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FACTOR ANALYSIS OF REPETITIVELY SCANNED SPECTRA IN GAS CHROMATOGRAPHY–MASS SPECTROMETRY

THE NUMBER OF COMPONENTS IN PARTIALLY RESOLVED PEAKS

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SUMMARY

Matrix rank analysis and principal components analysis have been employed to examine spectra recorded by repetitive scanning during computerized gas chromatography–mass spectrometry with a commercially available system. The number of significant components in partially resolved peaks is given by reference to internal standards. Peak shape need not be strictly maintained during the analysis and no prior knowledge of the pure component spectra is required. The method should allow the detection of hidden impurities, and the determination of the number of components is important for the mathematical resolution of their spectra when chromatography is only partially successful.

INTRODUCTION

The application of computerized gas chromatography–mass spectrometry (GC–MS) to the analysis of complex mixtures is becoming more common. In a number of commercially available systems, mass spectra are recorded in digital form at regular intervals during elution of the sample. The stored data are then a record of the spectral contents of the chromatogram and can be examined by using established techniques for the investigation of spectral series[1–5]. In general, such methods depend on the detection of maxima in plots of parent and fragment ion abundances, or matching of selected significant ions with a library of spectra, and are especially suitable for the analysis of partially resolved peaks. The latter are very commonly encountered during the GC analysis of biological extracts even when using capillary columns. In GC–MS, the purity of a peak is normally tested by comparing two or more fairly widely separated spectra, either visually or by subtraction of their normalized profiles via an interactive display terminal and subsequent examination of the difference spectrum.

An additional technique for the examination of partially resolved peaks in computerized GC–MS would be factor analysis, represented by the numerical meth-

ods of matrix rank analysis (MRA) and principal components analysis (PCA). Both techniques have been separately applied to the determination of the number of components in arrays of absorption spectra[6–14]. MRA was first applied to the mass spectra of mixtures by Monteiro and Reed[15], who investigated a simulated four-component mixture and suggested the application of this technique to mixtures partially fractionated by a number of means, including chromatography. Both MRA and PCA were used by Halket and Reed to calculate the number of components in several mixtures distilled within the mass spectrometer itself[16,17]. Davis *et al.*[18] have applied PCA to the mass spectra obtained during GC–MS of binary gas mixtures and such methods would appear to offer potential for the analysis of repetitively scanned and recorded data. The potential of factor analysis in GC–MS applications has also been emphasized by Ritter *et al.*[19], who employed PCA to determine the numbers of components in hydrocarbon mixtures pre-fractionated by distillation. Recently, both MRA and PCA have been used to analyse data arrays constructed from the mass spectra of liquid chromatographic fractions containing up to four steroids[20], and a simulation study of chromatographic–mass spectrometric data revealed that components having identical retention times or elution volumes can be distinguished in favourable instances.

In the present investigation, MRA and PCA have been applied to some arrays of repetitively scanned spectra such as might be obtained during a typical biomedical investigation using commercially available instrumentation. In each instance, the number of components in single peaks is given and the results can be checked by means of internal standards. The determination of the number of components is the first step in their possible mathematical resolution via the UNRAVL program[21] or earlier approaches[15,22].

EXPERIMENTAL

Reagents and sterols

All reagents were of analytical-reagent grade, obtained from E. Merck (Darmstadt, G.F.R.). Sterols were purchased from Serva (Heidelberg, G.F.R.) or Steraloids (Wilton, N.H., U.S.A.).

Derivatization

Samples were silylated by reaction with a mixture of pyridine, hexamethyldichlorosilane and trimethylchlorosilane for 1 h at room temperature. Excess of reagents was removed in a stream of nitrogen and the residues were sonicated with *n*-hexane for a short time. After centrifugation, the supernatants were taken and used directly for GC–MS analysis.

Gas chromatography–mass spectrometry

An LKB 9000S mass spectrometer was employed, having an ion source temperature of 290° and a separator temperature of 270° in all experiments. In all instances, an LKB 2130 data system was used to provide the repetitively scanned spectra in digitized form. Other conditions were as follows.

Packed column. A 2 m × 3.2 mm I.D. column containing 3% OV-101 on Chromosorb W HP (60–100 mesh) was used. The oven temperature was 240°, injec-

tion temperature 260°, ionization energy 22.5 eV and helium carrier gas flow-rate 30 ml/min. Spectra were recorded on magnetic tape every 15 sec in the range *m/e* 50–500 at a scanning time of 2 sec.

Two samples (internal standards and an algal extract) were injected under these conditions. The internal standards consisted of a mixture of approximately 500 ng each of 5α-cholestane and the trimethylsilyl ethers of cholesterol (5-cholestene-3β-ol), desmosterol (5,24-cholestadien-3β-ol) and fucosterol (24-ethyl-5,24(28) (E)-cholestadiene-3β-ol). The algal extract was subjected to silylation as above and was believed to contain mainly cholesterol and fucosterol, based on GC evidence. The amount injected was sufficient to give peaks of similar size to those obtained with the internal standards.

Wall-coated open tubular capillary column. This column (25 m × 0.35 mm I.D.) was coated with OV-101 (LKB, Bromma, Sweden). The oven temperature was 240°, ionization energy 70 eV and helium carrier gas flow-rate 2 ml/min, made up to 30 ml/min before the double-stage jet separator. "Falling needle"-type solid injection[23] was employed and spectra were recorded on magnetic disk every 4 sec in the range *m/e* 1–400, with a scan time of 1.5 sec. Approximately 500 ng of 5α-cholestane were injected under these conditions.

Data matrices

The formation of a data matrix from a series of repetitively scanned mass spectra is illustrated in Fig. 1.

The curve in Fig. 1 is the computer-reconstructed total ion current chromatogram of a GC peak and the matrix rows represent the repetitively scanned spectra. The convention of representing spectra by the rows of data arrays is adopted here. The basis of the factor analysis approach to mixtures analysis relies on the fact that under normal ion source pressure conditions, the electron-impact mass spectrum of a mixture consists of a linear combination of the pure component spectra. The relationship between the mixtures spectra and the component spectra in a partially resolved GC–MS peak may be represented by the matrix equation,

$$\begin{bmatrix} M_{11}M_{12}M_{13}\ldots M_{1m} \\ M_{21}M_{22}\ldots M_{2m} \\ M_{31}\ldots M_{3m} \\ \ldots \\ \ldots \\ \ldots \\ \ldots \\ \ldots \\ M_{s1}M_{s2}M_{s3}\ldots M_{sm} \end{bmatrix} = \begin{bmatrix} D_{11}D_{12}\ldots D_{1n} \\ D_{21}D_{22}\ldots D_{2n} \\ D_{31}\ldots D_{3n} \\ \ldots \\ \ldots \\ \ldots \\ \ldots \\ \ldots \\ D_{s1}D_{s2}\ldots D_{sn} \end{bmatrix} \times \begin{bmatrix} A_{11}A_{12}A_{13}\ldots A_{1m} \\ B_{21}B_{22}\ldots B_{2m} \\ C_{31}\ldots C_{3m} \\ \ldots \\ \ldots \\ N_{n1}N_{n2}\ldots N_{nm} \end{bmatrix}$$

or,

$$M_{s\times m} = D_{s\times n} \cdot C_{n\times m} \tag{1}$$

M is the matrix containing the superimposed spectra of the n pure components (A, B, C, ..., N) covering the mass range *m/e* 1–m. In this example, there are s spectra of mixtures.

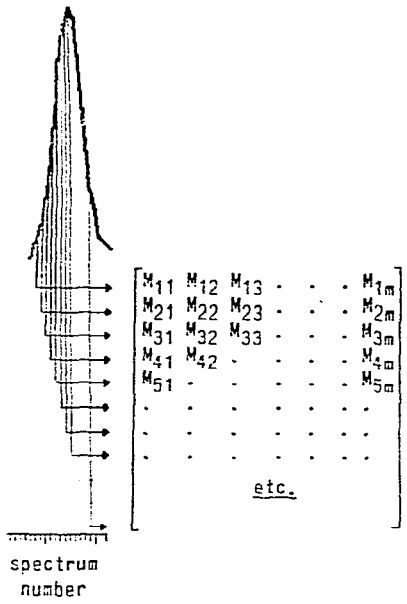

Fig. 1. Illustration of data matrix formation from repetitively scanned spectra in GC–MS.

D is the matrix containing the distributions of the components within the GC peak. The sizes of the elements of this matrix are determined by the relative amounts of the components, their instrumental sensitivities and their retention times.

C is the matrix whose rows are the normalized pure component spectra (n in number).

The matrix equation (eqn. 1) is then similar to that employed in mixtures analysis by mass spectrometry in the petroleum industry, as carried out more extensively before the introduction of chromatographic methods[24]. In this instance, matrices M and C were known together with the instrumental sensitivities, and a matrix related to D, the quantitative analysis, was calculated by matrix inversion. In the present experiments, only matrix M is obtained experimentally and application of factor analysis yields n, the number of pure component spectra.

Data preparation

Intensities (0–16,383 units) provided by the data system for the most significant ions in the successive mass spectra were punched on to cards for processing by the Telefunken TR440 computer of the University of Hamburg. The criteria for selection of m/e values were such as to ensure that the major spectral features were included: peaks higher than m/e 200 and having an abundance greater than 2% of the most intense peak. The higher mass ions ($m/e > 300$) were generally included without regard to abundance. The computer programs were originally written in Fortran IV for use with an IBM system[16]. Sub-routines for the calculation of covariance matrices and eigenvalues were kindly provided by Dr. W. Rehpenning, University of Hamburg.

Matrix rank analysis (MRA)

The arrays of mixtures spectra obtained by repetitive scanning were reduced by a process of gaussian elimination with pivoting[25,26], *i.e.*, the elements of the data array, M_{ij}, were transformed according to the matrix equation

$$M'_{ij} = M_{ij} - \frac{M_{i1} \cdot M_{1j}}{M_{11}} \tag{2}$$

where M' is the transformed array and M_{11} is the largest element (pivot element) in the original data array, pivoted to the leading position by interchange of the relevant rows and columns. The process is repeated for M', the largest element again being pivoted to the leading position (M_{11}). The elimination is repeated for M'', etc., until the absolute value of the pivot element becomes effectively zero, *i.e.*, reaches a value less than or equal to its error in the transformed array. The number of eliminations required to reach this stage determines the number of independent components (rank of the data matrix). For comparison purposes, an error matrix, S, is constructed whose elements are the estimated errors in the corresponding data matrix elements. During the gaussian elimination of M and M', etc., the error matrices are transformed according to a special propagation equation[27]. At each stage, the transformed data matrix elements may be compared with their corresponding errors, the error matrix being pivoted at the same time.

The error matrix, S, is constructed by consideration of the elements in the raw data matrix. This is particularly difficult with GC–MS data because the necessary rapid scanning demands high amplification ratios. As fewer ions are collected for the smaller peaks, their abundance measurements become unreliable[28]. Added to this difficulty are errors caused by the analogue-to-digital converter being used at high speed. Some attempts have been made at overcoming these problems.

It was considered that even a fairly crude method of calculating the errors would suffice for the initial GC–MS studies and the present scheme utilizes errors computed on a simple percentage basis, the smaller elements being assigned greater percentage errors than the larger elements. For data matrix elements having magnitudes greater than 500 units, the error taken in matrix S was 30%, for elements having 101–500 units 40%, for elements having 21–100 units 50% and for elements having less than 20 units 100%. The smaller elements are then assigned very large errors, and this has the effect of filtering away the less significant peaks in the data array. The scheme described here was found to give more satisfactory results than other combinations of errors having smaller percentages. The detection and elimination of large sporadic errors are discussed later.

Principal components analysis (PCA)

Principal components analysis was developed by Hotelling[29] for use in analysing psychological test data and Tatsuoka[30] has prepared a satisfactory introductory text explaining the technique and the concept of linear independence. Other authors have discussed chemical applications[11]. In simple terms, the data matrix is examined by statistical means and the number of eigenvalues found to account for its variance determines the number of components. In the present computer program, an error

comparison routine has not yet been incorporated because of the complexity of the errors associated with GC–MS data and PCA is used here as an independent check on the MRA results, the PCA error limit being determined empirically using internal standards. The PCA program also gives the correlation coefficients of the data matrix rows (spectra) and of the columns (mass profiles), and these have been shown to provide valuable information about both the composite spectra and the spectra of the pure components[22].

Analytical procedure

The present factor analysis scheme and method of comparing the results of two different techniques are illustrated in Fig. 2.

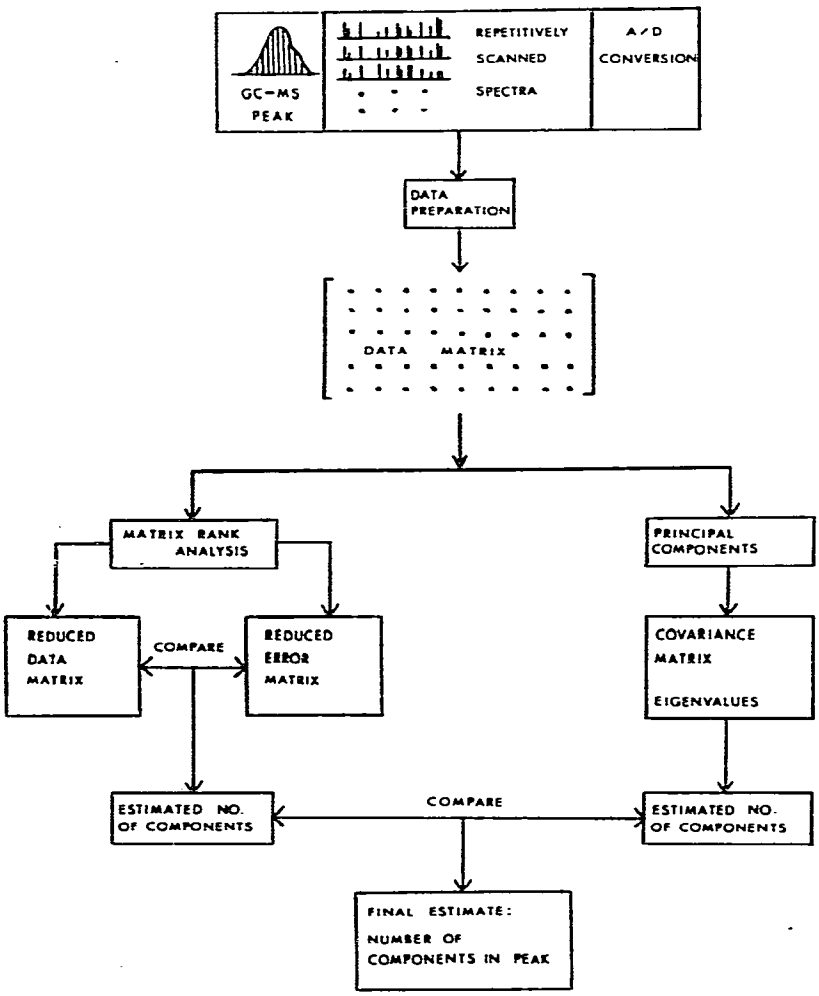

Fig. 2. Schematic representation of the present approach to the factor analysis of GC–MS data arrays by comparison of the results of two different techniques: matrix rank analysis and principal components analysis.

Data manipulation

In addition, the programs allowed rows or columns of the data arrays to be eliminated conveniently during processing in order to determine the effect of the elimination upon the number of components found, *e.g.*, the effect of removing peaks (matrix columns) originating from the column background.

RESULTS AND DISCUSSION

The computer-reconstructed total ion current chromatograms for both of the above packed column examples are shown in Fig. 3A and B, respectively, together with indications of the data matrices formed in each instance, I–VII.

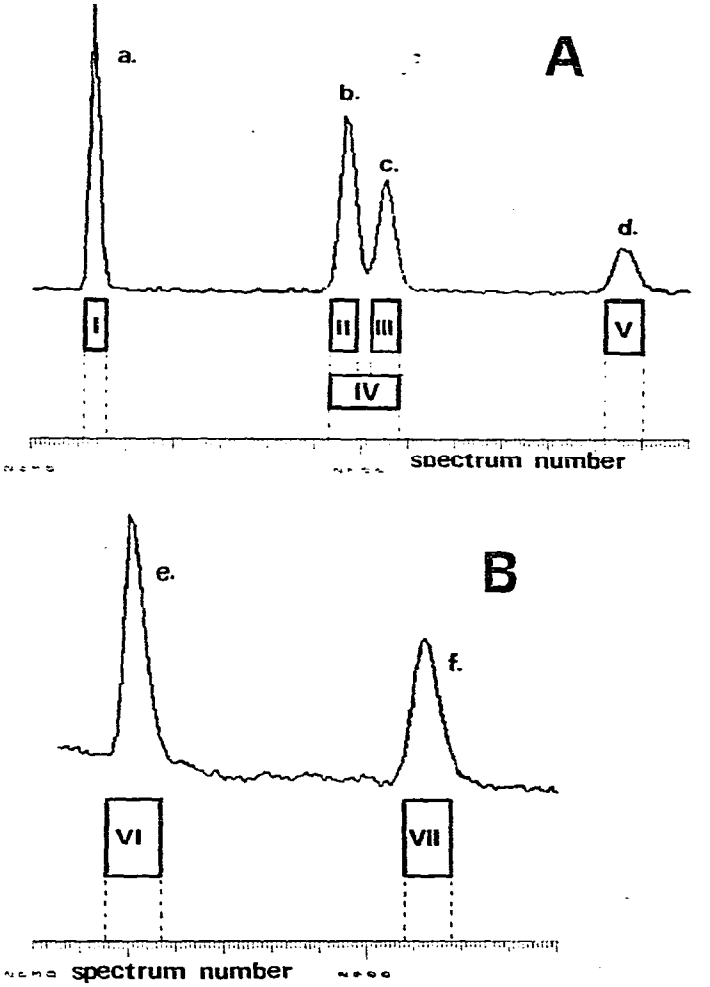

Fig. 3. Computer-reconstructed total ion current chromatograms with indications of data matrices formed in each instance. (A) Sterol standards; (a) 5α-cholestane and trimethylsilyl ethers of (b) cholesterol, (c) desmosterol and (d) fucosterol. (B) Algal extract; (e) cholesterol TMS, partially resolved peak; (f) fucosterol TMS.

The peaks in the selected spectra were chosen as described under *Data preparation*. The numbers of spectra and *m/e* values selected for the raw data matrices were as follows:

I cholestane 6 spectra and 23 masses
II cholesterol TMS 7 spectra and 40 masses
III desmosterol TMS 7 spectra and 37 masses
IV cholesterol TMS and desmosterol TMS 14 spectra and 20 masses
V fucosterol TMS 9 spectra and 23 masses
VI "cholesterol" TMS 13 spectra and 24 masses (algal extract)
VII fucosterol TMS 11 spectra and 24 masses (algal extract)
VIII cholestane 9 spectra and 15 masses (capillary column)

TABLE I

RESULTS OF MATRIX RANK ANALYSIS (MRA) AND PRINCIPAL COMPONENTS ANALYSIS (PCA) OF GC–MS PEAKS BEFORE AND AFTER BACKGROUND REMOVAL

Peak	*Background**	*Spectra × masses***	*MRA****				*PCA*§			
			0	*1*	*2*	*3*	*1*	*2*	*3*	*4*
I	(i)	6 × 23	7432	424	−211	—	52.28	*0.27*	0.04	—
			(2229)	(195)	(*741*)	—				
I	(ii)	6 × 20	7432	−247	—	—	*58.60*	0.04	—	—
			(2229)	(*738*)	—	—				
II	(i)	7 × 40	2650	249	157	—	8.75	*0.10*	—	—
			(795)	(211)	(*1030*)	—				
II	(ii)	7 × 36	2650	249	—	—	*9.57*	0.03	—	—
			(795)	(*1029*)	—	—				
III	(i)	7 × 37	995	340	257	—	1.47	*0.07*	0.02	—
			(298)	(211)	(*351*)	—				
III	(ii)	7 × 35	995	284	—	—	*1.57*	0.03	—	—
			(298)	(*341*)	—	—				
IV	(i)	14 × 20	2650	995	322	257	16.51	1.99	*0.24*	0.04
			(795)	(298)	(212)	(*351*)				
IV	(ii)	14 × 17	2650	995	284	—	19.26	*1.99*	0.05	—
			(795)	(298)	(*341*)					
V	(i)	9 × 23	667	240	180	—	1.61	*0.10*	0.02	—
			(200)	(212)	(*250*)	—				
V	(ii)	9 × 17	667	120	—	—	*1.57*	0.02	—	—
			(200)	(*210*)	—	—				
VI	(i)	13 × 24	1369	624	494	−224	4.56	0.94	*0.33*	0.03
			(410)	(191)	(154)	(*494*)				
VI	(ii)	13 × 20	1369	624	175	—	3.87	*0.60*	0.03	—
			(410)	(191)	(*610*)					
VII	(i)	11 × 24	909	435	243	—	3.20	*0.21*	0.03	—
			(272)	(166)	(*385*)	—				
VII	(ii)	11 × 21	814	167	—	—	*1.91*	0.04	—	—
			(244)	(*185*)	—	—				
VIII§§	(i)	9 × 15	6106	882	—	—	*94.15*	0.64	0.13	0.03
			(1831)	(*925*)	—	—				

* (i) Whole matrix; (ii) after removal of background masses.
** Number of spectra selected × number of masses selected.
*** Pivot elements after each elimination step with errors in parentheses.
§ Eigenvalues of the covariance matrix ($\times 10^{-5}$).
§§ Background insignificant, capillary column.

PCA and MRA results for these data matrices are summarized in Table I, both before and after elimination of the column background ions (OV-101) at *m/e* 207, 281 and 355 and their isotope peaks where appropriate.

The step at which the number of components is determined in each instance is indicated in Table I by italicization of the relevant pivot element (MRA) where it becomes less than its error, or of the eigenvalue (PCA) where it is greater than the eigenvalue which accounts for the error variance in the data. The latter is determined empirically in the present experiments. In cases I–VII, removal of the background ions caused the calculated number of components to be decreased by one. In peak VIII, the background was insignificant.

Matrix IV was included in order to provide an extra component in the array, although the components were well separated on the column. The relative significance of the second-largest eigenvalue for peaks IV indicates the high degree of resolution of the major components.

Peak VI was at first thought to contain only cholesterol. However, the mass spectrum recorded at its centre proved to be very different, revealing an excess of unidentified contaminant(s). The technique of mass chromatography was employed to detect the presence of cholesterol (TMS ether) in the peak and this is clearly shown in Fig. 4.

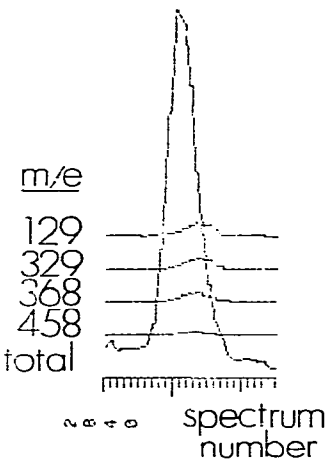

Fig. 4. Mass chromatographic detection of cholesterol (as its trimethylsilyl ether) in peak VI, from an algal extract.

The results for peak VI shown in Table I indicate the presence of two components after background removal, *i.e.*, showing that only *one* unidentified substance is present in significant amounts.

The level of significance of the eigenvalues in the examples studied here appear to lie at approximately 0.05 ($\times 10^5$) under the conditions employed. The tolerance for peak VIII (capillary column) lies at a higher level, a probable consequence of the increased magnitude of the mass spectral peaks and the increased scanning speed employed.

The data from the packed column (peaks I–VII) were not obtained with this study in mind but were selected from data files because a mixed peak had been

observed. A rather large interval (15 sec) had been chosen between the scans so that the amount of data obtained was limited. A smaller interval would have increased the reliability of the results by providing more data. In addition, an ionizing voltage of 22.5 eV had been employed in order to provide more abundant higher mass ions. The spectra obtained were observed to be very reproducible in spite of the fact that the ionization efficiency curve would be expected to be steep at this level. Further studies are being carried out to compare results obtained at different voltages.

Experimental errors and conditions

As the mass spectra become more noisy, perhaps as a result of greater amplification or faster scanning, the apparent number of components will increase and greater tolerances will be required to compensate. Eventually, a stage will be reached where the noise will blur the all-important real differences in the repetitively scanned spectra, making a satisfactory analysis impossible. Thus, certain experimental conditions must be satisfied, as follows.

(1) The basis of the method is the linear additivity of the pure component electron-impact mass spectra, so that these underlying patterns must be maintained throughout. This condition demands that the scanning speed be fast enough to ensure that only a negligible change in sample concentration or composition can occur during a scan. In addition, other factors that cause the spectral patterns to change must be kept constant, *e.g.*, ion source temperature and ionizing energy. Such conditions have been discussed by Beynon[31] in connection with mixtures analysis. Satisfaction of this condition may require very rapid scanning with a corresponding decrease in the reliability of the measurements.

(2) A sufficient number of spectra must be recorded during peak elution. Spurious peaks and larger system errors then have an improved chance of being detected and eliminated. The number of significantly different spectra recorded must be at least one greater than the number of pure components, otherwise the data matrix will not reduce and the true number of components will remain unknown. It is clear that the method has only the possibility of determining the components contained within the spectra and *m/e* values selected.

Minimization of these difficulties may necessitate the employment of a lower column temperature or carrier gas flow-rate. However, such changes may well be consistent with a higher degree of resolution of the components.

In practice, determination of the number of significant components by factor analysis would appear to be best approached by the use of internal standards.

Internal standards

Experimental difficulties may be much reduced by the analysis of internal standards under the same conditions as for the samples. A pure substance or mixture giving a known mixed peak eluting before the peaks of interest is injected and its known rank determined by the programs. The same routine and error treatments are then applied to unknown peaks having slightly longer retention times, where the rate of change of composition and concentration is less under isothermal column conditions. This is also a means of ensuring that the scanning speed is sufficiently fast, *i.e.*, that condition (1) above is satisfied. Of course, the number of spectra obtained for the unknown peaks may be insufficient, *i.e.*, equal to or less than the

number of partially resolved components. Such a situation is easily recognized because the calculated rank of the matrix will remain at a maximum, equal to the smaller data matrix dimension.

Detection of gross errors

The presence of undetected gross errors in the data matrix can seriously affect the results obtained. Some methods of detecting such errors are described here.

It is apparent that the neighbouring spectra repetitively scanned over a multicomponent peak should have fairly similar patterns if condition (1) above is satisfied, as compared with the patterns of widely separated spectra. A measure of the similarity of the spectral patterns is given by their correlation coefficients, so that neighbouring spectra should show very similar coefficients when measured from any one spectrum chosen as a reference. It would be reasonable to adopt the most intense spectrum as the reference, because the measurements will be less reliable at the peak extremes. As already mentioned, the correlation coefficients have been calculated by the PCA program and are to be found in the relevant row of the correlation matrix of the spectra in the data array. The listing of such coefficients allows fractionation profiles to be constructed for each peak. Such profiles are independent of the total ion current peak shape and even normalized spectra could be employed in their calculation. Fractionation profiles for peaks I, V, VI and VII are represented in Fig. 5, together with the corresponding total ion current profiles.

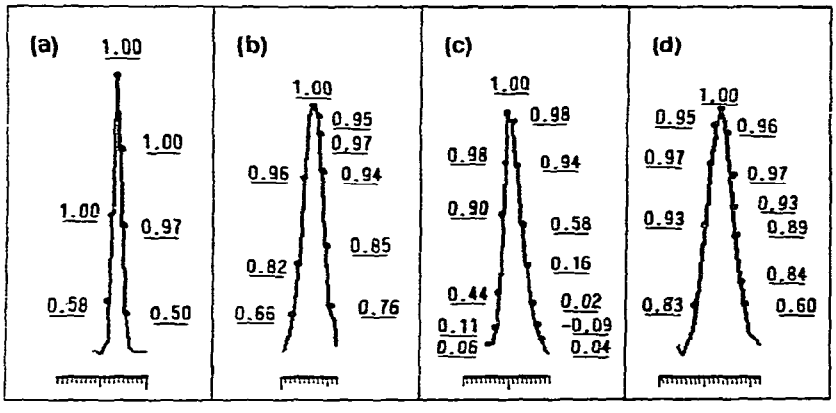

Fig. 5. Fractionation profiles for the mass spectra in (a) peak I (cholestane), (b) peak V (fucosterol TMS standard), (c) peak VI (partially resolved peak from an algal extract) and (d) peak VII (fucosterol TMS from an algal extract).

An idea of the information potentially obtainable from such profiles is shown in Fig. 5c, where the presence of at least one extra component in peak VI is indicated by the rapid fall in coefficient magnitude.

Examination of such profiles may allow spectra to be selected for inclusion in the data matrix as spectra containing gross errors are readily identified by a deviating coefficient. Unlike other methods for the examination of partially resolved GC peaks[32–34], the results obtained by factor analysis are independent of the peak shape, *i.e.*, the attenuation of the detector may be changed between spectra, provided

that the changing errors are monitored. Even when applying curve-fitting techniques to overlapping peaks, it is important to know their number[34].

A more precise method for the elimination of larger acquisition errors would be the examination of the fragment ion curves and the correction of deviations by curve-smoothing methods. However, this approach demands the maintenance of peak shape.

Capillary columns

The results obtained for peak VIII indicate that the methods will also give reasonable results with capillary columns, where the rate of change of concentration and composition may be much higher than with packed columns, necessitating faster scanning speeds. This problem may be partially offset by scanning over a more limited mass range.

Detection of impurities in GC peaks

The figures in Table I show that the column background can be readily distinguished from the major peak components. The background may be regarded as an impurity, although in several of the examples studied here, *e.g.*, peaks III, V and VII, one of the background fragment ions, *m/e* 207, was found to be of the same order of magnitude as the sterol fragments. In spite of this, the results indicate that the computer programs are capable of distinguishing minor components and further studies are being carried out to determine their limits of detection.

Analyses may be made of whole mass spectra or of selected ions but the maximal feasible matrix size has not been determined here. The methods may be regarded as numerical analysis of a mass chromatogram in array form where peak shape is not critical for component detection. Factor analysis should allow an index of the purity of GC peaks to be given. This would be a particularly important consideration in the GC–MS examination of industrial materials for the presence of small amounts of potent carcinogenic impurities which could seriously upset the results of carcinogenicity studies[35]. A similar application would be in the analysis of pharmaceutical preparations for potentially dangerous contaminants, co-eluting with the major component. The methods have many other potential applications, *e.g.*, to air contaminant profiles obtained by the thermo-gradient tube technique developed by Angerer and Haag[36], and such a study is in progress.

CONCLUSION

Two different methods are employed to determine the number of components in partially resolved GC peaks, one technique (principal components analysis) providing an independent check on the results of the other (matrix rank analysis). The results are promising and show that the techniques can be easily applied to repetitively scanned mass spectra from commercially available equipment. A more extensive study is being performed in order to evaluate the limitations of the methods with more complex peaks and over wider concentration ranges of the components.

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