CHROM. 17,313

# PYROLYSIS–MASS SPECTROMETRY INVESTIGATIONS OF REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STATIONARY PHASES

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(Received August 30th, 1984)

SUMMARY

   Pyrolysis–mass spectrometry has proved to be a valuable technique for characterisation of octadecylsilane chemically bonded phases for reversed-phase high-performance liquid chromatography. Information on both the functionality of the silane used for bonding and the deactivated (capped) status of commercial octadecylsilane phases was obtained. The complexity of the data demanded the use of chemometric (pattern recognition) procedures.

INTRODUCTION

   Reversed-phase high performance liquid chromatography (RP-HPLC) using chemically bonded stationary phases has enjoyed increasing popularity over recent years. In 1980, it was estimated[1] that 61% of all analytical chromatographic separations were carried out on columns of this type. Octadecylsilyl (ODS) bonded phases were responsible for 79% of these separations. These figures are thought to be conservative and perhaps 80% is more realistic for both values in 1984[2]. This popularity is evident from the number of commercial manufacturers marketing phases of this type. However, it is well known that considerable differences in chromatographic properties exist between commercial phases[3–5] and even between batches from a single manufacturer[6,7]. These differences are often difficult to explain without knowledge of the chemical character of the phases employed, information which is normally not supplied by the manufacturer and is difficult to obtain. This lack of characterisation has been suggested as a reason for RP-HPLC remaining a poorly defined separation method[8], although it has also stimulated numerous studies into the nature of the bonded phase. These studies have included simple chromatographic tests[9–13], instru-

mental techniques such as infrared[14,15] photoacoustic[15] and ultraviolet[17] spectroscopy, in addition to chemical analysis[18-20].

Recently pyrolysis techniques such as pyrolysis–gas chromatography (Py–GC) and Py–GC–mass spectrometry (Py–GC–MS) have been reported in the literature[21-23]. These techniques have revealed information both on the functionality of the silanising reagent used for bonding, and on the deactivated (capped) status of the phases studied. Both Py–GC and Py–GC–MS usually require 1–2 h for a complete analysis and are not necessarily reproducible over long periods of time, when column degradation phenomena can affect results[24]. An alternative analytical pyrolysis technique is direct pyrolysis–mass spectrometry (Py–MS), which has been shown to be of value in the rapid characterisation of many non-volatile materials, including micro-organisms and polymers[25]. The advantages of Py–MS over Py–GC techniques relate to speed of analysis (5 min), improved long-term reproducibility (no column) and the ease of presenting the data for computer analysis. In addition, modern pattern recognition data handling techniques can be used to extract chemical information from the pyrolysis mass spectra. In this study, the chemical nature of RP-HPLC ODS phases was investigated by Py–MS. Both a model system of chemically characterised phases and twelve commercial ODS phases were studied.

## MATERIALS AND METHODS

### Materials

ODS phases of known chemical character were provided by Shandon Southern Products (Runcorn, U.K.). These phases were prepared by treating silica with either octadecyltrichlorosilane (OTCS) or octadecyldimethylchlorosilane (ODMCS) followed by endcapping using trimethylimidazosilane (TMIS)[26]. Samples were provided before and after the capping reaction. Sample designation is given in Table I.

Twelve commercial ODS phases of "unknown" character were studied. Spherisorb ODS, ODS-2 (Phase Separations, Queensferry, U.K.), Partisil ODS, ODS-2, ODS-3 (Whatman, Maidstone, U.K.), Supelcosil LC-18, LC-18DB (Supelco, Bellefonte, PA, U.S.A.), $\mu$Bondapak $C_{18}$ (Waters Assoc., Northwich, U.K.), Hypersil ODS (Shandon Southern Products) Nucleosil $C_{18}$ (Machery, Nagel & Co., Duren, F.R.G.), Zorbax ODS (DuPont, Wilmington, DE, U.S.A.) and LiChrosorb RP-18 (E. Merck, Darmstadt, F.R.G.). Sample designations and batch numbers are given in Table II.

### Pyrolysis–mass spectrometry

This was carried out using a Pyromass 8-80 (VG Gas Analysis, Middlewich, U.K.). This instrument employs the Curie-point pyrolysis technique and is configured in a similar manner to the mass spectrometer described by Meuzelaar et al.[27], except that the Pyromass utilises a magnetic mass analyser rather than a quadrupole. A full description of the Pyromass has been given elsewhere[28], but the key operational parameters of the instrument were: mass range, $m/z$ 300–12, scanned exponentially; expansion chamber temperature, 150°C; scan time, 1.3 sec cycle$^{-1}$; number of scans, 35. Low voltage electron impact ionisation (16 eV) was used to minimise fragmentation of the pyrolysis products. Nevertheless, any mass in the spectrum may have a multiple origin, i.e. be derived from a number of pyrolysis products, a problem which makes chemical evaluation of the data difficult.

In this study small samples of phase (50 $\mu$g) were pyrolysed in the centre of 1 mm I.D. pure iron tubes (Goodfellow Metals, Cambridge, U.K.), guaranteeing a pyrolysis temperature of 770°C. Pyrolysis was maintained for 5 sec. Although thermogravimetric analysis (TGA) of the phases revealed that thermolysis occurred at *ca.* 300°C, it was necessary to use a higher pyrolysis temperature to give a sufficient yield of products to obtain reproducible spectra.

*Data handling*

The raw mass intensity data for triplicate analyses of each phase were recorded on floppy diskettes and were transferred to an IBM 3033 mainframe computer for analysis. The data were analysed using a batch GENSTAT programme which contains routines for principal components analysis, canonical variates analysis, and a type of "factor analysis" for interpretation of the chemical differences between the spectra. The first stage of the data analysis is a normalisation to total ion intensity to remove the effect of variations in sample size. Water (*m/z* 18) was removed from the data set because TGA showed that it was associated, up to levels of 10%, with some of the phases.

The data analysis is similar to that described by Windig *et al.*[29], in which principal components analysis is used as a data reduction technique and canonical variate analysis is applied to some or all of the principal components with non-zero variances, to attempt to distinguish groups of sample replicates. The "factor analysis" is a novel procedure based loosely on the work of Windig *et al.*[30,31]. A line from the centroid of the canonical variate is drawn through the centre of a group. This line makes an angle $\alpha$ with the first canonical variate axis and angle $\pi/2 - \alpha$ with the second. A rigid rotation of the two canonical variates axes through angle $\alpha$ is carried out so that the rotated first canonical variate axis corresponds to a line through the middle of the group. The group is differentiated from the centroid on the rotated first canonical variate axis and not on the rotated second canonical variate axis. The remaining axes are unchanged and provided they do not differentiate the group from the centroid, the rotated combined principal component/canonical variate loadings on the first two axes contain useful information on the chemical nature of the difference between that group and other groups. This information can be constructed into a "factor spectrum" showing positive and negative contributions to the discrimination of the group.

A fuller description of pattern recognition methods used for handling pyrolysis mass spectrometry data is given elsewhere[32].

RESULTS AND DISCUSSION

*Manual chemical characterisation*

Typical pyrolysis mass spectra are shown in Fig. 1. These spectra are essentially a homologous series of monoalkenes indicative of straight chain alkane thermolysis. In most cases the last detected alkene was octadecene (*m/z* 252).

Hansson and Trojer[21] state that information on the functionality of the silane used in the bonding reaction may be derived from comparision of the $C_{17}/C_{18}$ alkene peak heights after Py–GC analysis. This is confirmed by Mussche and Verzele[23]. In this study, comparison of the corresponding mass intensities, *m/z* 238 and 252, re-
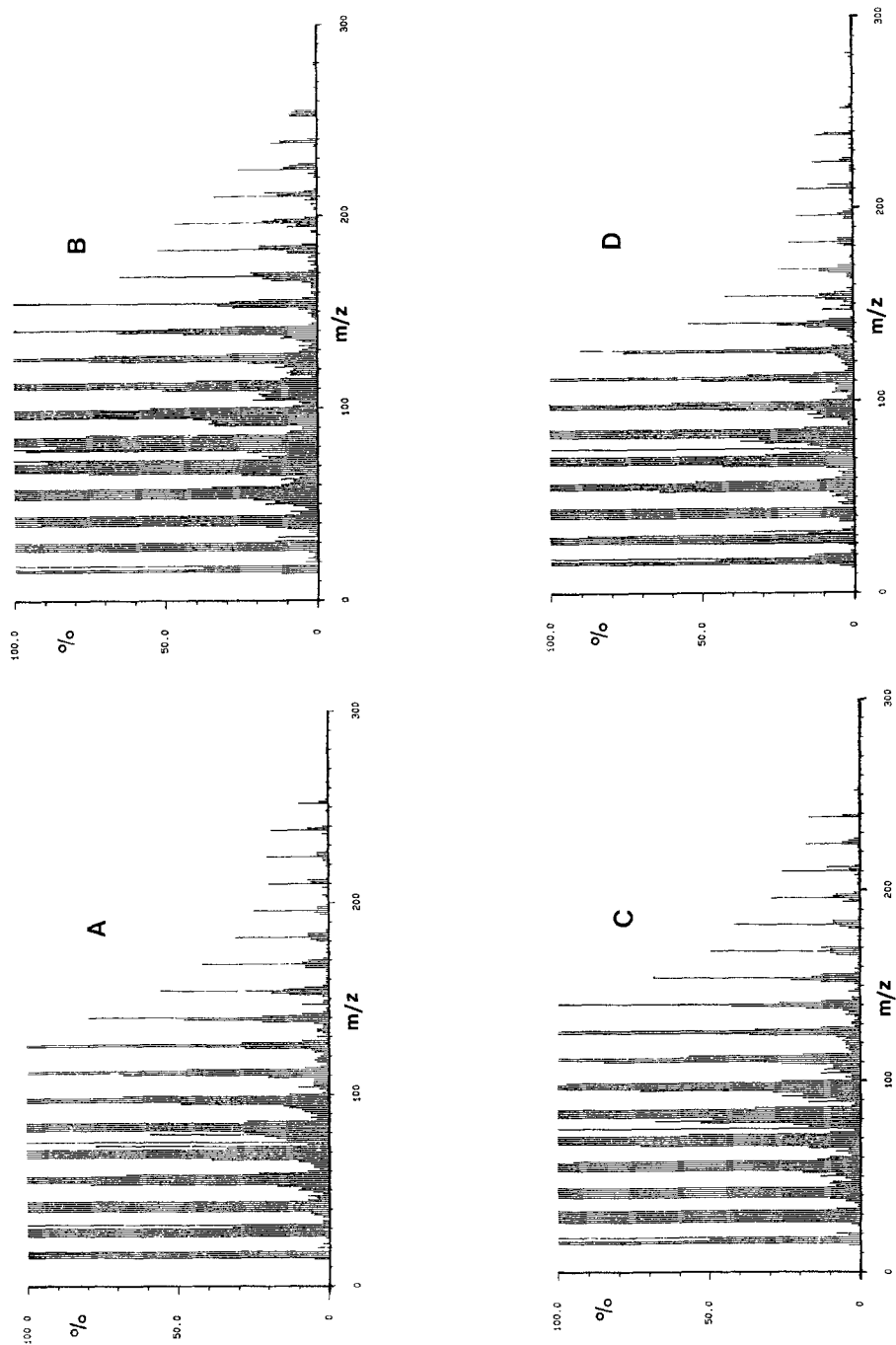
Fig. 1. Typical pyrolysis–mass spectra of ODS phases. A = μBondapak C₁₈; B = Zorbax ODS; C = Spherisorb ODS-2; D = Hypersil ODS. Py-MS conditions given in Materials and methods.

TABLE I

CHEMICALLY CHARACTERISED ODS PHASES: MANUAL CHARACTERISATION

| Sample | Phase character | Mass ratio | |
|---|---|---|---|
| | | $C_{17}/C_{18}$ | $C_1/C_4$ |
| A | ODMCS* | 3.0 | 0.28 |
| | | 3.8 | 0.38 |
| | | 2.2 | 0.26 |
| B | ODMCS + TMIS** | 3.0 | 0.37 |
| | | 2.2 | 0.37 |
| | | 3.9 | 0.40 |
| C | OTCS*** | 17.3 | 0.13 |
| | | 63.0 | 0.12 |
| | | 36.0 | 0.11 |
| D | OTCS + TMIS | 11.7 | 0.31 |
| | | 4.3 | 0.28 |
| | | 15.7 | 0.33 |

     \* Octadecyldimethylchlorosilane.
   \*\* Trimethylimidazosilane.
\*\*\* Octadecyltrichlorosilane.

spectively, while revealing a similar trend (Table I) did not allow conclusive assignment (see Table II).

The reproducibility of the data is poor owing to the low intensities measured. Additional fragmentation produced by the mass spectrometer and/or the pyrolysis conditions used may be responsible for these low observed intensities.

Hansson and Trojer also state that information on the deactivated (capped) status of the ODS phases may be obtained from comparison of the methane and butene peak heights. Comparison of the corresponding mass intensities, $m/z$ 16 and 56, reveals that while capping may be indicated by an increase in the mass ratio (Table I) only a tentative assignment may be given to the commercial phases without prior knowledge of the silane functionality (Table II). Methane will be produced by pyrolysis of chlorodimethyl- and dichloromethylsilanes in addition to the trimethylsilane (TMS) derivative used for capping. It is interesting to note that the monofunctional ODS phase may be capped on this basis, although it is reported that capping of such phases is unnecessary[4]. Verzele and Mussche also report capping of monofunctional ODS phases[33]. It is not clear how capping is achieved unless the silica was not fully derivatised before capping was attempted.

Visual inspection of the pyrolysis mass spectra reveals a significant mass ($m/z$ 75) in some of the samples. This mass is very characteristic of low ionisation mass spectra of TMS derivatives[34]. The presence of such a mass may be more indicative of capping reactions. It also indicates that silicon-containing compounds may be volatilised during pyrolysis, as suggested by other workers[23].

It is clear that manual interpretation of the complex mass spectral data is inadequate for conclusive chemical characterisation. The complexity of the data generated by Py–MS demands data handling (chemometric) procedures of equal sophistication[35], procedures which are best carried out by computers.

TABLE II

COMMERCIAL ODS PHASES: MANUAL CHARACTERISATION

| Sample | Packing material | Batch no. | Mass ratio | | Capping designation* |
|---|---|---|---|---|---|
| | | | $C_{17}/C_{18}$ | $C_1/C_4$ | |
| 1 | μBondapak $C_{18}$ | — | 2.04 | 0.63 | |
| | | | 1.93 | 0.45 | C |
| | | | 1.89 | 0.67 | |
| 2 | LiChrosorb RP-18 | NV 1249 | 2.00 | 0.24 | |
| | | | 5.10 | 0.26 | N |
| | | | 1.89 | 0.33 | |
| 3 | Spherisorb ODS | 19/91 | 6.36 | 0.17 | |
| | | | 80.0 | 0.29 | N |
| | | | 23.0 | 0.24 | |
| 4 | Spherisorb ODS-2 | 19/275 | 8.03 | 0.29 | |
| | | | 7.54 | 0.35 | N |
| | | | 6.76 | 0.35 | |
| 5 | Zorbax ODS | — | 1.72 | 0.45 | |
| | | | 2.09 | 0.43 | C |
| | | | 2.25 | 0.40 | |
| 6 | Hypersil ODS | 4/115 | 6.27 | 0.42 | |
| | | | 3.02 | 0.49 | C |
| | | | 5.75 | 0.47 | |
| 7 | Supelcosil LC-18DB | 0613 | 1.50 | 0.54 | |
| | | | 1.47 | 0.46 | C |
| | | | 1.31 | 0.49 | |
| 8 | Supelcosil LC-18 | 0636 | 2.09 | 0.17 | |
| | | | 2.15 | 0.32 | N |
| | | | 1.20 | 0.21 | |
| 9 | Nucleosil $C_{18}$ | 1101 | 5.13 | 0.19 | |
| | | | 96.0 | 0.22 | N |
| | | | 5.86 | 0.28 | |
| 10 | Partisil ODS | 100348 | — | 0.36 | |
| | | | — | 0.24 | N |
| | | | — | 0.34 | |
| 11 | Partisil ODS-2 | 100633 | — | 0.12 | |
| | | | — | 0.11 | N |
| | | | — | 0.08 | |
| 12 | Partisil ODS-3 | 100663 | — | 0.53 | |
| | | | — | 1.05 | C |

* C = capped; N = uncapped.

*Computer-aided chemical characterisation*

The amenability of Py–MS to automated data handling permits comparison between data sets using multivariate pattern recognition procedures. Such chemometric procedures are necessary when no gross differences in the data sets are apparent and differentiation between samples may well depend upon quantitative differences only[35].
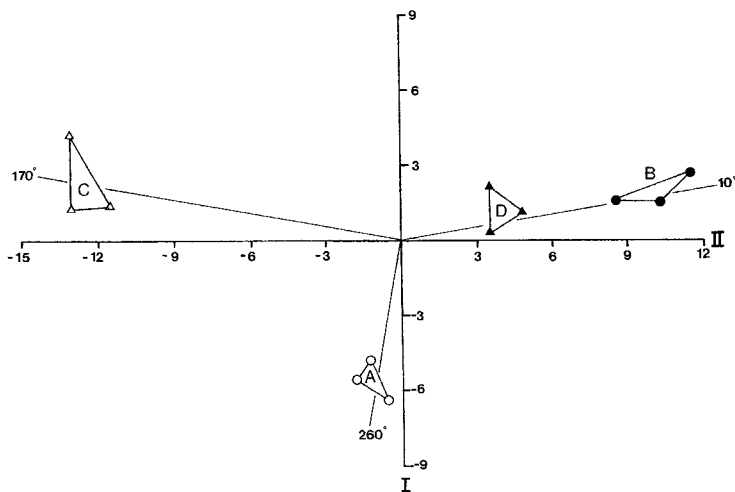
Fig. 2. Characterised data set canonical variate plot, variate I vs. II. Sample designation given in Table I.
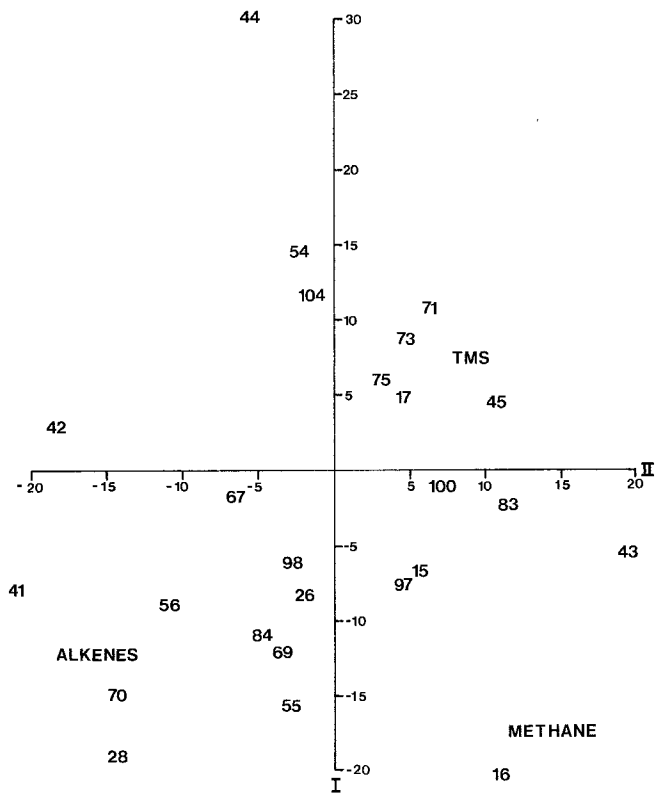
Fig. 3. Characterised data set combined variate loadings, variate I vs. II.

In view of the overall similarity of the pyrolysis mass spectra obtained, canonical variate analysis (CVA), one well established pattern recognition procedure, was carried out in an attempt to characterise chemically the ODS phases studied. The model, chemically characterised, phases and the commercial phases were treated as two separate data sets.

*Model data set*. Application of CVA to the model data set of chemically characterised phases resulted in discrimination of the samples: 96% of the variance within the data set could be expressed in the first two canonical variates (Fig. 2). The mass spectral components responsible for this discrimination are revealed by examination of the variate loadings (Fig. 3). By application of the "factor analysis" technique described, the components responsible for the discrimination of any sample may be derived.

A 10° rotation indicated samples B and D to be capped (Fig. 4). The discriminating masses in the positive spectrum may be assigned tentative structures derived from trimethylsilyl derivatives. This spectrum contains the characteristic $m/z$ 75 mass previously mentioned and the mass $m/z$ 73. These may be assigned the structures[34]:

$m/z$ 75      $(CH_3)_2Si = \overset{+}{O}H$
$m/z$ 73      $(CH_3)_3Si^+$
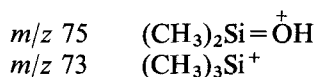
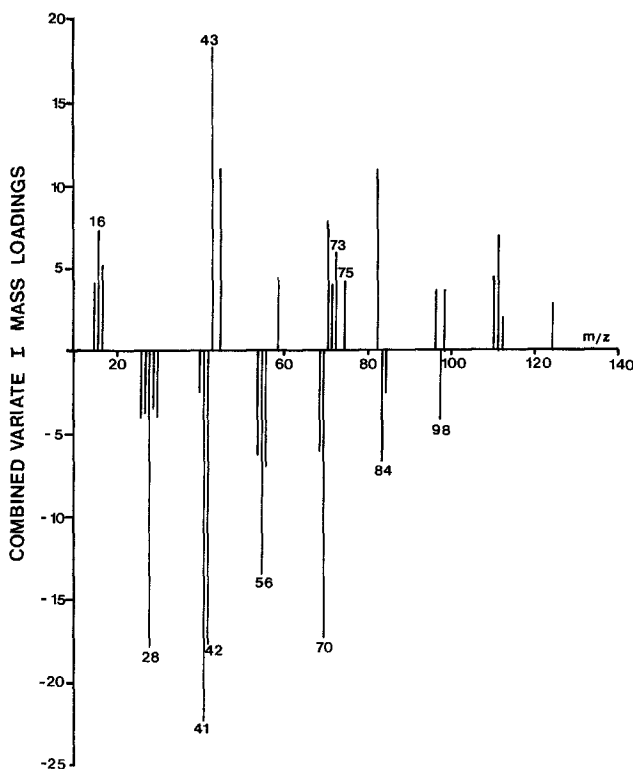Loss of methyl groups from the TMS derivative results in the mass group $m/z$ 15–17.



Fig. 4. Characterised data set. Factor spectra for samples B and D, 10° rotation. Full factor spectrum shown.

The intense mass $m/z$ 43 is attributed to the propyl radical, $C_3H_7$; a component produced by alkyl chain cleavage. This mass is produced by both capped and uncapped phases alike, but has been attributed solely to the capped phase. This problem has been encountered elsewhere with small data sets[35]. The negative factor spectrum contains predominantly masses derived from alkenes ($m/z$ 28, 41, 42, 56, 70, 84) characteristic of alkane thermolysis.

Factor spectra after 260° and 170° rotations contain essentially the same mass spectral components (Fig. 5). The significant differences, $m/z$ 15 and 16, are attributed to the methyl groups of the dimethylchlorosilane used for bonding (Sample A). The trichlorosilane derivative (Sample C) shows no such components.

*Commercial phase data set.* Application of CVA to the full data set of twelve commercial phases resulted in poor discrimination between the samples (Fig. 6). This poor discrimination was attributed to the Partisil phases (Samples 10, 11 and 12) which were revealed as outliers. It is thought that the particle size (10 $\mu$m) may in some way explain this behaviour. All the other phases analysed had a 5-$\mu$m particle size.

Removal of the Partisil phases resulted in good discrimination of the remaining 5-$\mu$m ODS phases: 82% of the variance within the data set was expressed by the first two canonical variates (Fig. 7). Factor analysis (1° rotation) revealed that the com-
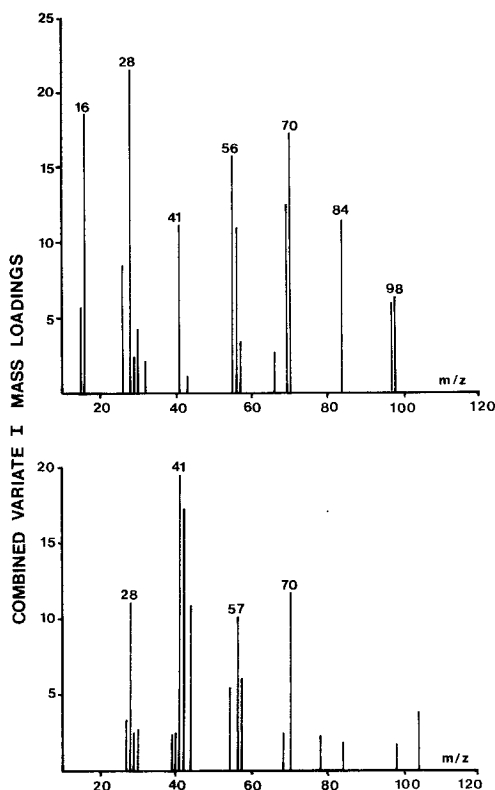


Fig. 5. Characterised data set. Factor spectra for samples A and C. Sample A, 260° rotation; sample C, 170° rotation. Positive factor spectrum only shown.
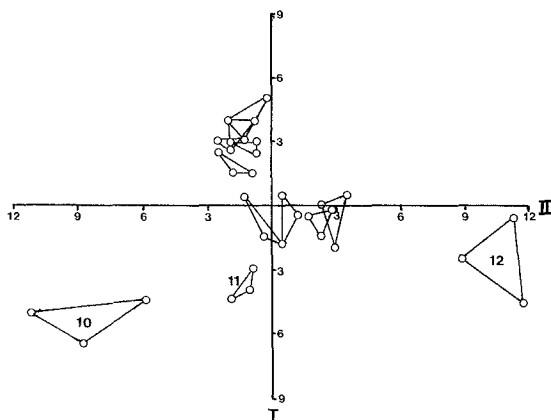
Fig. 6. Full commercial data set (twelve samples) canonical variate plot, variate I *vs.* II. Sample designation given in Table II.

ponents responsible for this discrimination could be attributed to TMS capping as previously discussed. In this case, no $m/z$ 43 mass was observed (Fig. 8). Samples 1, 4, 6 and 7 were thus designated as capped. This result confirms in most cases the tentative capping assignment given by manual characterisation. However, Spherisorb ODS-2 (sample 4), which was indicated to be uncapped by manual characterisation, is revealed as capped by computer-aided characterisation. Zorbax ODS (sample 5) lies intermediate between capped and uncapped phases. Factor analysis reveals that $m/z$ 15 and 16 are the predominant masses, while $m/z$ 73 and 75 do not significantly contribute. Zorbax ODS is designated as uncapped on this basis.

In order to obtain information on the silane functionality used for bonding, the capped (samples 1, 4, 6 and 7) and uncapped (samples 2, 3, 5, 8 and 9) phases
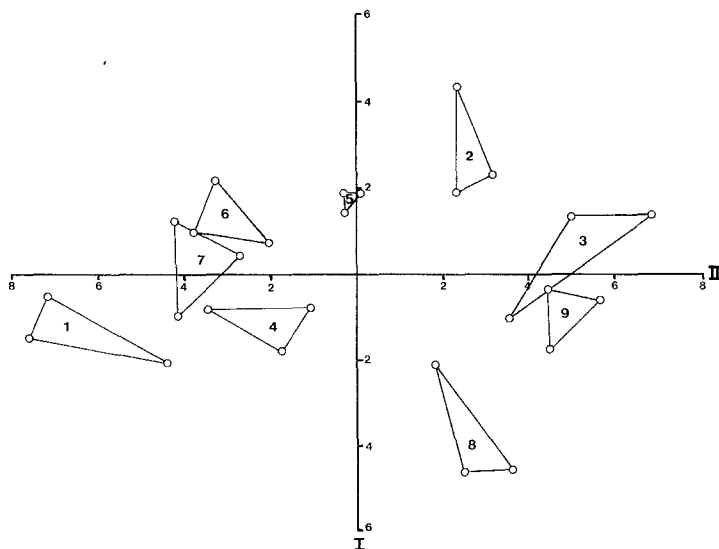


Fig. 7. Commercial data set (nine samples, Partisil phases removed). Canonical variate plot, variate I *vs.* II. Sample designation given in Table II.
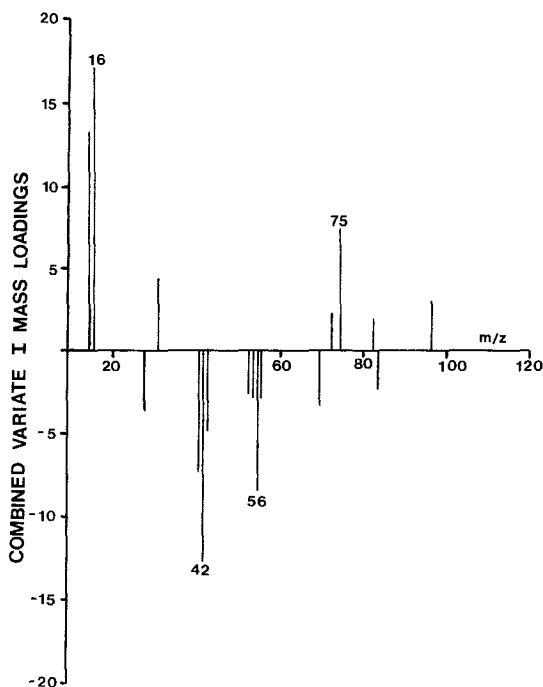
Fig. 8. Commercial data set (nine samples, Partisil phases removed). Factor spectra for capped phases, 1° rotation. Full spectrum shown.

were treated as two separate data sets. Factor analysis reveals that the predominant discriminating factors are $m/z$ 15 and 16, which may now be attributed solely to the methyl groups derived from the silane used for bonding since the influence of masses attributed to TMS has been removed. The intensity of the factors allows tentative assignment of the silane functionality, *i.e.* mono > di > tri (Table III). In some cases the assignment was confirmed by the manufacturer. Supelco phases (samples 7 and 8) were indicated to be difunctional; however, it is reported that these phases are prepared from monochlorosilanes and endcapped with TMS. Endcapping is required

TABLE III

COMMERCIAL ODS PHASES: COMPUTER-AIDED CHEMICAL CHARACTERISATION

| Sample | Brand name | Capping designation | Silane functionality designation |
|--------|-----------|--------------------|-------------------------------|
| 1 | $\mu$Bondapak $C_{18}$ | C | Di |
| 2 | LiChrosorb RP-18 | N | Di |
| 3 | Spherisorb ODS | N | Tri |
| 4 | Spherisorb ODS-2 | C | Tri |
| 5 | Zorbax ODS | N | Mono |
| 6 | Hypersil ODS | C | Tri |
| 7 | Supelcosil LC-18DB | C | Di |
| 8 | Supelcosil LC-18 | N | Di |
| 9 | Nucleosil $C_{18}$ | N | Tri |

since the reaction with the monochlorosilane does not result in a full coverage of the silica[36]. This incomplete coverage explains the difunctionality indicated for these phases, since the total number of methyl groups is less than anticipated for a fully covered monochlorosilane derivatised phase. It is interesting to note that, although both phases were reported to be endcapped, only sample 7 was indicated to be capped by this characterisation.

CONCLUSIONS

The work described in this paper indicates that Py–MS can be used as a rapid method for providing information as to the nature of the silane functionality and deactivated (capped) status of chemically bonded RP-HPLC materials. The interpretation of the complex mass spectral data produced by Py–MS demands the use of chemometric techniques since in most cases the significant differences between spectra are quantitative rather than qualitative. In this study it has proved possible to characterise commercial ODS phases although unambiguous assignments are not, at the present level of development, always possible. Further research on improving the discrimination through optimisation of the pyrolysis and mass spectrometry parameters is required.

ACKNOWLEDGEMENTS

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