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Short column gas chromatography–mass spectrometry and principal component analysis for the identification of coeluted substances in doping control analysis

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Abstract

The identification of four doping control substances in an artificial mixture, using short column gas chromatography–mass spectrometry (GC–MS) analysis was examined. Two chromatographic peaks were recorded in the chromatogram, using a short capillary column (1.8 m) at an oven temperature of 180°C. The first peak was associated with a mixture of a solvent derivative and an artifact. The second one corresponded to the mixture of four control substances. Principal component analysis was applied on a selected GC–MS data set of the latter peak to determine clear full spectra of pure substances from mixture spectra. The time of GC–MS analysis was significantly reduced to less than 1 min from 30 min which is a typical GC–MS analysis time, using standard methods of doping control analysis. © 1998 Elsevier Science B.V.

Keywords: Principal component analysis; Doping control; Dextromethorphan; Methadone; Dextropropoxyphene; Cocaine

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) analysis is used among other techniques in doping control analysis [1]. The routine GC–MS analysis is time consuming and this is particularly critical when a large number of samples has to be examined. In addition, there are cases where two or more coeluted substances have fully overlapped chromatographic peaks. These can only be resolved by using either a different column or a different oven temperature profile. This results in an increase in the overall time of analysis. Consequently, the development of methods that could reduce the analysis time without sacrificing the analytical information is of practical interest. More generally, reduction of time of analysis can be done through the methods and the techniques of fast GC analysis (high speed GC) [2–6] and more particularly through the use of short chromatographic columns [7]. It should be emphasized that in most cases reported in the literature for high speed GC the basic focus is on preserving the GC resolution by optimizing the chromatographic conditions and/or developing specific instrumentation. However, when the focus of the analysis is on reducing the time without using a specific instrumentation, then methods for data analysis could be a tool for resolving overlapped GC peaks. Short column GC–MS analysis can significantly reduce the time of analysis but it may result in low quality mass

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spectra (mixture spectra), derived from overlapped GC–MS peaks. Multivariate data analysis (MDA) techniques have been used for resolving complicated mixture spectra [8–10] such as those of coals [11] and biological samples [12–14] and for detecting and resolving overlapped peaks [15,16]. The isolation of pure spectra in GC–MS analysis by means of mathematical methods has been reported [17,18]. In this work a GC–MS analysis, using a short capillary chromatographic column was performed as an alternative to the routine one, on a mixture sample containing four control substances, possibly found in doping control analysis. Principal component analysis (PCA), an MDA technique, was tested as an alternative method for resolving overlapped components eluted in a single chromatographic peak.

2. Theoretical

PCA is in principle, a data reduction technique applied on a number of variables. Data reduction is achieved by finding linear combinations of the original variables which account for as much of the original total variance as possible. The successive linear combinations are extracted in such a way that they are uncorrelated with each other and account for successively smaller amounts of the total variance. Each successive component accounts for as much as possible of the variation not accounted for by the previous components. The mathematical basis of PCA is discussed elsewhere [19,20].

In this work PCA combined with graphical rotation was used for finding the real dimensions of the data and extracting ''PC spectra'' which can be attributed to the mass spectra of the chemical components. Graphical rotation [21] is a technique which transforms the abstract mathematical solution of PCA into chemically meaningful results. It consists of a systematic-stepwise rotation of PCs till a recognizable pattern of mass spectra occurs.

A special type of diagram (VarDia) [9,22] was used to provide with indications (maxima) for directions in PCs' space with highly correlated variables. The rotated ''PC spectra'' in these directions might coincide with the pure mass spectra of the substances under investigation. Some times, pure spectra are recognized in directions not shown by the VarDia. In these cases the VarDia is used as a platform for graphical rotation.

3. Experimental

3.1. Apparatus

A gas chromatograph (HP-5890 series II) equipped with an electron impact (EI) mass selective detector (HP-5972) was used. The chromatographic column used was an ULTRA2 (1.8 m×0.2 mm I.D., 0.33 μm film thickness; Hewlett–Packard). A 0.5 μl volume of the sample was injected each time in the column. The carrier gas was helium with a flow-rate 1.2 ml/min at 70°C (determined experimentally by injection of a sample of air) and the split ratio was 1/40. The inlet temperature was 250°C and the transfer line GC–MS temperature was 280°C. The EI source was tuned at 70 eV. The scan duration was 2.6 scans/sec. The mass range used for MS data acquisition was 40–310 amu.

3.2. Materials

The following stock solutions of four control substances in methanol were used: dextromethorphan hydrobromide (9200 ppm), methadone hydrochloride (3400 ppm), dextropropoxyphene hydrochloride (4100 ppm) and cocaine hydrochloride (4300 ppm). All the solutions were chemically pure.

3.3. Procedure

A mixture sample of the above four control substances was prepared by diluting each of the above stock solutions in methanol and then mixing, giving final concentrations as follows: 50 ppm dextromethorphan hydrobromide, 50 ppm methadone hydrochloride, 100 ppm dextropropoxyphene hydrochloride and 50 ppm cocaine hydrochloride. A low oven temperature profile (isothermal at 130°C) was firstly used in GC–MS analysis to separate each control substance. In this way the experimental full clear spectra were acquired (total time of analysis 5 min). A higher oven temperature profile (isothermal at 180°C) was used to get the unresolved chromatographic peak corresponding to the mixture of the

four control substances with total time of analysis less than 1 min.

3.4. Software used

The mathematical analysis of the spectrometric data was carried out using the multivariate data analysis software PONTOS [23]. This program runs under the Microsoft Windows environment. Currently, the program includes the techniques of PCA, discriminant rotation and canonical correlation analysis. A ''Wiley138'' library search system was used complementary to other references [24] for the identification of the recorded mass spectra.

4. Results and discussion

In Fig. 1, the short column GC–MS chromatograms of the mixture of four control substances at 130°C and 180°C oven temperature respectively are presented. At low temperature (Fig. 1a) all the four control substances were separated and the corresponding full mass spectra of each control substance was clearly identified using retention times and the significant mass peaks suggested [24]. This was also reconfirmed by the library search system of the GC–MS instrument. In the same figure the two additional chromatographic peaks correspond to a solvent derivative (retention time 0.05 min) and an artifact (retention time 0.39 min). GC–MS analysis at 180°C (Fig. 1b) drastically reduced the time and resolution and as a result only two chromatographic peaks were recorded. The first peak corresponds to the solvent derivative and the artifact and the second one corresponds to the mixture of the four control substances. The mass spectrum at retention time 0.366 min, which is the second peak of the chromatogram presented in Fig. 1b, is given in Fig. 2a. This is a mixture spectrum having mass peaks attributed to dextromethorphan (e.g. peaks 59, 271, 150, 214, 42, 171), methadone (e.g. peaks 72, 91, 223, 165), dextropropoxyphene (e.g. peaks 58, 117, 208, 115, 91, 130) and cocaine (e.g. peaks 82, 182, 83, 105, 303, 77, 94). In order to reconfirm the

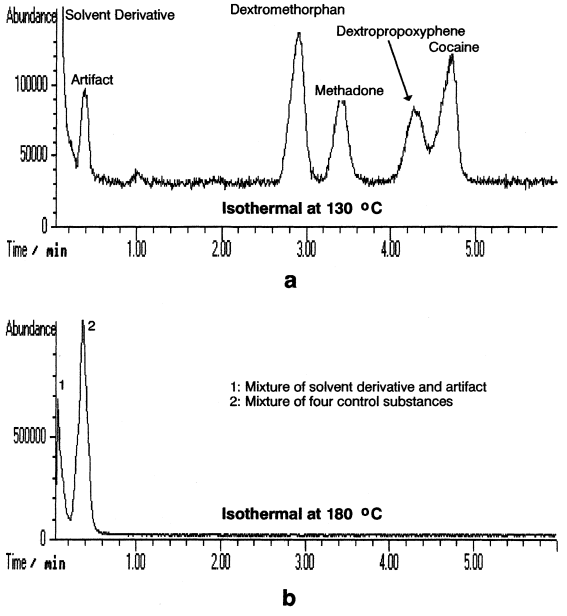

Fig. 1. Short column GC–MS chromatogram of the mixture of the four control substances: (a) in a low isothermal oven temperature profile; (b) in a higher isothermal temperature profile.

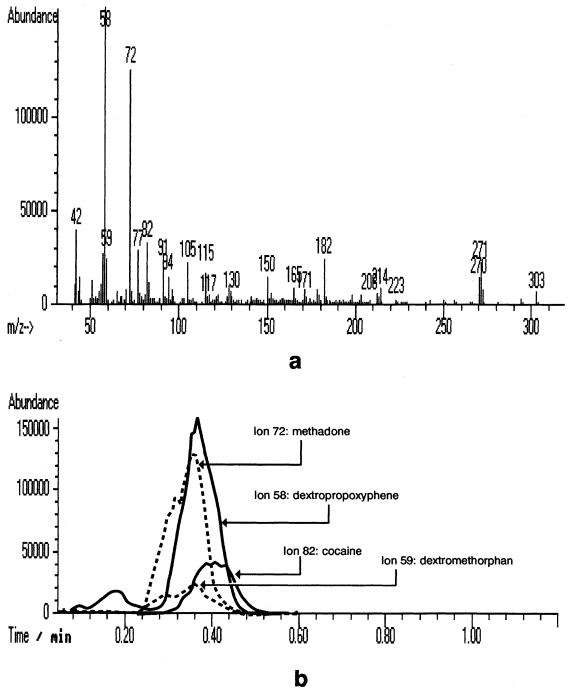

Fig. 2. (a) Representative mass spectrum at 0.366 min of the short column GC–MS analysis. (b) Extracted ion chromatograms of characteristic masses corresponding to each of the four control substances

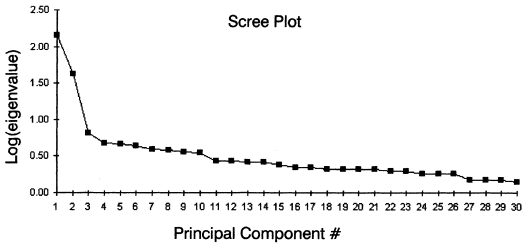

Fig. 3. Logarithmic scree plot.

coelution of all the above control substances, the extracted ion chromatograms are given in Fig. 2b. It should be noticed that there is only a small shift among the extracted ion chromatograms. This small shift is a basic prerequisite for the application of PCA.

PCA was used to investigate if clear full spectra could be extracted from mixture spectra in the time range 0.239 to 0.506 min (41 spectra) of the second unresolved chromatographic peak of Fig. 1b. The raw data matrix (41×271) consisted of 41 objects (scans 30 to 70) and 271 variables (masses 40 to 310). The data matrix was autoscaled prior to PCA (i.e. subtraction of the mean value and division by the standard deviation of each variable was performed). The eigenvector calculations were then done on the so-called correlation around the mean dispersion matrix. By presenting the eigenvalues of the PCs in logarithmic scale and using the scree plot criterion [20] the first four PCs were selected, together explaining 72% of the total variance (Fig. 3).

PC spectra presented below were produced by multiplication of the resulted PC loadings with the standard deviation of each variable.

In Fig. 4a the VarDia diagram of PC2/PC3 space is presented. The rotated PC2/PC3 space at 285°

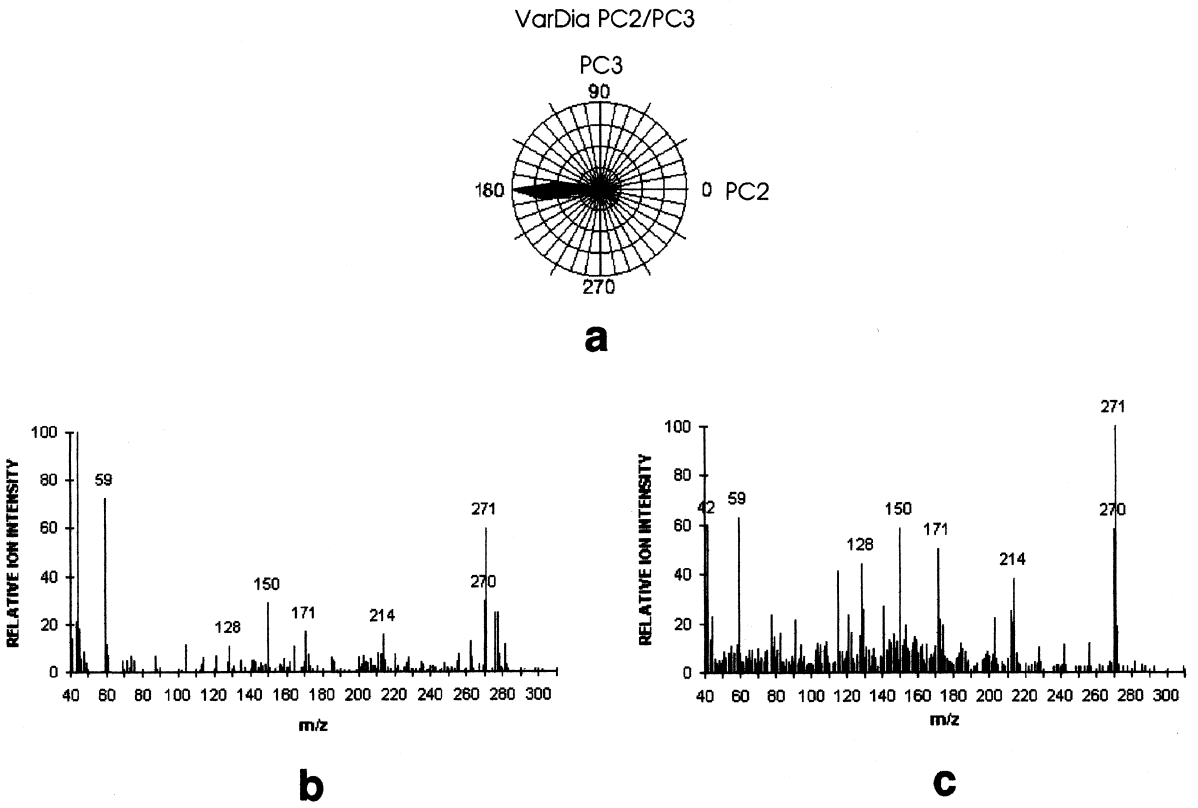

Fig. 4. (a) VarDia diagram of PC2/PC3 space. (b) Principal component spectrum PC2 (positive part) of the rotated PC2/PC3 space at 285°. (c) Mass spectrum of pure dextromethorphan extracted experimentally.

produced the PC2 ''PC spectrum'' which appears in Fig. 4b. In Fig. 4c the experimentally recorded mass spectrum of dextromethorphan is presented for comparison. It should be noticed that this direction (285°) is not clearly shown on the VarDia. It was determined by scanning the PC2/PC3 space and rotating the original PCA solution. However, mathematical methods exist for the determination of pure components [25]. In Fig. 5a and Fig. 6a the VarDia diagrams of PC2/PC4 and PC3/PC4 spaces are presented respectively. By similarly rotating properly, ''PC spectra'' matching to the other three control substances were found. More specifically, rotation of PC2/PC4 space at 260° produced the rotated PC2 spectrum whose positive part (Fig. 5b) shows similarity with the experimentally recorded clear mass spectrum of cocaine (Fig. 5c). The PC3 spectra extracted by rotation of PC3/PC4 space at 100° (Fig. $6b_1$) and 300° (Fig. $6b_2$) are correlated with the experimentally recorded pure mass spectra of dextropropoxyphene (Fig. $6c_1$) and methadone (Fig. $6c_2$) respectively.

5. Conclusions

PCA as applied in this work provided clear full spectra, for a short column GC–MS analysis of a mixture of substances possibly found in doping control analysis. It should be emphasized that these substances are generally important in drug analysis. By using a short GC column a dramatic reduction in time of analysis was achieved compared to routine GC–MS analysis. The method is especially useful if documentation with full mass spectra of the control substances is necessary. Yet, it should be mentioned that the method is currently used as an exploratory one and further research should be carried out to

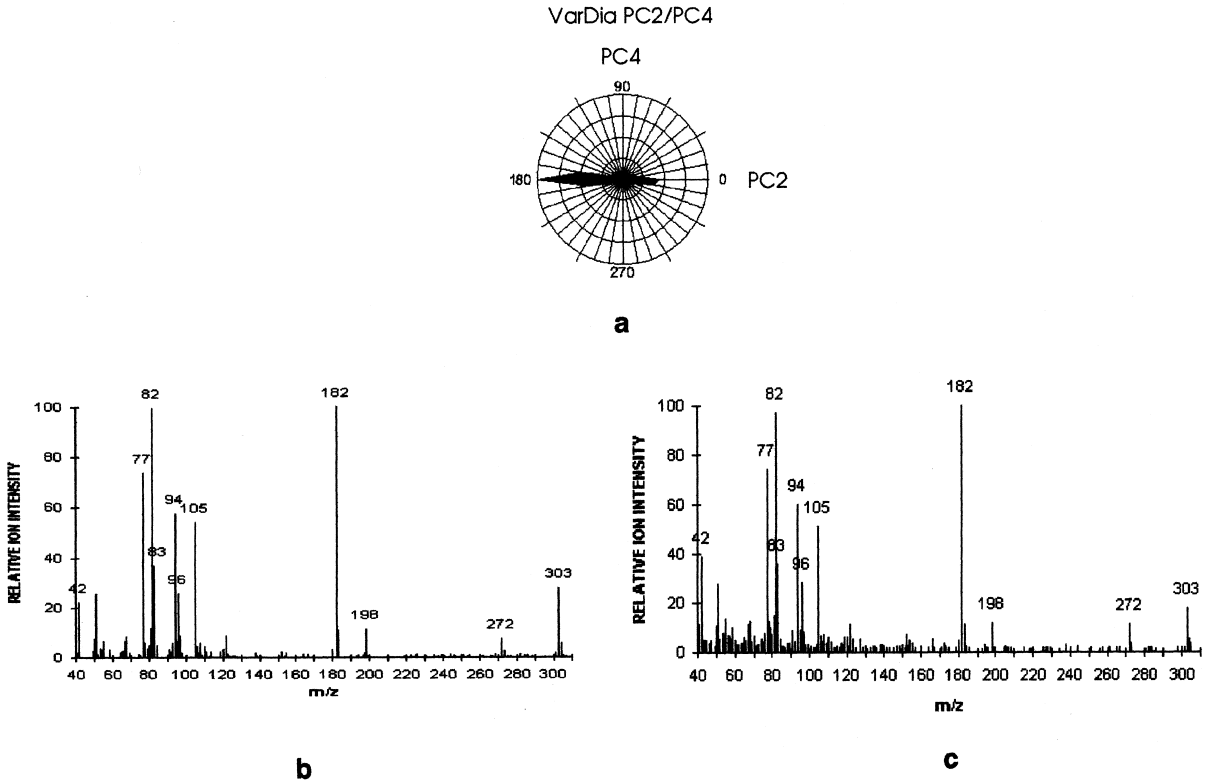

Fig. 5. (a) VarDia diagram of PC2/PC4 space. (b) Principal component spectrum PC2 (positive part) of the PC2/PC4 space rotated at 260°. (c) Mass spectrum of pure cocaine extracted experimentally.

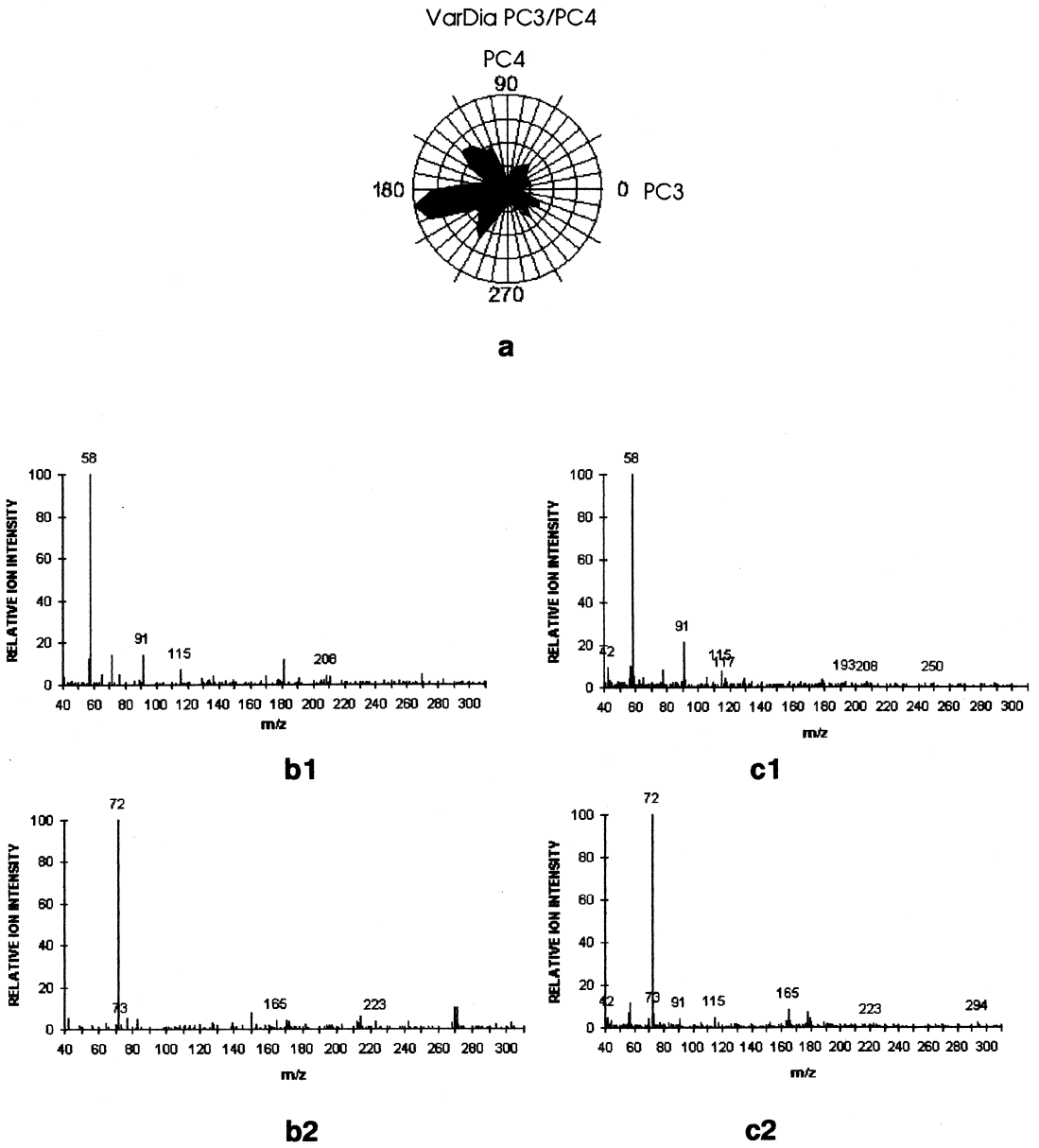

Fig. 6. (a) VarDia diagram of PC3/PC4 space. (b_1) Principal component Spectrum PC3 (positive part) of the rotated PC3/PC4 space at 100°. (b_2) Principal component spectrum PC3 (positive part) of the rotated PC3/PC4 space at 300°. (c_1) Mass spectrum of pure dextropropoxyphene extracted experimentally. (c_2) Mass spectrum of pure methadone extracted experimentally.

automate the extraction of the pure components' spectra. Among those methods are the automated comparison of the extracted ''PC spectra'' with a set of target mass spectra and/or the searching of ''PC spectra'' in commercial electronic libraries of mass spectra. From the analytical point of view the determination of the detection limit of the proposed method compared to the method of selective ion monitoring is also of high interest and is under investigation.

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