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FORENSIC ASPECTS OF HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

This paper reviews the applications of high-pressure liquid chromatography (HPLC) to forensic problems, and discusses some of the developments that have taken place in the use of the technique in the Metropolitan Police Laboratory. Preparation of octadecyltrichlorosilane-modified silica is described and some of the chromatographic characteristics of this material are investigated. Applications of HPLC to the analysis of cannabis, opium alkaloids, amphetamine-related materials, LSD and polynuclear hydrocarbons are described.

INTRODUCTION

As far as the forensic chemist is concerned there are four discrete areas of chemical analysis which are of importance, *viz.* (1) The detection and characterisation of drugs, their metabolites and, less frequently, poisons. (2) The comparative analysis of a variety of materials such as paint, glass, soil, etc., which arise as contact traces. (3) The quantitative analysis of alcohol in biological fluids. (4) The characterisation of solvents, particularly in arson debris. At the Metropolitan Police Laboratory high-pressure liquid chromatography (HPLC) is only being applied in the first two areas and in particular in the drug field. Within the two areas a great diversity of sample type and size arises. In the drug context, for example, sample size can range from kg amounts of materials (*e.g.*, large cannabis seizures, illicit drug hauls, etc.) down to μg or ng amounts of drugs if syringe washings or toxicological material require analysis. For contact traces, samples associated with a suspect (*e.g.*, material on clothing or footwear) are compared with those taken from the scene of a crime, and in the majority of cases samples are very small. The analytical challenge of forensic science is very great and could perhaps be described as being inversely proportional to sample size.

When HPLC studies first commenced at the Metropolitan Police Laboratory in 1971, it was felt essential that if the technique was to make a useful contribution it had to fulfil certain requirements, *viz.* (1) It should be applicable to problems which could not be adequately dealt with using existing techniques. (2) For many analyses it was necessary that HPLC should display high sensitivity and selectivity. (3) It should be relatively inexpensive and reliable in operation. As a consequence of the

second and third requirements it was decided to initiate research activities which were peripheral to the main applications objectives. Thus studies on the preparation of packing materials, column packing techniques, and instrument design were made in order to enable efficient columns to be prepared in the laboratory and used on equipment of minimum cost. It is our philosophy that a liquid chromatograph should, as far as is economically possible, be "dedicated" in its application. The use of isocratic solvent conditions has considerable practical and economic advantages over a gradient elution system. The problems of gradient reproducibility and re-equilibration of the column at the end of each run are serious and have been commented upon by workers attempting to use such techniques to separate complex mixtures of drugs of abuse[1]. Chromatographs with gradient elution facilities are more expensive than those which operate isocratically and, provided the latter are low in cost, it is often preferable to use two isocratic systems to achieve a desired result rather than one gradient elution system. The considerable improvements in column performance in the last few years also mean that it is often possible to achieve separations isocratically which would formerly have required gradient elution.

All the chromatographs we have in routine use (currently five instruments) operate isocratically, usually at pressures below 2000 p.s.i. By using low-priced pumps with laboratory-made injection ports and columns each analytical system need only cost a little over £1000 and most of this cost lies in the detector (the cost of the recorder has not been included).

EXPERIMENTAL

Packing materials

Although the packing materials used for particular analyses will be mentioned later, the development of HPLC packing materials is an important activity. We commenced working with commercially available pellicular materials, but have now abandoned their use in favour of microparticulates. It had been our experience that adsorption-based separations on microparticulate silica were very difficult to reproduce if non-polar solvents were used. Although the common expedient of incorporating a small proportion of a polar modifier in the solvent can help, it does not provide a complete answer to the problem. This prompted an interest in chemically modifying silica, particularly with octadecyltrichlorosilane (ODS), but it is interesting to note that events have turned full circle, for many of our best separations are now achieved on silica, although not in an adsorption mode.

Modification of silica with ODS

Numerous commercial packing materials based on the reaction of silica with ODS have been available for some years. In the case of microparticulate materials, however, it was the policy to sell only ready-packed columns and in all cases these were very expensive. We found that the reaction is not difficult to control and once microparticulate silica became commercially available it was convenient to carry out our own preparation of the ODS-modified material[2,3].

The loading of bonded organic material can be varied by simple adjustment of the reaction conditions. For example, starting with the same batch of Partisil 5 (a microparticulate silica available from Whatman Ltd., Springfield Mill, Kent.

Great Britain) which had been dried overnight at 100°, it was possible to introduce different levels of organic material by using the following general procedure:

Six grams of silica, 40 ml of solvent (sodium dried), and 5 ml of ODS were refluxed for 1 h under anhydrous conditions with magnetic stirring throughout. The reaction mixture was centrifuged and the modified silica vigorously washed by decantation with 5 × 40 ml of hexane (sodium dried). The resulting material was dried in a vacuum oven and lightly sieved before use.

The packing materials so produced had the levels of organic loading determined by ashing at 600°. The results are shown in Table I.

TABLE I

LEVELS OF ORGANIC LOADING ON ODS MODIFIED SILICA

Packing	*Solvent*	*Reaction temperature (°C)*	*Weight loss at 600° × 100 / Residue weight*
A	hexane	20 (*i.e.*, ambient)	12.0
B	hexane	69	16.8
C	isooctane	99	30.7
D	xylene	139	32.5

Higher loadings can often be obtained by first subjecting the silica to acid hydrolysis, presumably resulting in an increase in surface silanol groups.

The performance of an ODS-modified silica is very dependent upon the amount of bonded organic material and packing materials compared in dimensionally identical columns under isocratic solvent conditions display two significant characteristics: (1) The retention volume of an eluting compound undergoing a reversed-phase liquid–pseudoliquid partition process increases with increasing organic loading of the packing material (see Fig. 1 and Table II). (2) The viscous drag of the packing material increases with increasing organic loading, hence under identical conditions of flow-rate and solvent the column back pressure is highest for packings of highest organic loading.

By having the ability to bond different amounts of ODS on to silica, packing material with an ODS loading that is optimum for a particular separation can be prepared. In practice, we use two different materials for the forensically important separations developed here, *i.e.*, a loading of about 30% for separating polynuclear hydrocarbons and a loading of about 15% for cannabis separations. In the case of polynuclear hydrocarbons it is possible to achieve virtually identical chromatograms on packings of different organic level merely by altering the solvent composition and the choice of a high-loaded packing is based on the advantage of the lower pressure drop arising from the use of almost pure methanol as the solvent. In the case of cannabis, however, the separation varies with the ODS loading of the packing material and it is not possible to achieve such useful separations on higher-loaded packings even with adjustments of the solvent composition.

The separations we have studied on ODS-modified silicas lead us to believe that only occasionally can the separation mechanism be described in simple terms.

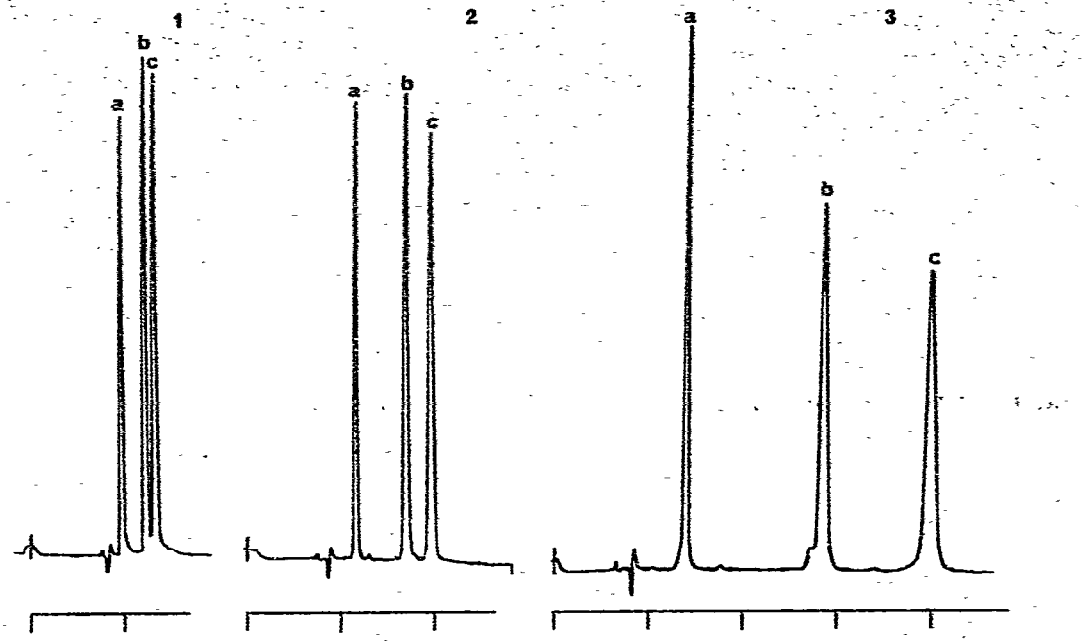


Fig. 1. Influence of ODS loading on the reversed-phase separation of polynuclear hydrocarbons on ODS-modified Partisil 5 of different ODS loadings. Column, 25 cm × 4.9 mm I.D., packed with A, B and C (see Table I), *i.e.*, 1, 2 and 3, respectively; solvent system, methanol–water (9:1); flow-rate, 1 ml/min; pressure drops, 1100, 1200 and 1400 p.s.i. for 1, 2 and 3, respectively; detector, UV at 254 nm; time scale marked in 5-min intervals. a = Biphenyl; b = pyrene; c = 1,2-benzanthracene.

TABLE II

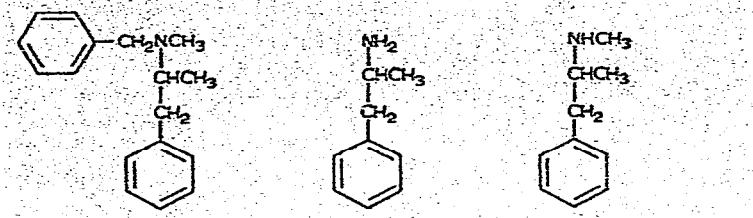
VARIATION OF THE ELUTION VOLUME OF PYRENE AS A FUNCTION OF THE SOLVENT COMPOSITION AND THE LEVEL OF ODS ON THE SILICA

The values in parentheses show the pressures in p.s.i. required to pump the solvent at 1 ml/min down a 25-cm × 4.9-mm-I.D. column packed with the ODS-modified silicas using a standard slurry-packing technique.

*Packing**	*Solvent (% methanol in water)*			
	100	*95*	*90*	*80*
A	—	—	6.0 ml (1000)	9.4 ml (1500)
B	—	6.2 ml (900)	8.4 ml (1200)	16.8 ml (1600)
C	6.8 ml (750)	9.0 ml (1100)	14.2 ml (1400)	—
D	7.4 ml (1000)	—	18.4 ml (1600)	—

* See Table I for a description of the packing materials.

Even with high loadings of ODS it is improbable that all the active sites on the silica surface are reacted and, if the molecules being separated can interact with these sites, it is probable that a mixed separation mechanism will result. Consider, for example, the separation of the three basic drugs shown below.



Benzphetamine Amphetamine Methyl amphetamine

We now know that such compounds can be separated very effectively on silica using aqueous buffer–methanol solvents. In Fig. 2 the separation on silica is compared with that obtained on the various ODS-modified silicas. Table III shows the variation in capacity factor as a function of ODS loading. It is apparent that once ODS is present it begins to have an influence on the separation, presumably because solvent–

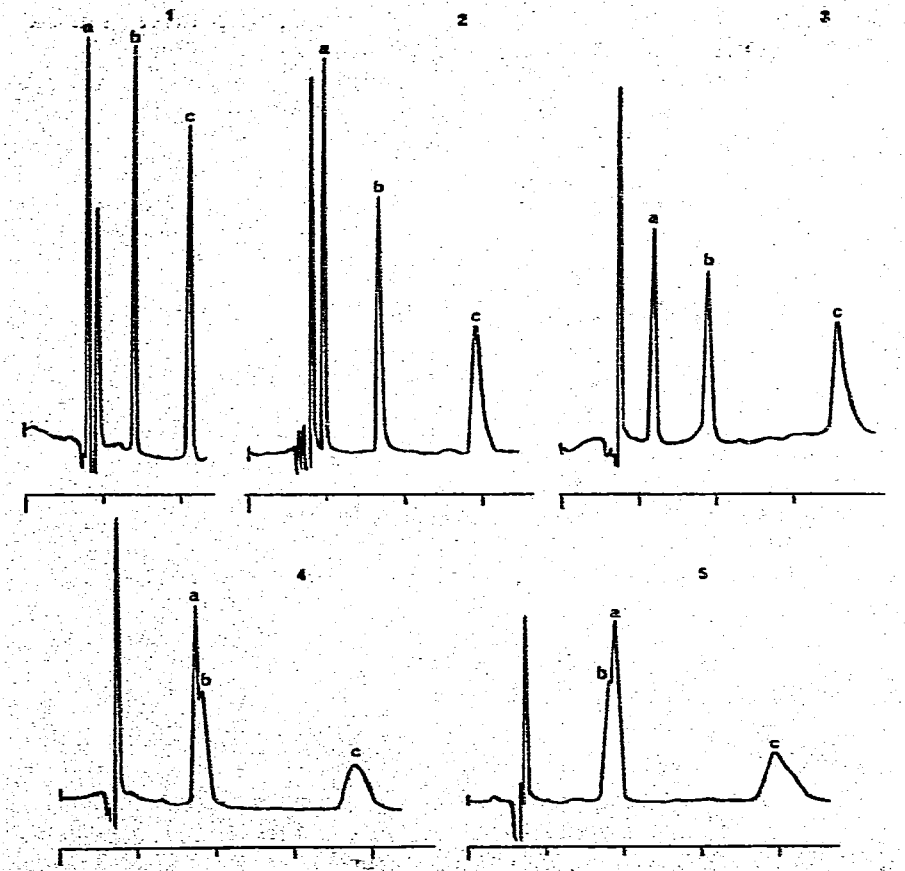


Fig. 2. Separation of basic drugs on Partisil 5 and on ODS-modified Partisil 5 of different ODS loadings. Column, 25 cm × 4.9 mm I.D., packed with Partisil and packings A, B, C, and D (see Table I), *i.e.*, 1, 2, 3, 4, and 5, respectively; solvent system, methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (27:2:1); flow-rate, 1 ml/min; pressure drops, 1000, 1000, 1100, 1300, and 1500 p.s.i. for 1, 2, 3, 4, and 5, respectively; detector, UV at 254 nm; time scale marked in 5-min intervals. a = Benzphetamine; b = amphetamine; c = methyl amphetamine.

TABLE III

VARIATION OF CAPACITY FACTOR k' AS A FUNCTION OF ODS LOADING

The columns, 25 cm × 4.9 mm I.D., with packing materials as described in Table I. Solvent system, as in Fig. 2.

Column packing	k'		
	Benzphetamine	*Amphetamine*	*Methyl amphetamine*
Silica	0	0.7	1.6
A	0.3	1.3	2.8
B	0.3	1.5	3.7
C	1.4	1.5	4.1
D	2.0	1.7	5.1

pseudosolvent partition begins to play a part. However, the effect of this process does not influence each compound in the same way and appears to be most marked in the case of benzphetamine, which is non-retained on silica and strongly retained on the ODS-modified material. This type of behaviour is perhaps indicative of the fact that several different separation mechanisms contribute to the final result. In general, because of the appreciable peak broadening which occurs if basic drugs are separated on ODS-modified packings, we feel that reversed-phase separations of such materials should be avoided. Indeed, if a separation has been achieved it is worthwhile checking to see if an improved separation would not result by using silica with the same solvent system. Studies on a commercially available ODS-modified silica also showed that the column efficiency was particularly poor for basic drugs and thereby detracted from its usefulness[4].

Vinyl modification of silica

The relatively poor performance of ODS-modified silica for the separation of basic drugs prompted me to look at other ways of modifying silica to produce chemically bonded materials of value as packings. The subsequent success of silica used with aqueous buffered solvents has to a large extent negated the value of the packing materials produced during this programme of work although the novelty of the approach will, I hope, prompt others to explore its potential. The experimental details have been reported[5].

Microparticulate silica as packing material

As pointed out earlier, there are practical problems in using silica for liquid–solid adsorption based HPLC separations. The complex structure of silica, however, is such that even when it is used under conditions where adsorption mechanisms are unlikely to occur it can still achieve separations. Jane[6] has shown that a wide variety of basic drugs can be separated on microparticulate silica using mixtures of methanol and aqueous buffer solutions. Using packed columns prepared here he has achieved some of the most efficient separations that have so far been reported in the chromatographic literature and the various applications are discussed later in this text. It has not been possible, however, to explain adequately the separation mechanism. Ion-exchange, hydrogen bonding, and perhaps exclusion processes may all contribute to the final separation, but, regardless of the mechanism, the efficiency of columns

packed with microparticulate silica (nominally of 5-μm particle size) for separating basic drugs is very high (typically 8000–16,000 theoretical plates in a 25-cm column). This means that it is often possible to separate quite complex mixtures isocratically on silica, whereas gradient elution would be required if less efficient columns were used.

Injection techniques

During the early stages of our work on microparticulate materials we often encountered apparently random variations in chromatograms. These variations were manifested in the form of "double peaks" (*i.e.*, shoulders before, or after a major peak), tailing, peak broadening, and the sudden inability of a column to achieve erstwhile satisfactory results. We now attribute these phenomena to injection problems.

As a result of much experimental work we consider that the following factors are of significance if the maximum efficiency of an analytical HPLC column is to be achieved: (1) The injection volume should be kept as small as possible. (2) The injection should be made as close to the central axis of a column as possible. (3) The injection should be made as close to the top of the packing material as possible. (4) The packing material must not be disturbed by injections. (5) The injection solvent must be as dense or denser than the eluting solvent.

To fulfil these requirements we use a modified ball valve as a stop–flow injection port[2], and the centralised injection is made on to a piece of wire mesh with 8-μm holes which is pressed into contact with the packing material. The wire provides a rigid but permeable barrier which prevents disturbance of the packing material and enables trouble-free injections to be made over a long period of time.

The injection solvent can play a critical part in HPLC. If its density is lower than that of the eluting solvent, diffusion in the solvent above the packing material can lead to band spreading. The polarity of the injecting solvent will also influence the sharpness of the band being deposited on the top of the column and it is always advisable to study the effect with the compounds to be separated, since it will vary from one type of sample to another.

Fluorimetric detection

Although the UV detector has adequate sensitivity for many HPLC analyses, the forensic scientist frequently finds that it is necessary to analyse either very small samples, or that he has to seek low levels of materials in a limited amount of sample. In such circumstances it is advantageous to have a much more sensitive detection system and the fluorimetric detector fulfils this requirement. Although several such detectors are now available commercially, this was not the case when our work commenced, so initially we adapted a commercial fluorimeter[7] and subsequently, built our own instrument[2]. It is probably worthwhile briefly mentioning some of the details because a well designed fluorimetric detector can offer a 10- to 100-fold increase in sensitivity over a UV detector, and its use is described in some of the applications mentioned later.

There are many practical advantages in using a right-angled optical configuration for fluorimetric detection and this provides the basis for most commercial spectrofluorimeters. To adapt a commercial instrument to perform as an HPLC de-

tector it is necessary to design a flowcell which can be mounted in the optical centre of the sample compartment, and to ensure that the relatively high background light levels from such a flow cell can be dealt with by the electronics of the instrument. If the path length of the flow cell and the intensity of the illuminating system are fixed, as they usually are by the optical features of the original instrument, designing a suitable cell involves establishing a compromise between fluorescence from the silica of the flow-tube, light scatter, and cell volume. This has been the subject of much empirical study at the Metropolitan Laboratory and the conclusions that have been reached are as follows: (1) The background fluorescence of silica tubings varies greatly. The best of those we have tested is Supersil (Heraeus Quartz Fused Products, Shepperton, Great Britain), which has very low natural fluorescence. (2) There does not appear to be any marked advantage to be gained by using silica tubing of square cross-section as opposed to circular. (3) A tube of about 2 mm I.D. and 3.5 mm O.D. represents an effective compromise between internal volume, mechanical strength, and light-scattering properties.

A typical flow cell is shown in Fig. 3, which is capable of giving the performance described in Applications. A paper dealing with the general applications of fluorimetric detection to the HPLC analysis of drugs has been published[8].

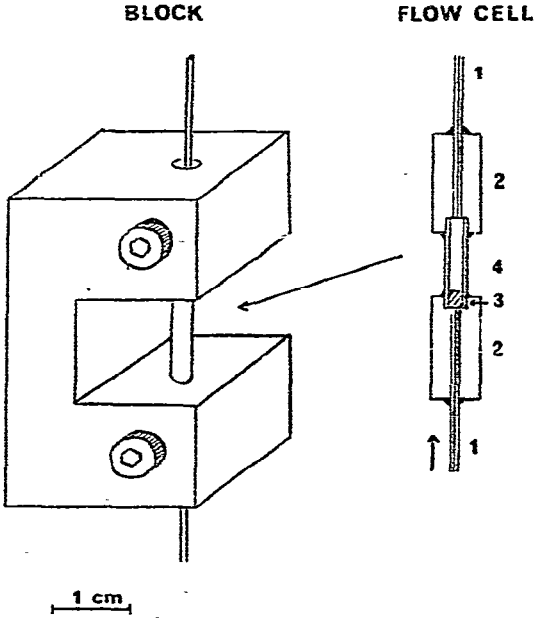


Fig. 3. Flow cell for use in the fluorimetric detection of HPLC eluates. 1 = Stainless-steel tubing 0.254 mm I.D. and 1.59 mm O.D. brazed into 2. 2 = Stainless-steel rod machined to suitable dimensions. 3 = Porous PTFE insert. 4 = Quartz tube 3.5 mm O.D., 2 mm I.D., and 17.5 mm in length sealed into 2 with epoxy resin.

APPLICATIONS

If the experience in the Metropolitan Laboratory is typical, the major application of HPLC in forensic work currently lies in the analysis of drugs of abuse. To a

large extent this can be accounted for by the sensitivity and ease of operation of the UV detector. Most drugs of abuse display moderate to strong UV absorption and frequently occur in milligram quantities in samples —particularly illicit preparations. The UV detector, therefore, can readily detect such materials after HPLC separation without the necessity for prior concentration. In the toxicological area, however, where the drug levels are often much lower, many more problems arise. If the amount of sample is limited, the use of a UV detector to characterise toxicological levels of drugs and their metabolites becomes extremely difficult because one is forced to operate very close to the detection limits. In the contact trace area, where samples are again small, similar limitations occur.

As the potentialities of HPLC become more widely appreciated, however, the trends will be towards the detection of lower amounts of materials. The fluorimetric detector is of particular value where sensitivity is essential and methods have already been developed involving its use in toxicological analysis. If a compound displays natural fluorescence or can be induced to fluoresce by chemical means, it becomes possible to analyse for such materials even when the starting sample only contains nanogram to microgram levels. In the contact trace area, a number of samples containing polynuclear hydrocarbons are encountered and the combination of HPLC and fluorescence detection provides a powerful analytical technique.

Cannabis

The identification of cannabis constitutes a numerically important aspect of the work of the drug sections in many forensic science laboratories. The material is encountered in its resin, herbal or liquid forms but identification using a combination of thin-layer chromatography (TLC) and specific colour reactions is relatively straightforward. A more complex problem, however, is the comparison of cannabis samples to discriminate between samples of different origin. TLC and gas chromatography (GC) have been used to help in comparison cases, but quite frequently samples which are known to be different cannot be distinguished by these methods. This prompted a programme of work here in which a variety of techniques were studied, and after considerable developmental work it was found that HPLC was a very powerful analytical method for cannabis comparison, and indeed was superior in its discrimination to other chromatographic techniques[3].

After preliminary studies with pellicular materials, we finally found that reversed-phase separations on ODS-modified microparticulate silica offered the most effective way to separate complex mixtures of cannabinoids. As mentioned earlier, the ODS loading of the packing material was found to have a marked influence on the separation and was optimum at about the 15% level. A detailed study of HPLC for cannabis comparison was made on such columns and although the chemical identity of the various cannabinoids that were being detected was not known, it became apparent that: (1) Replicate analyses of the same cannabis extract gave identical chromatograms. (2) Analysis of different fragments from the same slab of resin or the same batch of herbal material gave very similar chromatograms. (3) Different samples from the same country were usually capable of being discriminated, although chromatograms displayed some similarity. (4) Samples from different countries usually gave very different chromatograms. (5) Samples which gave identical gas chromatograms often displayed marked differences on HPLC.

This combination of desirable properties in one relatively simple analytical procedure led to its adoption for case-work, which in turn prompted further work to identify the compounds being separated. Following some elegant work by Smith[9], this task has now been largely completed. All the major cannabinoids are separated and detected, with the neutral cannabinoids eluting before their acidic analogues. The capability of HPLC in providing a simple means of analysis for the acidic cannabinoids is important, for these compounds are decarboxylated under GC conditions, unless derivatised.

A typical cannabis chromatogram is shown in Fig. 4 and the comparative value of the method is illustrated in Fig. 5. Selection of the UV wavelength for monitoring is important in the HPLC of cannabis as there are large differences in the UV spectra of the various cannabinoids—254 and 220 nm are the wavelengths we use routinely.

The preparative application of HPLC to the separation of cannabis metabolites has been described[10], and it has been shown that dansyl derivatives can be readily prepared and separated using gradient elution separations on silica[11]. Loeffler *et al.*[12]

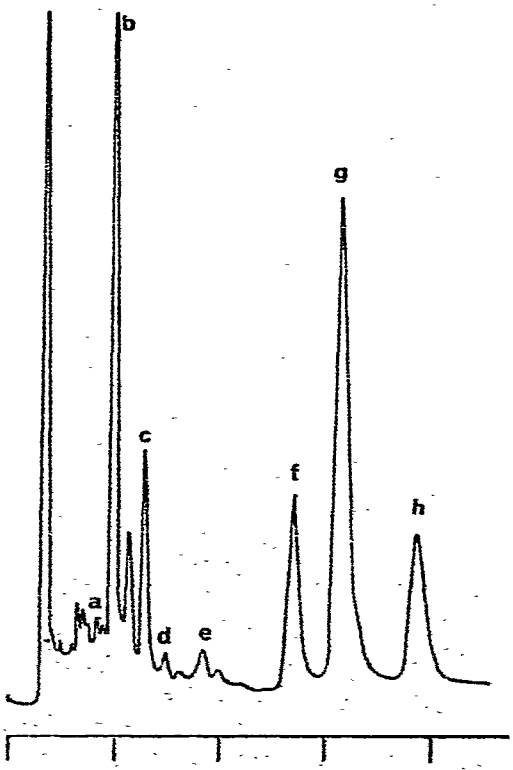


Fig. 4. HPLC separation of cannabis on ODS-modified Partisil 5. Column, 25 cm × 4.9 mm I.D., packed with ODS-modified Partisil 5 of approximately 15% ODS loading; solvent system, methanol–0.02 *N* sulphuric acid (4:1); flow-rate, 2 ml/min; pressure drop, 3000 p.s.i.; detector, UV at 254 nm; time scale marked in 5-min intervals. a = cannabidiol; b = cannabidiolic acid; c = cannabinol; d = Δ^9-tetrahydrocannabinol; e = cannabichromene; f = cannabinolic acid; g = Δ^9-tetrahydrocannabinolic acid; h = cannabichromenic acid.

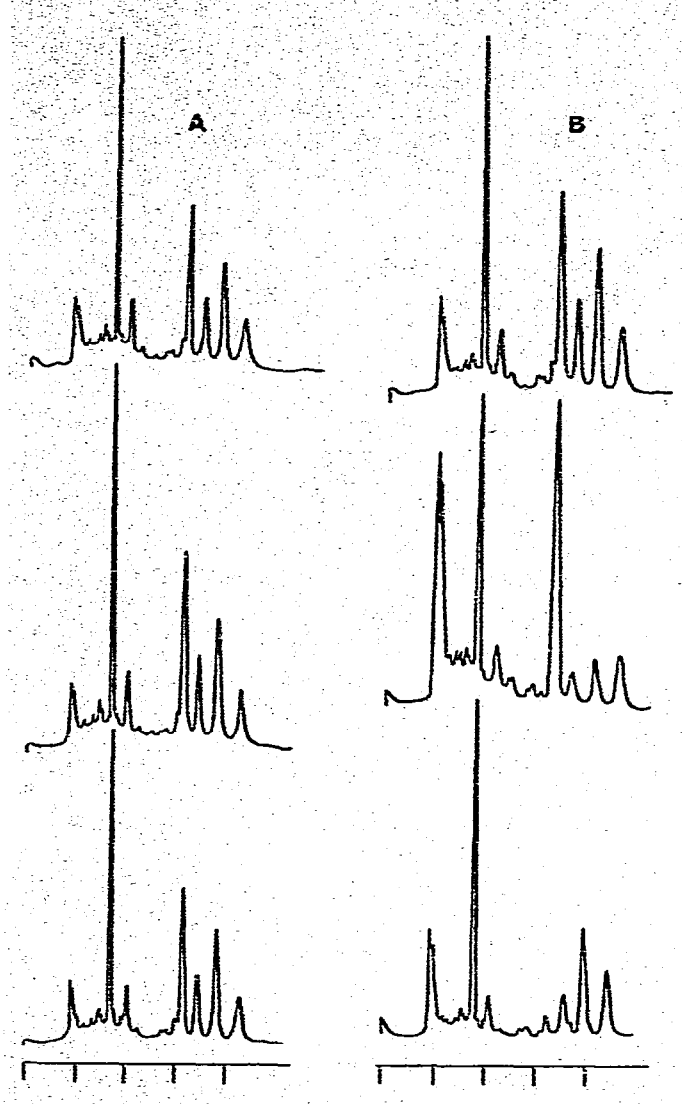


Fig. 5. Discrimination of cannabis samples by HPLC. Chromatographic system, as in Fig. 4, except flow-rate 1 ml/min. (A) Extracts of resin samples from the same seizure; (B) extracts of resins from different seizures. In both cases all the resins are of Pakistani origin. Time scale marked in 10-min intervals.

used dansylation–HPLC–fluorimetric detection to detect cannabis and its metabolites in faeces. There seems little doubt that the high sensitivity afforded by fluorimetric detection will eventually result in the forensic use of HPLC to detect cannabis and its metabolites in body fluids following fluorigenic labelling of some kind.

Lysergic acid diethylamide (LSD)

LSD is almost always encountered forensically in the form of some illicit preparation—in Great Britain the so-called "microdot" is a common form. The pres-

ence of diluents and adulterants in illicit preparations can pose a problem and the high activity of LSD means that low doses are used. The typical "microdot" tablet is unlikely to contain more than 100–200 μg of active ingredient and the thermal instability of the compound precludes the use of GC for its direct detection. TLC is commonly used to analyse for LSD but there are many advantages to be gained by using HPLC for the rapid screening of samples. Wittwer and Kluckhohn[13] described an HPLC method for LSD analysis involving a separation on silica with acetonitrile–diisopropyl ether solvent mixtures. Although the procedure gave adequate separation of LSD from chemically related ergot alkaloids, it was complicated by the use of a UV detector. This necessitated a preliminary column clean-up to remove UV absorbing tablet excipients followed by the concentration of the LSD before HPLC. The high natural fluorescence of LSD and the absence of fluorescent excipients in most illicit preparations means that fluorimetric detection coupled with HPLC offers both selectivity and sensitivity. Heacock *et al.*[14] pointed out the advantages to be gained by using fluorimetric detection, but their chromatographic system left much to be desired in the way of resolution. A very simple HPLC procedure was developed here[15] based on a reversed-phase separation, but this was rapidly abandoned once the potential of using silica in an "ion-exchange" mode[6] was appreciated. In retrospect, it seems likely that the separation we were achieving was largely attributable to reaction of silanol groups on the packing rather than any partition process involving the ODS.

The procedure that is now in routine use here is as follows: A portion of the illicit sample is crushed with methanol and an aliquot of the supernatant material is injected into the following HPLC system: column, 25 cm × 4.9 mm I.D., packed with Partisil 5; solvent system, methanol–0.3% ammonium carbonate (60:40); flow-rate, 1 ml/min; detector, fluorimetric, LDC Model 1209. Under these conditions the elution volume of LSD is approximately 7 ml and the detection limit about 10 ng (*i.e.*, injected).

The intense fluorescence of LSD can provide a basis for very sensitive HPLC analysis. It is necessary, however, to utilise a detector operating under optimum excitation and emission conditions and with an intense light source. The flow cell mentioned earlier has been used to modify a spectrofluorimeter (Perkin-Elmer MPF 2A) to provide a detector capable of measuring down to 10–100 pg of LSD when used in conjunction with the above chromatographic system. This detector has been used to detect LSD in urine samples from individuals thought to have taken the drug[16].

Opium alkaloids

In virtually all samples the opium alkaloids or their derivatives are mixed with other materials, and some form of separation is essential if the active components are to be identified and quantified for forensic purposes. Morphine in particular requires derivatisation if GC is to be used as the separation technique and HPLC offers a potentially useful way of avoiding the thermal limitations of such compounds.

Trinler and Reuland[17] have shown how HPLC can be used to rapidly screen for street drugs using a reversed-phase system, but their separation of cocaine, heroin, methadone, and morphine is inferior to the separation achieved on silica shown in Fig. 6. Cashman and Thornton[18] achieved a successful separation of morphine, monoacetylmorphine and heroin on Porasil T using chloroform–methanol as the eluent, and applied the method to authentic samples. Cation-exchange separations of the

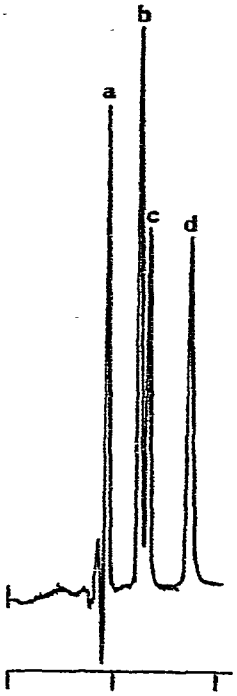


Fig. 6. Separation of some commonly abused drugs on silica. Column, 25 cm × 4.9 mm I.D., packed with Partisil 5; solvent system, methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (27:2:1); flow-rate, 1 ml/min; pressure drop, 900 p.s.i.; detector, UV at 254 nm; time scale marked in 5 min intervals. a = Cocaine; b = heroin; c = methadone; d = morphine.

same compounds on Zipax SCX (DuPont) were achieved by Knox and Jurand[19] under both isocratic and gradient elution conditions, and a modification of their procedure has also been applied to the analysis of illicit preparations[20]. In our experience the best system currently available for separating these compounds is that based on silica[6]. As mentioned earlier the exact separation mechanism is not clearly understood but the elution sequence is in the order heroin, monoacetylmorphine, and finally morphine. This is the same sequence obtained on silica columns operating in an adsorption mode[18] but the reverse of that found on Zipax SCX[19].

The HPLC system used at the Metropolitan Laboratory for the separation of opium alkaloids is as follows: column, 25 cm × 4.9 mm I.D., packed with Partisil 5; solvent system, methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (27:2:1); flow-rate, 1 ml/min; detector, UV at 254 and 278 nm. Under these conditions all the major alkaloids in opium elute within 10 min. Increasing the concentration of aqueous buffer in the eluent leads to more rapid elution and reduced resolution. The columns are stable for long periods.

On the above system the alkaloids present in raw opium can be readily separated, although only morphine, codeine, thebaine, papaverine and narcotine are present at sufficiently high levels to be detectable without prior concentration. The chromatograms obtained from different samples can be used for opium comparison in a manner analogous to that used for cannabis (see Fig. 7). Heroin, frequently encounter-

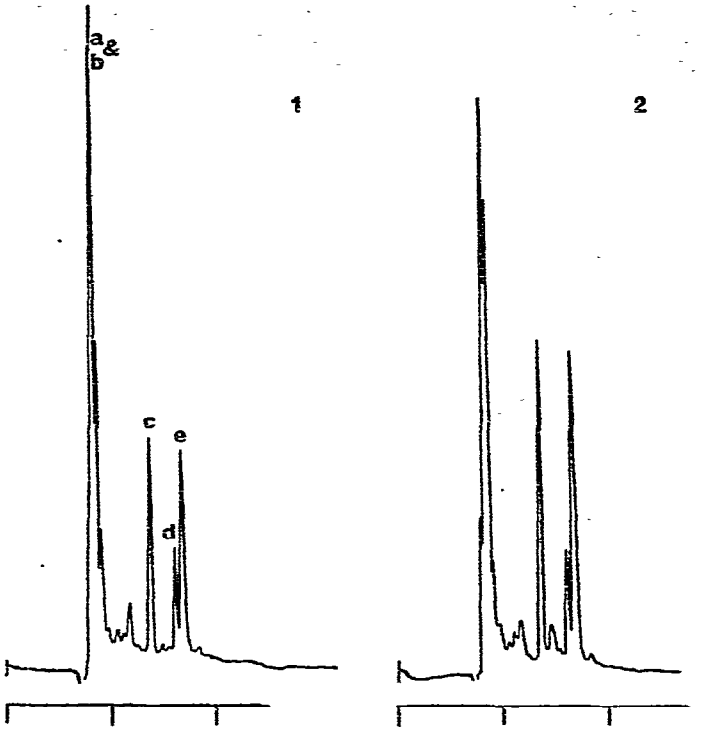


Fig. 7. Separation of opium alkaloids on silica: use in opium comparison. Column, 25 cm × 4.9 mm I.D., packed with Partisil 5. Chromatographic conditions, as in Fig. 6. Samples 1 and 2 are different opium extracts. a = Papaverine; b = narcotine; c = thebaine; d = codeine; e = morphine.

ed in the form of the so-called "Chinese Heroin", can be readily separated from adulterants such as caffeine, strychnine and quinine by the same HPLC method. For analysis the sample is merely dissolved in the eluting solvent before injection. Injections made before and after alkaline hydrolysis provide a simple chemical confirmation of the presence of heroin (see Fig. 8). The HPLC method can be used for the rapid screening of samples, quantitation of the active ingredients and as a clean-up method if fractions are collected prior to identification by spectroscopic means. Chromatograms showing the presence of different adulterants also have potential value for "Drugs Intelligence" activities.

HPLC can also be used to monitor for the presence of morphine in biological fluids. Jane and Taylor[21] have developed a sensitive method based on the conversion of morphine to pseudomorphine. The latter material, which is highly fluorescent, is then separated and detected fluorimetrically. The method is particularly interesting for the ingenious incorporation of a reactive internal standard, which compensates for the influence of coextracted material on the primary reaction.

Phenylethylamines

Sympathomimetic phenylethylamines such as amphetamine and methylamphetamine are frequently abused drugs, and are increasingly appearing in the form of illicit preparations.

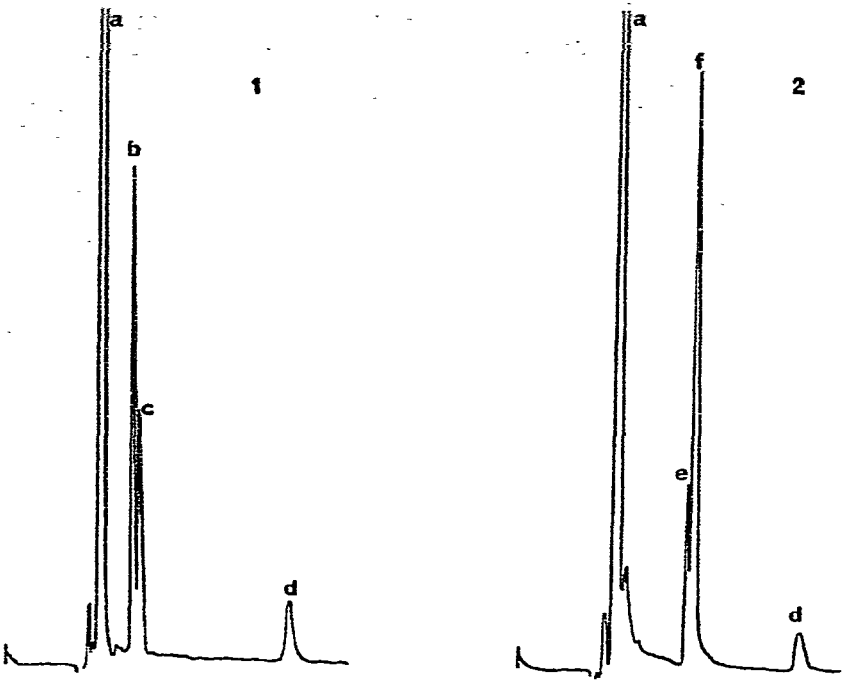


Fig. 8. Analysis of a "Chinese Heroin" sample on silica. Column and chromatographic conditions, as in Fig. 6. (1) "Chinese Heroin" sample: a = caffeine; b = heroin; c = monoacetyl morphine or monoacetyl codeine; d = strychnine. (2) Same sample after treating with alkali: e = codeine; f = morphine.

Cashman *et al.*[22] studied the HPLC of these compounds on DA-X4, a strong anion-exchange resin, and showed that under isocratic conditions it was possible to obtain good separations in both acidic and alkaline solvents. He also studied the adsorption-based separation on Corasil II (*i.e.*, a pellicular silica) and concluded that to achieve separations comparable to those obtained with the ion-exchange system it would be necessary to use gradient elution. Once again we find that the separations on silica using methanol–aqueous buffer solvents[6] are the most useful. The chromatographic conditions used for opium alkaloids are also capable of separating all the commonly abused phenylethylamines and adulterants occurring in illicit mixtures. Chromatograms can be used for qualitative and quantitative analysis and the separation can be used as a clean-up procedure if required.

Barbiturates

HPLC separations applicable to pharmaceutical preparations have been described[23–25] and a method based on the fluorigenic labelling of barbiturates with dansyl chloride applicable to toxicological levels has been reported[26].

Benzodiazepines

This class of compound is a problem area analytically. The widespread use of drugs in this category means that inevitably the forensic scientist is called upon to ana-

lyse such materials quite frequently. Although the characterisation of various benzodiazepines in pharmaceutical preparations poses no problems the screening of blood and/or urine for their presence is less satisfactory. HPLC methods have been reported for the former type of analysis[27–29], but no methods of wide applicability to toxicological samples have appeared.

Cocaine

This drug usually appears in illicit preparations mixed with other drugs and adulterants. GC or TLC provides an adequate method of examining such mixtures, but HPLC could be used although no detailed methods have been reported. On silica columns solvents such as methanol–ammonium nitrate enable cocaine to be retained and could provide the basis for an analytical procedure. The analysis of body fluids for cocaine and its metabolites is a more difficult problem and it is possible that HPLC might have a part to play. Some form of derivatisation procedure would probably be necessary if HPLC were to be used, for the major cocaine metabolite ecgonine does not absorb strongly in the UV region normally used for detection.

"Contact trace" analysis of samples containing polynuclear hydrocarbons

The widespread occurrence of polynuclear aromatic hydrocarbons (PAHS) inevitably means that they will on occasion appear in samples of forensic significance. Lloyd[30–32] carried out a detailed study to identify the various compounds of this type which occur in forensic samples, particularly used engine oils, and showed how synchronous scanning fluorimetry could be used for comparative analysis. He enhanced the evidential value of the results by carrying out traditional column chromatography before fluorimetric analysis.

Although used engine oils cannot be considered to be particularly important forensically, they are difficult samples to compare by most analytical techniques and it was felt that it would be worth extending Lloyd's work and attempt to develop an HPLC method for two reasons: (1) PAHS have ideal properties for HPLC detection in that they are strongly UV absorbing and in most cases are highly fluorescent. Used oils containing such materials therefore provide excellent model systems with which to acquire experience of HPLC and fluorimetric detection. (2) The experience gained in separating the PAHS in used oils could serve as a basis for the comparison of a variety of other samples such as bitumens, greases, soots, creosotes, soils etc. A study was made of the problem at the Metropolitan Laboratory and a routine HPLC procedure has been developed.

We found at a very early stage that although HPLC was potentially a very powerful way of separating PAHS, there were many practical difficulties. The irreversible adsorption of water and other polar materials on to silica from samples such as engine oils means that it is very difficult to maintain a liquid–solid adsorption column at constant activity, and this is of course an essential requirement for successful sequential comparative analysis. The problem can be to some extent overcome by subjecting the samples to a rigorous clean-up before HPLC, but the best solution is to use a reversed-phase separation on packings such as the ODS-modified silicas described earlier[2,7]. Another practical difficulty arises from the inherent nature of the PAH contamination in used oils. In general, all used oils seem to contain the same mixture of PAHS, but they differ in the amounts and hence relative proportions pre-

sent. To discriminate oils effectively by HPLC it is essential to use columns which provide sufficient resolution to enable semi-quantitative comparisons to be made. A column with an efficiency of about 5–10,000 theoretical plates is necessary for this purpose and the following system is adequate for routine analysis: column, 25 cm × 4.9 mm I.D., packed with ODS-modified Partisil 5 to give an organic loading of about 30%; solvent system, methanol–water (95:5 or or 90:10); flow-rate, 1 ml/min; detector, UV at 254 nm; sample, oil dissolved in dichloromethane to give a concentration of 10–100 mg/ml, 2 μl injection made. Under the above conditions used oils give chromatograms which are reproducible and can act as "fingerprints" of the samples; typical chromatograms are shown in Fig. 9. The various peaks in the chromatograms correspond to the PAHS and other UV absorbing materials present in the oil. Unused oils do not display such peaks, for the paraffinic material constituting the major part of the sample is non-UV absorbing and is therefore undetected. It appears from the samples that we have studied that the PAH concentration of used

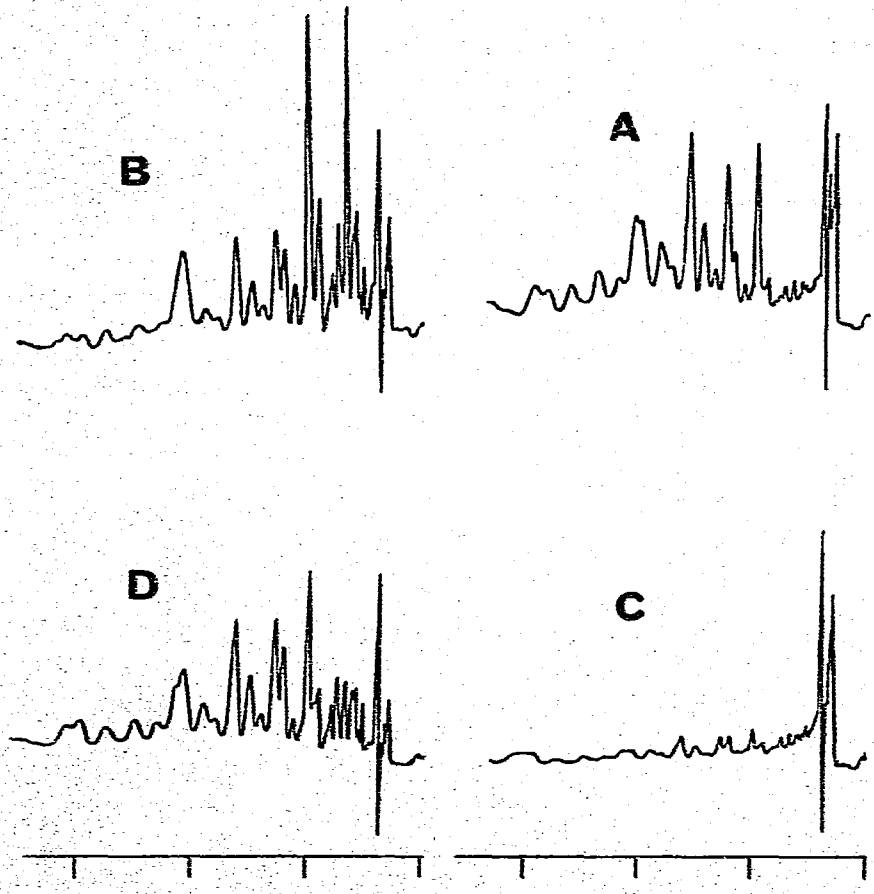


Fig. 9. HPLC of used engine oils: Comparison of different oils. Four different oils at a concentration of 100 mg/ml in dichloromethane were chromatographed under the conditions described in the text. Time scale marked in 5-min intervals.

oils increases in proportion to the extent that they are used, but the rate of formation of individual compounds is subject to a much more random process. We have made no attempt to identify the eluted compounds chemically; this aspect has already been studied by Lloyd, and is irrelevant as far as comparative analysis is concerned, but the chromatographic system is sufficiently reproducible to enable retention time data to provide a basis for qualitative analysis should this be required. The HPLC method of used oil comparison is easy to use and despite the absence of any clean-up stage in the method we have found that column life is acceptably long.

Although HPLC with UV detection can frequently provide an adequate method of oil comparison there is an overall similarity between chromatograms reflecting the quantitative rather than qualitative variations in the samples. As is so frequently the case in forensic science, the non-similarity of samples can be readily ascertained but the HPLC method often yields chromatograms that are not unique and apparently identical chromatograms do not necessarily indicate that samples have the same origin. It is possible to increase the specificity of the technique by: (a) carrying out separations on more than one column system, (b) increasing the chromatographic efficiency of the column used, or (c) using a more selective detector.

We have opted for the third alternative since this is the only one which does not necessarily involve some increase in analysis time.

Because of the complexity of the PAH mixtures found in used engine oils, the columns used do not adequately resolve every component. Many of the PAHS, however, display fluorescence, and it is possible to impart additional selectivity to the analysis by using a fluorimetric detector that can be wavelength tuned. Such a detector can be coupled in series with a UV detector to give two simultaneous traces, or two such detectors operating at different excitation and emission wavelengths can be similarly coupled. Under these conditions, multi-component peaks produced by the relatively non-selective UV detector can often be adequately resolved by using a fluorimetric detector. In comparative analysis terms it means that several different chromatograms can be produced by monitoring the same eluate (Fig. 10 shows a typical example) thereby improving the discrimination of the method without increasing the analysis time. In addition, fluorimetry provides a way of increasing the sensitivity of the analysis, for under optimum conditions it can impart a 10- to 100-fold increase. In a recent paper Lloyd[35] has shown how the sensitivity of a fluorimetric detector to nitrogen heterocycles and PAHS can be enhanced by using a packed flow cell, and he has applied this innovation to the monitoring of forensically important samples.

One interesting area that we have had little time to study concerns the use of HPLC for soil comparison. Soils contain fluorescent components, which may well be PAHS, and extracts subjected to chromatography under the above conditions give chromatograms which may be of value for comparative analysis. Fig. 11 shows a typical chromatogram produced by injecting 2 μl of the supernatant solution obtained from a 1 g/ml suspension of soil in dichloromethane.

Plasticisers

Forensic scientists are called upon quite frequently to compare plasticised polymeric material and, for phthalates in particular, HPLC offers a sensitive and rapid analytical method. A review has been published on the GC and HPLC analysis of

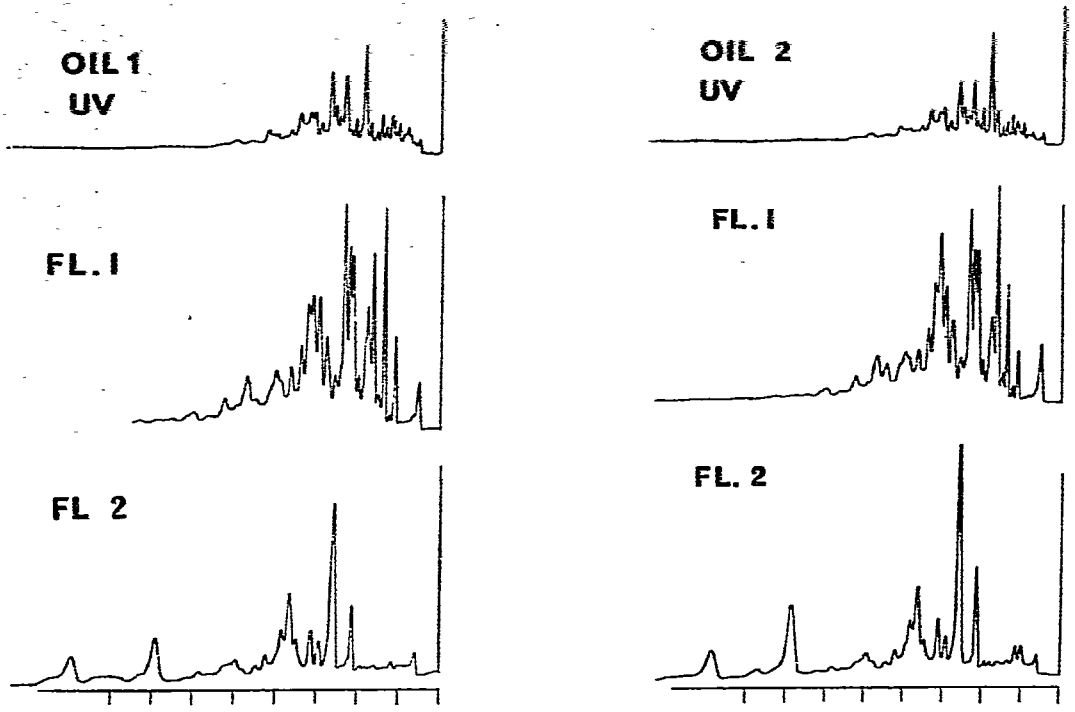


Fig. 10. HPLC of used engine oils. Comparison of UV absorption and fluorimetric detection for oil discrimination. Two different oils were chromatographed under identical conditions on ODS-modified Partisil 5 of *ca.* 30% ODS loading. Chromatographic conditions, as described in the text. Detection conditions: UV = UV detection at 254 nm; FL 1 = fluorimetric detection with excitation at 275 nm and emission at 320 nm; FL 2 = fluorimetric detection with excitation at 360 nm and emission at 460 nm.

phthalates[34] and more recent papers on the same subject include that of Funasaka *et al.*[35]. It is very debatable whether HPLC has any advantage over GC for this type of analysis, and it may be that the ubiquitous HPLC separations of phthalates that appear in advertising literature reflect the ease of availability of an homologous series with desirable UV absorbing properties rather than any real step forward in analytical chemistry. Reversed-phase HPLC on columns of ODS-modified silica is a convenient way to separate such materials, and the same type of column system can be applied to the HPLC separation of antioxidants.

Explosives

The utility of HPLC for analysing thermally labile compounds suggests that it has a part to play in the examination of explosives. Several papers have appeared on this subject[36–38] and it appears that HPLC has value for comparing explosives, *e.g.*, to establish a common origin, but the relatively poor detection limits for some explosive components appears to restrict the application of the technique in the contact trace area.

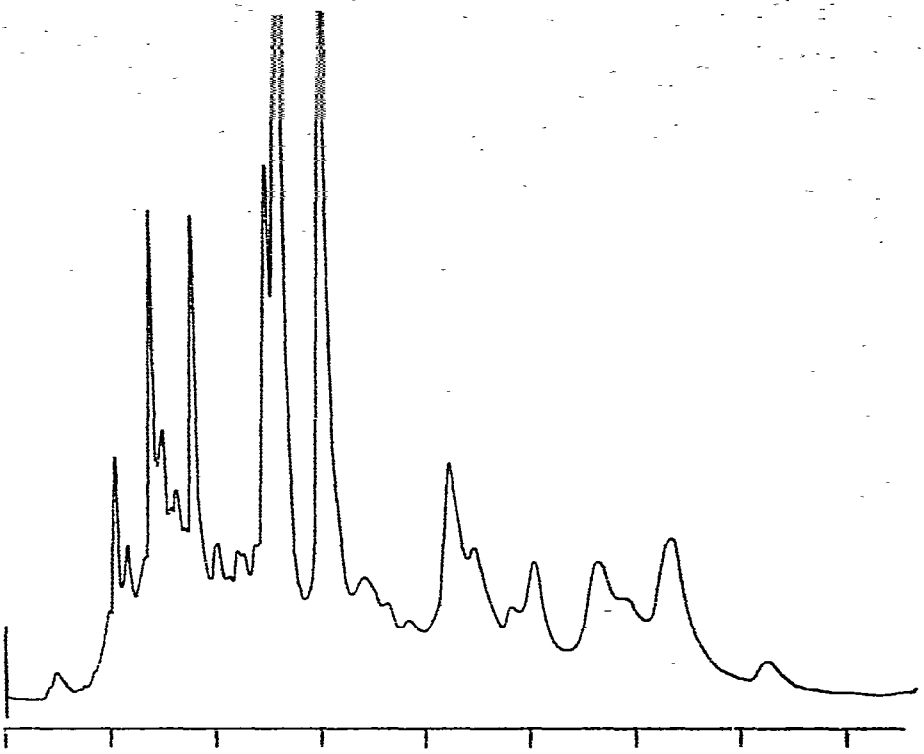


Fig. 11. HPLC of soil extract on ODS-modified Partisil 5 of *ca.* 30% ODS loading. Chromatographic conditions, as described in the text. Fluorimetric detector operated with excitation at 280 nm, emission at 420 nm. Time scale marked in 5-min intervals.

CONCLUSIONS

It is very easy for a specialist review to give a deceptive impression of the significance of a particular analytical technique. The current status of HPLC in the Metropolitan Police Laboratory is that it is a well established method being used routinely for a number of analyses to which it is particularly well suited. However, the importance of qualitative analysis in forensic drug work means that HPLC, which is particularly advantageous where quantitation is required, is considerably less important than TLC. Similarly, there seems little possibility that HPLC will oust GC in areas where the latter technique performs adequately.

In the future, it is probable that the relative importance of the various chromatographic techniques may well change. The use of HPLC in combination with fluorimetric detection will undoubtedly increase in the area of drug metabolite analysis, and if the procedure becomes more widely applicable to trace metal analysis, *e.g.*, separating fluorescent metal chelates, there would be almost unlimited potential for its expansion. It is often suggested that the direct linkage of HPLC with a mass spectrometer is the ultimate growth area, but economic considerations and the ease of using HPLC in a preparative mode with subsequent spectroscopic examination of

fractions may mean that this development will not have the impact that it is theoretically capable of having. Nevertheless the next ten years will prove to be very exciting for the analytical chemists working in HPLC.

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REFERENCES

1 M. L. Chan, C. Whetsell and J. D. McChesney, *J. Chromatogr. Sci.*, 12 (1974) 512.
2 B. B. Wheals, C. G. Vaughan and M. J. Whitehouse, *J. Chromatogr.*, 106 (1975) 109.
3 B. B. Wheals and R. N. Smith, *J. Chromatogr.*, 105 (1975) 396.
4 P. J. Twitchett and A. C. Moffat, *J. Chromatogr.*, 111 (1975) 149.
5 B. B. Wheals, *J. Chromatogr.*, 107 (1975) 402.
6 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
7 C. G. Vaughan, B. B. Wheals and M. J. Whitehouse, *J. Chromatogr.*, 78 (1973) 203.
8 B. B. Wheals, *Proc. Symp. Appl. HPLC Clin. Chem., Kings College Medical School, London, 1975*, to be published.
9 R. N. Smith, *J. Chromatogr.*, 115 (1975) 101.
10 *Technical Information Sheet*, Waters Associates, Milford, Mass.
11 S. R. Abbott, A. Abu-Shumays, K. Loeffler and I. S. Forrest, *Res. Commun. Chem. Pathol. Pharmacol.*, 10 (1975) 9.
12 K. O. Loeffler, D. E. Green, F. C. Chao and I. S. Forrest, *Proc. West. Pharmacol. Soc.*, 18 (1975) 363.
13 J. D. Wittwer and J. H. Kluckhohn, *J. Chromatogr. Sci.*, 11 (1973) 1.
14 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
15 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
16 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.* 120 (1976) 496.
17 W. A. Trinler and D. J. Reuland, *J. Forensic Sci. Soc.*, 15 (1975) 153.
18 P. J. Cashman and J. I. Thornton, *J. Forensic Sci. Soc.*, 12 (1972) 417.
19 J. H. Knox and J. Jurand, *J. Chromatogr.*, 87 (1973) 95.
20 P. J. Twitchett, *J. Chromatogr.*, 104 (1975) 205.
21 I. Jane and J. F. Taylor, *J. Chromatogr.*, 109 (1975) 37.
22 P. J. Cashman, J. I. Thornton and D. L. Shelman, *J. Chromatogr. Sci.*, 11 (1973) 7.
23 R. W. Roos, *J. Pharm. Sci.*, 61 (1972) 1979.
24 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
25 J. E. Evans, *Anal. Chem.*, 45 (1973) 2428.
26 W. Dunges, G. Naundorf and N. Seiler, *J. Chromatogr. Sci.*, 12 (1974) 655.
27 C. G. Scott and P. Bommer, *J. Chromatogr. Sci.*, 8 (1970) 446.
28 D. J. Weber, *J. Pharm. Sci.*, 61 (1972) 1797.
29 D. H. Rodgers, *J. Chromatogr. Sci.*, 12 (1974) 742.
30 J. B. F. Lloyd, *J. Forensic Sci. Soc.*, 11 (1971) 83.
31 J. B. F. Lloyd, *J. Forensic Sci. Soc.*, 11 (1971) 153.
32 J. B. F. Lloyd, *J. Forensic Sci. Soc.*, 11 (1971) 235.
33 J. B. F. Lloyd, *Analyst (London)*, 100 (1975) 1193.
34 L. Fishbein and P. W. Albro, *J. Chromatogr.*, 70 (1972) 365.
35 W. Funasaka, T. Hanai and K. Fujimara, *J. Chromatogr. Sci.*, 12 (1974) 517.
36 J. O. Doali and A. A. Juhasz, *J. Chromatogr. Sci.*, 12 (1974) 51.
37 R. A. Henry, *Applications Lab. Rep. No. 7201*, DuPont Instrument Products Division, Hitchin.
38 R. W. Dalton, C. D. Chandler and W. T. Bolleter, *J. Chromatogr. Sci.*, 13 (1975) 40.