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Note

Separation and analysis of acetylthiophene isomers

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Acetylthiophenes are used as intermediates in organic and pharmaceutical syntheses. For some applications 2-acetylthiophene is required essentially free of the 3-acetyl isomer.

Chromatographic methods were investigated with the aim of developing a method capable of detecting 1% or less of 3-acetylthiophene in the 2-acetyl isomer. No specific separations of these two compounds have been reported previously.

Gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) were used. An HPLC method using the polar bonded phase Spherisorb nitrile was selected as being the most applicable.

EXPERIMENTAL

Gas chromatography

A Perkin-Elmer Model F-11 gas chromatograph fitted with a single flame-ionisation detector was used. Nitrogen was used as the carrier gas and the inlet system was provided with a sample splitter.

Perkin-Elmer stainless-steel support-coated open-tubular (SCOT) and wall-coated open-tubular (WCOT) columns were used. The stationary phases studied and the chromatographic conditions employed are summarised in Table I.

TABLE I

COLUMNS AND CONDITIONS FOR GLC OF ACETYLTHIOPHENES

Column type	*Stationary phase*	*Length and bore*	*Nitrogen pressure (p.s.i.)*	*Nitrogen flow-rate (ml/min)*	*Temperature (°C)*	*Retention time (min)*	
						3-Isomer	*2-Isomer*
SCOT	Apiezon-L	50 ft. × 0.02 in.	15	6.9	86	22.2	23.4
	Diethylene glycol succinate	50 ft. × 0.02 in.	15	5.7	86	46.6	47.8
	Carbowax 20M	50 ft. × 0.02 in.	10	4.1	100	36.8	38.5
WCOT	UCON, LB-550X	100 m × 0.01 in.	60	4.0	112	41.8	42.7
	Tris(β-cyanoethoxy)-propane	50 m × 0.01 in.	60	6.0	86	36.0	39.5

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Liquid chromatography

A Waters Assoc. 6000A liquid chromatography pump was used with a Cecil 272 variable-wavelength UV spectrophotometric detector, set at 244 nm, an absorption maximum for 3-acetylthiophene. It was fitted with a 10-μl flow-cell. For quantitative analyses a Columbia Scientific Industries Supergrator computing integrator was employed.

Stainless-steel columns (100 and 150 mm length, 5 mm I.D.) were used with an on-column, needle-through-septum injection system as described by Bristow[1]. Injections were made into a layer of glass beads separated from the column packing by a stainless-steel mesh disc. Flow was interrupted during injection, unless stated otherwise. Care was taken to preserve separation efficiency by the use of the minimum length of microbore tubing (800 × 0.15 mm I.D.) and minimum dead-volume connections between the column end and the detector flow-cell.

The following column packings were used: Spherisorb 5SW (Phase Separations, Queensferry, Great Britain), Spherisorb nitrile (hetp, Wilmslow, Great Britain) and ODS Hypersil (Shandon Southern, Camberley, Great Britain). All were porous microspheres of nominal 5-μm diameter.

Slurry-packing techniques were employed[2]. Quantities (2 or 2.5 g) of packing (for 100 or 150 mm columns, respectively) were dispersed in 10 ml of support medium using an ultrasonic bath. Support media were methyl iodide–methanol (85:15) for the Spherisorb and Spherisorb nitrile, and methanol–0.1% (w/v) aqueous sodium acetate solution (80:20) for the reversed-phase ODS Hypersil packing.

After dispersal the slurries were shaken and quickly poured into a 400 × 5 mm I.D. filling column attached to the top of the analytical column with a bored-out union. The Waters Assoc. pump was connected to the top of the filling column and the slurry driven into the analytical column with methanol, or with a mixture of methanol–0.1% aqueous sodium acetate solution (50:50) for the ODS Hypersil, at the maximum flow-rate (9.9 ml/min). Pumping was maintained for about 30 min, the flow-rate was dropped as required to keep a backpressure of between 4000 and 5000 p.s.i.

The Spherisorb nitrile packing was found to need a much higher flow-rate than the other materials, to give a well packed column. This was achieved by coupling the maximum flow from each of two Waters Assoc. pumps without solvent programmer control through a tee-piece to the top of the filling column. A combined flow of 19.8 ml/min was obtained in this way.

When packed, the columns were left undisturbed for 10 min then fitted with mesh discs and injection systems. After conditioning with eluent flowing at 1 ml/min for 1 h they were tested using test solutes suggested by Bristow and Knox[3]. The results obtained for the columns are summarised in Table II.

Chemicals

All eluents used were of HPLC grade. Hexane and 1-chlorobutane were from Fisons (Loughborough, Great Britain); methanol and dichloromethane were from Rathburn Chemicals (Walkerburn, Great Britain).

Samples of 2-acetylthiophene were obtained from Croda Synthetic Chemicals (Oldbury, Warley, Great Britain). 3-Acetylthiophene was obtained from Cilag-Chemie (Schaffhausen, Switzerland) and had a melting point of 57–59° (ref. 4: 57°). Acetothienone was obtained from Hopkin & Williams (Chadwick Heath, Great Britain)

TABLE II

TEST RESULTS FOR LIQUID CHROMATOGRAPHY COLUMNS

N = Number of theoretical plates; h = plate height.

Column packing	*Effective length (mm)*	*Eluent (at 1 ml/min)*	*Solute*	*k'*	*N (theoretical plates)*	*h (μm)*
Spherisorb S5W	145	Hexane + 0.5% methanol	Nitrobenzene	1.53	13000	11.2
			Acetophenone	2.43	8270	17.5
Spherisorb nitrile	95	Hexane + 10% methylene chloride	Nitrobenzene	0.90	7260	13.1
			Acetophenone	3.80	6125	15.5
ODS-Hypersil	95	60% Methanol + 40% water	Cresol	0.99	9430	10.1
			Anisole	2.25	8210	11.6
			Phenetole	4.04	8100	11.8

RESULTS AND DISCUSSION

Gas chromatography

The 2- and 3-acetylthiophene isomers have different physical forms; 2-acetylthiophene is a liquid and 3-acetylthiophene a crystalline solid. However, their reported boiling points[4,5] are very close together (2-acetyl-, 213.5°/760 mm Hg and 3-acetyl-, 208–210°/748 mm Hg). Separation by GLC seemed likely to be difficult in view of this and the similar functionality of the compounds.

The SCOT and WCOT columns listed in Table I were studied. Column temperatures were adjusted to give retention times of between 20 and 40 min with the carrier gas inlet pressures and flow-rates as shown in the table.

Not surprisingly, no separation was obtained on the non-polar Apiezon-L stationary phase. More polar stationary phases gave varying degrees of separation, the best being obtained with a WCOT tris(β-cyanoethoxy)propane column. Peak tailing prevented complete resolution of a mixture of equal amounts of the two isomers, but as 3-acetylthiophene was eluted first this was less of a problem when mixtures containing only minor amounts of the 3-isomer were separated. Fig. 1 shows the chromatogram obtained from technical grade 2-acetylthiophene which contained slightly over 1% of the 3-acetyl isomer.

This separation could have been developed as a quantitative analysis, but total elution of the components of the mixture took more than 50 min. HPLC was expected to provide a more rapid analysis.

Liquid chromatography

Development of separation. Absorbtion chromatography on silica is usually recognised as the technique most likely to separate mixtures of compounds having the same chemical functionality which are positional isomers, such as the acetylthiophenes.

Attempts were made to separate the acetylthiophene isomers on the 5-μm Spherisorb silica column packing using hexane as the major eluent. Injections of 5 μl of a solution containing 0.1 mg/ml of each of the isomers in hexane were used.

When such silica adsorbents are used in HPLC, it is common practice to add a small quantity, usually 0.5%, of methanol or acetonitrile to dry non-polar eluents such as hexane to avoid the necessity of equilibrating eluents and the column packing

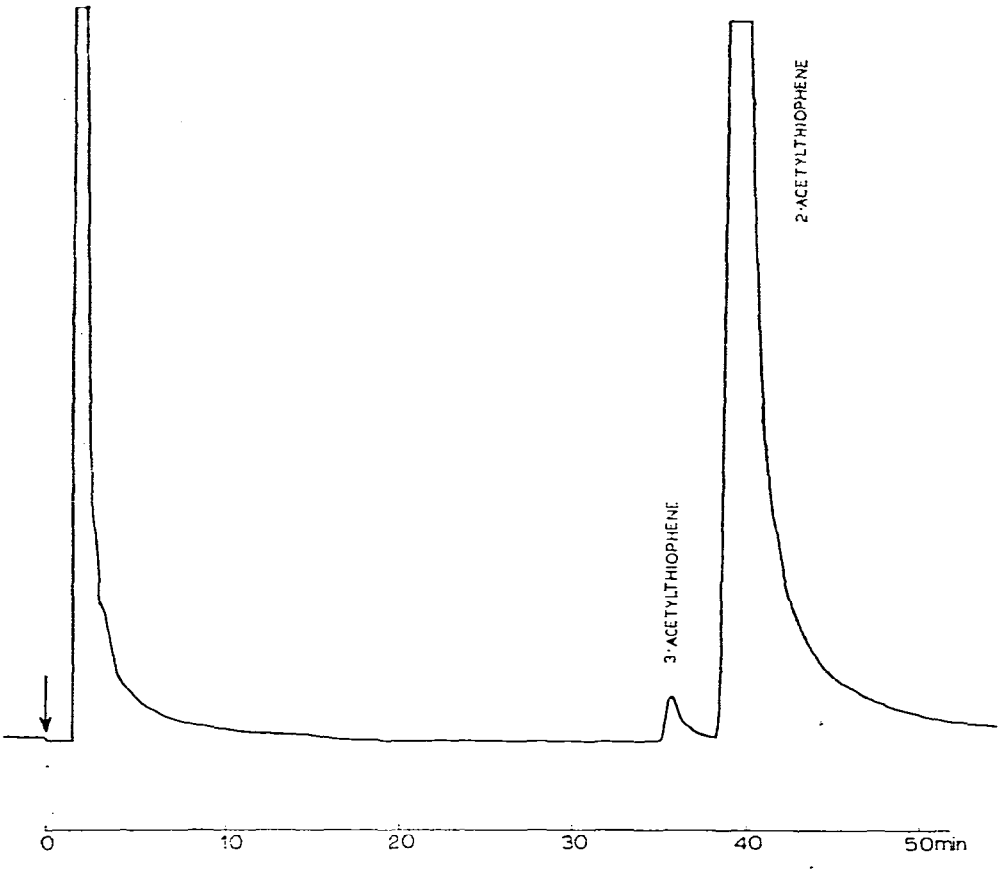

Fig. 1. Chromatogram showing the GLC separation of 1.4% 3-acetylthiophene in 2-acetylthiophene. Injection, 1 μl (1% in ether). Column, 50-m WCOT tris(β-cyanoethoxy)propane, 60 p.s.i. (6 ml/min) nitrogen, 86°, attenuation 4 × 10.

to standard degrees of water-saturation and activity, respectively. (This procedure had been adopted for the testing of the column's efficiency with nitrobenzene and acetophenone solutes.) However, it was found that the presence of this amount of methanol in the hexane was sufficient to remove the selectivity of the silica for the acetylthiophenes. Both isomers were eluted with the same retention time. Reduction of the methanol content to 0.1% allowed incomplete resolution.

A reversal of the order of elution found on GLC had occurred. The latter peak was caused by 3-acetylthiophene. Very good resolution was therefore required for this analysis by HPLC since the 3-acetylthiophene minor component would be found as a small peak on the tail of the larger peak from the 2-acetylthiophene.

Following the failure of this quick method of controlling silica activity, optimisation of the separation on silica would require standardised water-saturated elu ents and a careful control of the degree of activation of the silica after the methods of for example, Kirkland[6] or Thomas[7]. This exacting procedure was not attempted, ther kinds of column packing were examined instead.

Injections of a solution containing the acetylthiophene isomers in 50% aqueous methanol were made onto the ODS Hypersil reversed-phase column. They were eluted with various mixtures of methanol and water at a flow-rate of 1 ml/min. No separation was observed until at 10% methanol–90% water a shoulder appeared but no resolution of peaks took place. The elution time for the composite peak was 30 min. The use of even more dilute methanol eluents might improve this separation, but ODS Hypersil is considered unlikely to be useful for this analysis.

The Spherisorb nitrile column packing promised the best chance of success following the problems encountered with the normal silica and the poor separation obtained on the reversed-phase packing. Spherisorb nitrile is a chemically modified silica with most, if not all, of its surface hydroxyl groups "capped", removing the need for any special concern for water-content of eluents or the need for methanol addition to standardise activity. Its bonded surface layer, unlike that of ODS Hypersil has a polar character and so can be used in "normal" rather than "reversed-phase" chromatography.

The separation of the acetylthiophenes was investigated on this packing using the same solutions as for the Spherisorb silica. Mixtures of 1-chlorobutane or dichloromethane with hexane were studied as eluents.

Good separations with well shaped peaks were obtained. Complete baseline resolution was obtained with chlorobutane–hexane (20:80) and dichloromethane–hexane (5:95), at a flow-rate of 1.5 ml/min. The analysis time was reduced to 10 min by increasing the flow-rate to 2 ml/min. Good resolution was retained with the chlorobutane–hexane mixture but with dichloromethane–hexane the resolution deteriorated at the higher flow-rate. As a consequence chlorobutane–hexane (20:80) was chosen, at 2 ml/min as the preferred eluent for this analysis.

For this separation to be useful in the analysis required, the resolution of the 3-acetylthiophene had to be maintained when eluted in the presence of a 100-fold excess of the 2-acetyl isomer. An injection of 10 μg of technical-grade 2-acetylthiophen overloaded the column with respect to the resolution of the minor peak. The resolution was restored when the loading was reduced to 2 μg. Fig. 2 shows the chromatogram obtained. Retention times were: acetophenone (internal standard), 5.21 min, 2-acetylthiophene, 7.70 min and 3-acetylthiophene, 9.17 min.

Quantitative analysis. The calibration of the detector response for 3-acetylthiophene was carried out in the presence of the 100-fold excess of the 2-acetyl isomer. The peak shape of the minor 3-acetyl component could be influenced by the large 2-acetylthiophene peak immediately preceeding it.

No 2-acetylthiophene free from traces of the 3-acetyl isomer was available, calibration was made by "spiking" known amounts of 3-acetylthiophene on top of that already present in the sample of technical-grade 2-acetylthiophene. Acetophenone was used as an internal standard.

Solutions in hexane were made up by diluting stock solutions to give 0.4 mg/ml 2-acetylthiophene, 0.1 mg/ml acetophenone and 0, 0.002, 0.004 and 0.008 mg/ml 3-acetylthiophene added.

The solutions were randomised and triplicate injections (5 μl) made, without interrupting the eluent flow.

Linear regression of the added quantities of 3-acetylthiophene (as x, in nanograms) against the response (as y, the ratio of the area of the 3-acetylthiophene peak

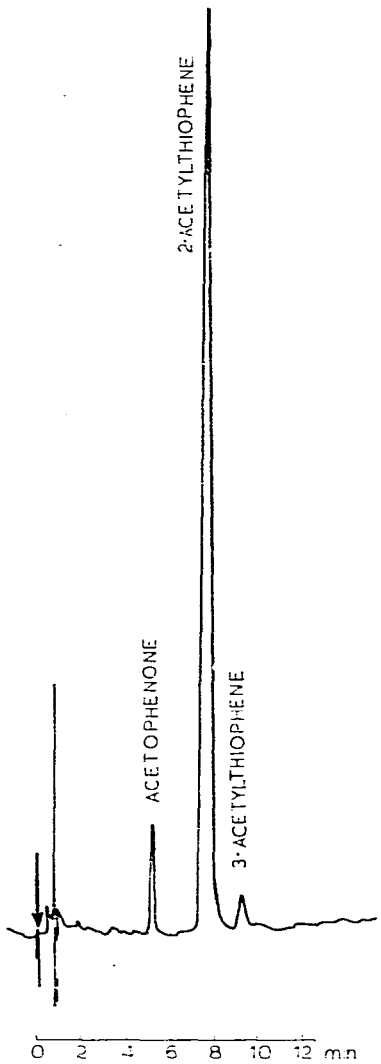

Fig. 2. Chromatogram showing the HPLC separation of 1.4% 3-acetylthiophene in 2-acetylthiophene. Acetophenone present as internal standard. Injection, 5 μl (2 μg of 2-acetylthiophene in hexane). Column, 100 × 5 mm Spherisorb nitrile, 2 ml/min 1-chlorobutane–hexane (20:80). Detector, UV at 244 nm, 0.1 a.u.f.s.

to the area of the acetophenone peak) gave: $y = 50.278x - 28.096$ (correlation coefficient = 0.9705, S.D. intercept = 1.22, S.D. slope = 1.25).

The intercept represents the concentration of the 2-acetylthiophene in the sample of technical grade 2-acetylthiophene used. This was 28.1 ng in the 2000 ng injected, *i.e.* 1.4%; 95% confidence limits were $\pm 0.04\%$.

The same calibration equation was applied to estimate the percentage of 3-acetylthiophene present in purer samples of 2-acetylthiophene, by extrapolation to below the range of concentrations studied. Levels of 3-acetylthiophene down to 0.8% ($\pm$ 0.16%) were estimated by this method, corresponding to 15 ng of 3-acetylthiophene injected.

CONCLUSIONS

The 2- and 3-acetylthiophene isomers can be separated by GLC or HPLC. 3-Acetylthiophene can be resolved in the presence of a 100-fold excess of the 2-acetyl isomer. GLC requires the use of elutions times in excess of 50 min with WCOT columns.

Analysis by HPLC on the polar bonded phase Spherisorb nitrile can be achieved in 10 min using 1-chlorobutane–hexane (20:80) as eluent. Nanogram quantities of the 3-acetylthiophene can be analysed in the presence of the excess of the 2-acetyl isomer.

ACKNOWLEDGEMENT

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