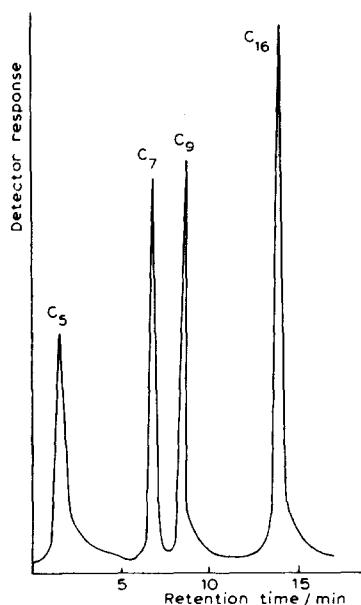
Fig. 11. Separation of C_5, C_7, C_9 and C_{16} hydroxamic acids (as chelates) in presence of a surfactant. Conditions as in Fig. 9, with the addition of 0.001 M methylsulphonic acid to the mobile phase.

Therefore, iron(III) chloride (0.01 M) was added to the mobile phase and gave results which support the postulate. Resolution of a series of hydroxamic acids was obtained by gradient elution with a water–methanol–iron(III) chloride (aq.) mobile phase to which 1% acetic acid was added to keep the pH below 3.5, since it was found that resolution was lost above pH 3.5 (see Figs. 9 and 10 for the gradient used). The addition of an ion-pairing agent (0.001 M methylsulphonic acid) was found to improve both peak shape and resolution still further, presumably by stabilising the iron(III) hydroxamate chelate (Fig. 11). The reproducibility of the system was determined by replicate analyses and measuring the standard deviation of the retention time (δ_{RT}) and peak area (δ_A) shown in Table VII for the n-C_4, C_8, C_{10}, C_{12} and C_{14}

TABLE VII

REPRODUCIBILITY DATA FOR A MIXTURE OF HYDROXAMIC ACIDS

A_S = asymmetry factor. δ_{RT} and δ_{PA} = standard deviation of the retention time and peak area. N = number of theoretical plates for each peak.

Hydroxamic acid	A_S	t_R (min)	δ_{RT}	δ_{PA}	N
C_4	0.8	6.4	3.0	0.4	12 750
C_8	0.88	13.4	4.0	1.1	6118
C_{10}	0.88	37.6	2.7	2.4	4113
C_{12}	0.9	40.4	2.1	3.8	3522
C_{14}	0.6	41.4	1.0	1.6	3124

hydroxamic acids. The asymmetry factors (A_s) for these peaks are acceptable (except for C_{14} which tails badly) as are the number of theoretical plates (N) calculated for each peak, bearing in mind the complex nature of the retention mechanism involved. The detection limit for the system is approximately 0.5 ng on-column $(2 \cdot 10^{-8}$ g ml$^{-1})$ for a typical hydroxamic acid.

CONCLUSIONS

It has been shown that infrared detection of lipid-type molecules can be used in both normal-phase and reversed-phase chromatography but the detection limit is relatively high (*ca.* 1 μg for a typical lipid molecule).

The use of a solid phase catalyst for the conversion of "lipids" into their corresponding hydroxamic acids and their subsequent separation as the iron(III) hydroxamates formed by on-column derivatisation is also demonstrated. This method gives higher sensitivity (*ca.* 0.5 ng) with high specificity and should be useful for lipid mixtures as well as for hydroxamic acids.

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REFERENCES

1 S. G. Perry, R. Amos and I. Brewer, *Practical Liquid Chromatography*, Plenum Press, New York, 1972.
2 A. P. Tulloch, *J. Chromatogr. Sci.*, 13 (1975) 403.
3 A. P. Tulloch, *Cosmetics Perfumery*, 89 (1974) 53.
4 H. K. Lichtenthaler, *Lipids and Technical Lipid Derivatives, CRC Handbook of Chromatography*, CRC Press, Florida, 1979.
5 E. W. Hammond, *J. Chromatogr.*, 203 (1981) 397.
6 K. Aitzetmüller, *J. Chromatogr.*, 79 (1973) 329.
7 K. S. J. Atkin, R. J. Hamilton, S. F. Mitchell and P. A. Sewell, *Chromatographia*, 15(2) (1982) 97.
8 R. F. Goddu, N. F. LeBlanc and C. M. Wright, *Anal. Chem.*, 27 (1955) 1251.
9 J. W. Munson, D. Papadimitriou and P. P. Deluca, *J. Pharm. Sci.*, 68 (10) (1979) 1333.
10 K. A. Connors and J. W. Munson, *Anal. Chem.*, 44(2) (1972) 336.
11 K. A. Connors and J. W. Munson, *J. Pharm. Sci.*, 61(2) (1972) 211.
12 W. P. Jencks, *J. Amer. Chem. Soc.*, 80 (1958) 4581.
13 W. P. Jencks, *J. Amer. Chem. Soc.*, 80 ((1958) 4585.
14 P. A. Sewell and L. R. Hindley, *A Study of Silica Supported Nickel Catalysts, Project Report*, Liverpool Polytechnic, Liverpool, 1969.
15 *Infra-Red Transmission of Solvents, Applications Report No. 2*, Wilks Scientific Corporation, 1976.
16 A. Bhati, M. Benbouzid, R. J. Hamilton and P. A. Sewell, *Chem. Ind.*, 10 (1986) 70.
17 S. Mizukami and K. Nagata, *Coord. Chem. Rev.*, 3 (1968) 267.
18 C. H. Lochmüller and H. H. Hanagac, *J. Chromatogr. Sci.*, 20 (1982) 171.