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Complex mixture analysis based on gas chromatography–mass spectrometry with time array detection using a beam deflection time-of-flight mass spectrometer

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ABSTRACT

A beam deflection time-of-flight mass spectrometer was developed in conjunction with an integrating transient recorder to provide time array detection, permitting high mass spectral scan file acquisition rates for complex mixture analysis by capillary gas chromatography–mass spectrometry (GC–MS). Results are presented for the analysis of a urinary organic acid mixture by GC–MS at a scan file acquisition rate of 10 scan files per second (sf/s), showing the advantages of such data collection in the deconvolution of partially resolved components. The reconstructed total ion current (RTIC) chromatogram available from data acquired at this scan file generation rate is shown to be comparable to the profile obtained from a flame ionization detector in representing the chromatography performed under identical experimental parameters. The RTIC chromatogram available from the database obtained at 10 sf/s is compared with that available from a database obtained at 1 sf/s, the latter representing that scan rate typically used with most GC–MS instruments. The advantages of the higher scan file acquisition rate in representing the chromatographic profile and in allowing mass spectral data to be obtained for components in the complex mixture that are unresolved chromatographically are discussed.

INTRODUCTION

This paper focuses on problems associated with complex mixture analysis by gas chromatography–mass spectrometry (GC–MS), in the context of capillary GC. A solution to problems with this technology is discussed by presenting time-of-flight mass spectrometry (TOF-MS) as the mass spectrometric detection technique of choice for this work. With such instrumentation, in which mass spectra can be recorded at exceeding high rates when required, high-quality mass spectra can be obtained for all components in the mixture, and these data can be used to improve the results of GC beyond those preivously reported in the context of conventional GC–MS.

Advances in high-resolution GC in the past decade are manifested in the development of narrow-

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bore chromatographic columns, which, with their tens of thousands of theoretical plates, provide chromatographic peaks of the order of 2 s or less in duration. Extremely high-efficiency capillary columns have seen limited use in GC–MS applications owing to limitations in the scan rates available with the quadrupole and magnetic sector mass spectrometers commonly used in GC–MS instrumentation [1]. Scan rates of a quadrupole instrument are limited by the transit time of the ions through the mass filter. In order to obtain unit mass resolving power, the radio frequency (r.f.) and dc potentials applied to the quadrupole must be held at fixed values for a sufficient length of time (milliseconds) to allow ions of a given mass-to-charge ratio (m/z) to pass through the device. Magnetic sector instrument scan rates are limited by the rate at which the magnetic field can be swept.

Another problem in capillary GC–MS experiments that employ quadrupole or magnetic sector devices is that the mass spectra obtained from these scanning mass spectrometers can be distorted by the changes in the partial pressure of an analyte as it elutes from the capillary column; the distortion manifests itself as a skewing of the relative mass spectral peak intensities. The degree of this skewing is dependent on the time necessary to acquire a mass spectrum and the rate at which the partial pressure of the analyte changes in the ion source (sharpness of the GC peak).

We have proposed the use of TOF-MS for GC–MS instrumentation [1,2] to overcome the limitations of scanning mass spectrometers. Time-of-flight mass spectrometers produce as many as 10 000 mass spectra every second, which, if ion currents at all m/z values were collected for each of these transient mass spectra, permits an increase in the data acquisition rate for GC–MS. Importantly, time-of-flight mass spectrometers operated in this way are not "scanning" instruments such as a quadrupole or magnetic sector instrument. The mass spectra produced by TOF-MS result from the sampling of ions produced in the ion source at a given time interval. This process produces mass spectral transients (100-μs duration), in which the relative peak intensities are unskewed regardless of changes in analyte partial pressure (on any time scale) in the ion source [1].

We have developed time array detection (TAD) for TOF-MS by using an integrating transient recorder (ITR) [3]. The ITR is a data system that can continuously collect up to 10 000 transient mass spectra per second, as produced by TOF-MS. Each transient mass spectrum is digitized at 200 MHz (one data point every 5 ns). Many transient mass spectra are summed by the ITR (typically 500–1000), creating a scan file that contains the resultant (summed) mass spectrum. The number of transient mass spectra summed into each scan file determines the scan file generation rate. For example, if 5000 transient mass spectra are produced every second in TOF-MS and a scan file generation rate of 10 scan files per second (sf/s) is desired, 500 consecutive mass spectral transients are summed and stored as a single mass spectrum in each scan file. The scan file generation rate can be adjusted easily to meet the needs of the chromatographic analysis. Ideally, at least ten scan files should be generated in a time period corresponding to the temporal width of the sharpest chromatographic peaks. If 50 sf/s are necessary to represent the chromatography accurately, the ITR data system can easily accommodate this.

An instrument for beam deflection time-of-flight mass spectrometry (BDTOF-MS) has been developed that provides transient mass spectra to the ITR with a mass-resolving power sufficient for GC–MS applications [4,5]. The advantages of the BDTOF-MS over conventional (pulsed-source) TOF-MS instruments have been described [6]. In BDTOF-MS, a continuous ion beam is focused into the flight tube where it passes between two parallel plates. Each of the parallel plates in the beam deflection assembly has a time-dependent voltage applied to it, producing an electric field between the plates. Most of the time, the continuous ion beam is deflected away from the center axis of the flight tube by the electric field between the parallel plates; under these conditions, no ions reach the detector at the end of the flight tube. Reversing the sign of the electric field between the parallel plates sweeps the continuous ion beam from one side of the flight tube to the other, allowing a portion of the continuous ion beam, containing all m/z values, to strike the detector in a time-dependent manner. BDTOF-MS has produced a mass-resolving power ($m/\Delta m$) of 1400, which, when coupled with the performance of the ITR, provides a powerful system for GC–MS applications.

This paper describes the results of analyses using the GC–BDTOF-MS–ITR system in the context of metabolic profiling of urinary organic acids. Metabolic profiling is a method for the quantitative and qualitative analysis of metabolites in complex mixtures extracted from physiological fluids such as urine or plasma [7]. The coupling of GC and MS has played a crucial role in the development of methodologies for such analyses. The metabolic profile of urinary organic acids reflects metabolic status and allows for the identification of genetic disorders that perturb homeostasis. The metabolic profile, which is a chromatogram of the components of the mixture, portrays the relative amounts of these metabolites as derived from intermediary metabolism of carbohydrates, amino acids and lipids in the individual. Improvements in metabolic profiling have been limited in the same way as have analyses of other complex mixtures. Even with capillary column technology, chromatographic resolution is not sufficient to separate every component in the mixture and some components in these complex mixtures may therefore go undetected.

This paper compares the results of the GC-only analysis of a urinary organic acid mixture using flame ionization detection (FID) with those obtained with the GC–BDTOF-MS–ITR system at data acquisition rates of 1 and 10 sf/s. The advantages of the higher scan file acquisition rate are demonstrated, both in terms of accurately representing the chromatographic profile and in providing a database from which mass spectra can be obtained for closely eluting components.

Two points should be made clear. First, there are quadrupole and magnetic sector instruments available today that can obtain several mass spectra per second. However, these faster scanning speeds cannot be used without sacrificing sensitivity. The TOF instrument as described also has a sensitivity *versus* scan file generation rate trade-off, although all TOF mass spectra are unskewed. The second point is that the scan file generation rate of 10 sf/s is not the limit of this instrument, but the optimum for the chromatography used in the application. If the chromatography was improved to generate chromatographic peaks with temporal dimensions of 0.2 s, the instrument could generate 50 sf/s, giving ten scan files across each chromatographic peak. Such mass spectral generation rates are unavailable when scanning instruments are used. At a scan file generation rate of 50 sf/s, each scan file represents the sum of 100 mass spectral transients; thus, a scan file generation rate greater than 50 sf/s is possible, but with a sacrifice in sensitivity.

EXPERIMENTAL

The BDTOF-MS instrument in these laboratories is a highly modified Bendix 12-101 TOF-MS instrument. A schematic diagram of the basic components is shown in Fig. 1. The ion source has been adapted from a JEOL DM303 mass spectrometer. The pulsing circuitry for the beam deflection assembly was designed and built in-house. The beam deflection plates are constructed from two copper-coated PTFE circuit boards. The detector is a Galileo FTD-2003 (Galileo Electro-Optics, Sturbridge, MA, USA) in which the standard multi-channel plates (MCP) were replaced with high-output (HOT) MCPs. The preamplifier was made in-house. The GC and BDTOF-MS instruments are connected via a modified JEOL HX110 aluminum block interface, heated to 260°C. The gas chromatograph is a Hewlett-Packard Model 5790. The urinary organic acid mixture was separated using a short DB-5 capillary column (6 m × 0.2 mm I.D.) (J&W Scientific), 0.4-μm film thickness, using a column head pressure of 13 p.s.i.g. with helium as the carrier gas, splitless injection with an injector temperature of 275°C and temperature programming from 50 to 325°C at 20°C/min. The combination of the

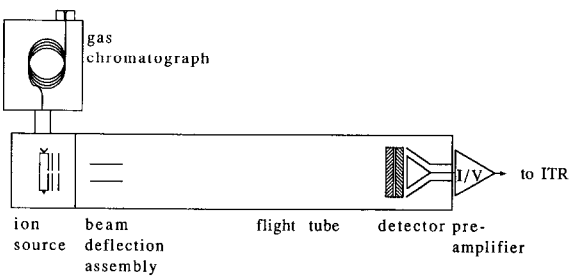

Fig. 1. Schematic diagram of beam deflection time-of-flight mass spectrometer interfaced to a gas chromatograph. Shown are ion source, beam deflection assembly, flight tube and detector. The flight tube is 2 m in length. The current output from the detector is amplified and converted to a voltage that is digitized by the integrating transient recorder (ITR).

short column with the fast temperature program reduces the normal analysis time by a factor of four.

The urinary organic acid mixture also was analyzed on a Hewlett-Packard Model 5890 gas chromatograph with a flame ionization detector. The FID output was digitized by an HP 3392A printing integrator, with the peak width response set to 0.01 min, which digitizes the data at a 20-Hz sampling rate.

The number of theoretical plates for the capillary column used for these analyses was determined on the GC–FID instrument. A two-component hydrocarbon mixture containing nonane and decane was analysed isothermally at 120°C. The average value for the number of plates as determined by measurements on each of the two peaks was 51 000.

The method of urinary organic acid extraction has been reported previously [8]. The acidic metabolites, isolated from 1 ml of a control urine sample, were derivatized by mixing 25 ml of pyridine and 100 ml of bis(trimethylsilyl)trifluoroacetamide–trimethylchlorosilane and heating at 80°C for 1 h. A 1.0-μl aliquot of the trimethylsilylated (TMS) urinary organic acids was analyzed by GC–MS with data acquisition rates of 1 and 10 sf/s, with the same column and GC conditions.

RESULTS AND DISCUSSION

Comparison of chromatographic profiles from GC–FID and GC–BDTOF-MS–ITR

A comparison of the chromatograms obtained from analysis of a urinary organic acid mixture by GC–FID and by GC–MS with reconstruction of the total ion current (RTIC) is shown in Fig. 2. The RTIC chromatograms represent the analysis of the same amount of this mixture and were generated from data obtained at 1 and 10 sf/s on the GC–BDTOF-MS–ITR system. A 3 min delay between injection of the mixture and initiation of data collection was used. The database obtained at 1 sf/s (providing only one RTIC point per second with which to reconstruct the chromatogram) was generated by summing 5000 consecutive mass spectral transients into each scan file, producing 600 scan files during the 10-min analysis. This database, obtained at 1 sf/s, represents that typically obtained with commercially available GC–MS instruments, which produce one spectrum per second. Another database, obtained at 10 sf/s, was generated by summing 500 consecutive mass spectral transients into each scan file, producing 6000 scan files during the 10-min analysis. Table I lists several of the TMS-derivatized organic acids identified from data collected at 10 sf/s, with their retention times and relative amounts for each component. The components in the mixture were identified by comparison of retention times and mass spectra of each component to those in a reference library previously compiled in our laboratory using another GC–MS instrument. Components that are yet to be identified have been labeled as unknowns. Quantification was achieved based on the area in the RTIC represented by 36 ng of the TMS derivative of tropic acid (peak marked with an asterisk in Fig. 2c) used as the internal standard.

The goal of any GC–MS analysis is to obtain useful information for all of the components in a mixture represented by the RTIC chromatogram. Ideally, the RTIC chromatogram provides an accurate representation of the chromatographic profile for retention times and quantification of the components in the mixture. The database from which the RTIC chromatogram is generated should provide information to distinguish those components that are not fully resolved chromatographically. In capillary GC–MS, it is difficult to meet these criteria when scan files are generated at 1 sf/s or less. Scan file acquisition rates of 10 sf/s or greater are necessary to generate RTIC chromatograms that accurately represent the resolution achieved by the capillary GC column used in this analysis [9]. The RTIC chromatogram in Fig. 2c accurately represents the chromatographic peak shapes and areas as verified by comparison with the chromatogram obtained by GC–FID in Fig. 2a.

Selected segments of the complete RTIC chromatograms (shown in Fig. 2) are shown in more detail in Fig. 3. The RTIC chromatogram from the database collected at 1 sf/s (Fig. 3a), typical of GC–MS analyses using scanning mass spectrometers, clearly does not accurately represent the chromatographic profile when compared with either the GC–FID or the GC–BDTOF-MS–ITR RTIC traces generated from the database collected at 10 sf/s (Fig. 3b). Regions where there are two or more closely eluting components are represented by a single peak in the RTIC chromatogram in Fig. 3a ow-

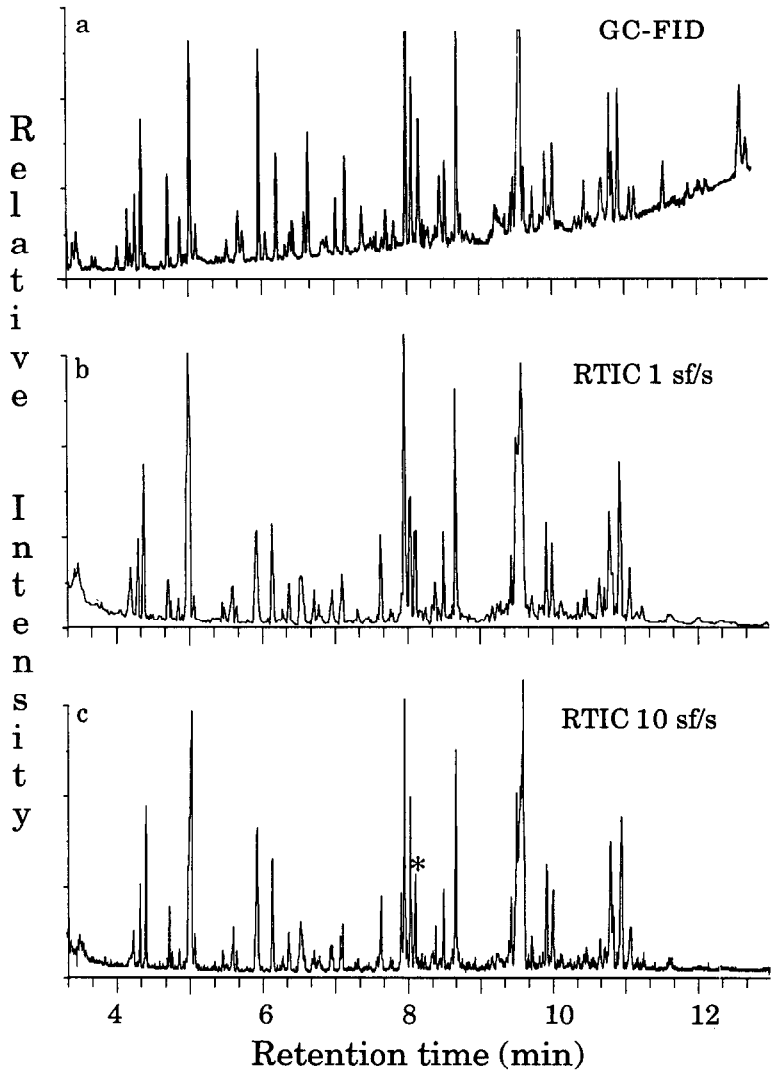

Fig. 2. (a) Gas chromatogram obtained using FID. (b) Reconstructed total ion current (RTIC) chromatogram generated from the GC–MS database collected at 1 sf/s. (c) RTIC chromatogram generated from the GC–MS database collected at 10 sf/s. The peak marked with the asterisk represents 36 ng of tropic acid (injected) as the internal standard.

ing to the small number of data points available with which to reconstruct the chromatographic peak profiles. For example, the segment marked A in Fig. 3a suggests the presence of three components, whereas the data in Fig. 3b clearly show that at least five components are eluting in this time period. Region B in Fig. 3a may represent baseline noise; however, the data in Fig. 3b show a chromatographic peak in that time window (see arrow). Similarly, region C becomes a chromatographic doublet (or possibly a triplet) when the mass spectra database allows for adequate reconstruction of the chromatographic resolution (Fig. 3b). If the RTIC chromatogram in Fig. 3a were the only one available, an analyst would incorrectly interpret apparent "single peaks" as representing single components. However, the mass spectrum obtained by averaging the scan files across the peak, as is common-

TABLE I

URINARY ORGANIC ACIDS IN MIXTURE

Quantitative results assuming identical ionization efficiencies for all compounds. The table contains information on compounds p at greater than 2.0 μg/mg of creatinine.

Retention time (min) (± 0.002 min)	Scan file number	Acic (or other compound)	Relative response (μg/mg creatinine)
4.328	798	Lactic	27.0
4.412	848	Glycolic	59.7
4.728	1038	Oxalic	25.9
4.767	1061	Glyoxylic oxime	7.1
4.863	1119	Unknown	8.8
4.928	1157	Unknown	3.6
4.988	1194	*p*-Cresol	17.6
5.035	1222	Sulfuric	182
5.080	1249	4-Hydroxybutyric	14.4
5.460	1477	3-OH-isovaleric	6.6
5.573	1545	Urea	9.4
5.605	1564	Unknown amino acid	18.8
5.653	1593	Benzoic	7.2
5.937	1763	Phosphoric	91.1
5.958	1776	Unknown amino acid	7.4
6.143	1887	Succinic	31.7
6.155	1894	Tri-TMS-glycine	28.2
6.285	1972	Pyrocatechol	6.4
6.365	2020	Glyceric	13.4
6.507	2105	4-Deoxyerythronic	8.7
6.542	2125	Unknown amino acid	22.1
6.560	2137	4-Deoxythreonic	10.8
6.708	2225	Unknown	12.8
6.958	2375	3-Deoxytetronic	30.2
7.092	2455	2-Deoxytetronic	42.7
7.432	2594	Unknown	6.2
7.642	2786	Pyroglutamic	39.6
7.768	2862	5-Hydroxymethyl-2-furoic	8.5
7.915	2950	Erythronic (isomer)	46.6
7.967	2981	Erythronic	147
7.998	3000	Threonic (isomer)	11.8
8.048	3030	Threonic	89.2
8.117	3071	Internal standard, tropic (36 ng)	55.6
8.208	3125	3-Hydroxyphenylacetic	3.5
8.358	3215	Furan-2,5-dicarboxylic	6.4
8.393	3237	4-Hydroxyphenylacetic	15.1
8.445	3267	2-Aminoadipic	4.2
8.502	3302	Tartaric	37.6
8.672	3404	2-Deoxyribonic	116
9.403	3843	Arabinonic	32.6
9.435	3862	Ribonic	58.4
9.565	3940	Hippuric	297
9.615	3970	Citric	39.6
9.720	4032	Methylhydroxyphenylhydracrylic	17.0
9.922	4153	Deoxyhexonic lactone	49.9
9.843	4106	Gluconolactone	40.3
10.357	4415	Ascorbic	9.8
10.657	4594	Glucuronic	23.2
10.803	4683	Gluconic	81.0
10.837	4703	Gluconic	31.5
10.953	4773	Glucaric	111
11.073	4845	Galacturonic	39.3

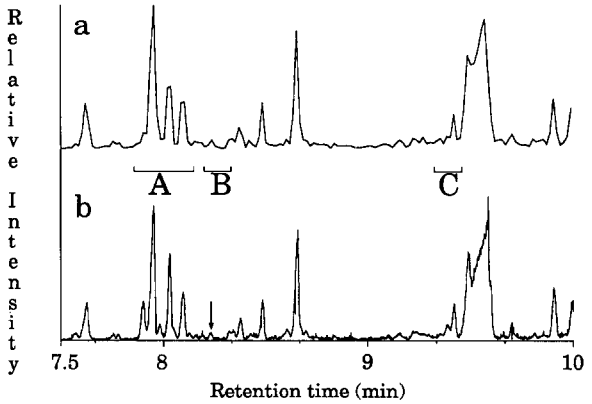

Fig. 3. (a,b) Selected segments of the complete RTIC chromatograms generated at 1 and 10 sf/s as shown in Fig. 2b and c, respectively. The region marked A in (a) suggests the presence of three components, whereas the data in (b) clearly show that at least five components are eluting during this time period. Region B in (a) may represent baseline noise, but the data in (b) show a chromatographic peak (arrow). Similarly, region C becomes a chromatographic doublet when the mass spectral database (acquired at 10 sf/s) allows for adequate reconstruction of the chromatographic resolution.

ly done for data presumed to represent a single component, would be the sum of mass spectra from two or more components, thus making mass spectral interpretation difficult, if not impossible. Minor components in the mixture are also difficult to distinguish from the baseline in Fig. 3a, again owing to the insufficient number of data points (scan files) with which to reconstruct the chromatographic peak profiles.

Mass spectral deconvolution: improving "chromatographic resolution" and compound identification from the mass spectral database

An important difference between the RTIC chromatogram in Fig. 2c and the GC–FID trace (Fig. 2a) is that with the RTIC chromatogram, additional dimensions of information are available in the mass spectra database. The mass spectral database can be used in two ways. Deconvolution of chromatographically unresolved components is possible by plotting the ion current at specified m/z values *versus* scan file number, generating a mass chromatogram. Mass chromatograms suggest which scan files should contain mass spectral information for particular components, allowing qualitative and quantitative information to be obtained, even for overlapping components. The success of this approach depends on the scan file generation rate of the mass spectrometer. For example, a sufficient number of scan files must be generated during a chromatographic doublet for the mass spectral database to be adequately time-resolved for identifying and deconvoluting overlapping components. The result of mass spectral interpretation is only as good as the quality of the mass spectra, which can be compromised owing to skewing of peak intensities by scanning mass spctrometers, or to spectral interferences from co-eluting components. Because the BDTOF-MS–ITR system can generate many unskewed mass spectra each second, mass spectral quality is outstanding and interferences are relatively easy to identify and remove.

One of the advantages of generating a mass spectral database at 10 sf/s, rather than at 1 sf/s, is demonstrated in Fig. 4. A portion of the RTIC chromatogram generated from the database collected at 1 sf/s shown in Fig. 4a has one "peak" in the vicinity of scan file 125 that appears to result from a single component in the mixture. The 5-s peak width might be interpreted as resulting from overloading the column, and the scan files 123–128 of the RTIC chromatogram would be averaged and the resulting mass spectrum (mis)interpreted. In comparing Fig. 4a with Fig. 4b (reconstructed from the database collected at 10 sf/s), it is apparent that more than one component is eluting during this time period, with very different elution profiles.

Differential comparison of adjacent mass spectra collected for a GC peak may help to discern the number of components present. For example, Fig. 5a and b show the mass spectra in scan files 1197 and 1220, respectively, from the region of the RTIC chromatogram in Fig. 4b. The mass spectrum in Fig. 5a has a number of peaks not observed in Fig. 5b; the most obvious are at m/z 165 and 180. Other peaks, such as those at m/z 73, 147 and 227 are found in both scan files. Because there are peaks that are not found in both scan files, one can assume that there is more than one component represented by the "peak" in the RTIC chromatogram in Fig. 4b. The ratio of relative intensities at m/z 147 and 227 in Fig. 5a and b remains constant, which indicates that both mass spectral peaks represent the

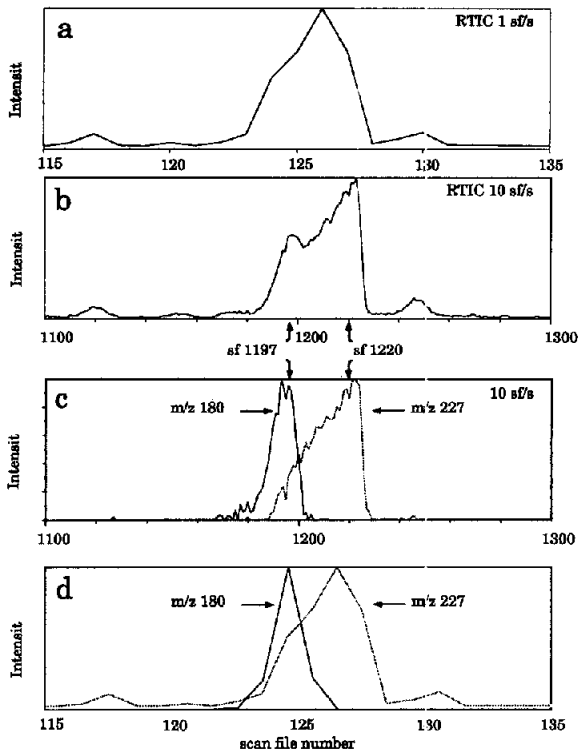

Fig. 4. (a,b) Comparison of a 20-s region of the RTIC chromatograms generated from databases collected at 1 and 10 sf/s. Mass chromatograms at *m/z* 180 (solid lines) and *m/z* 227 (broken lines).

same component. Also, the mass chromatograms at each of these *m/z* values correlate with each other. By plotting the signal intensities at *m/z* 180 and 227 *versus* scan file number, the elution profiles of the two components can be resolved, and these individual profiles realized as shown in Fig. 4c. Clearly, the second component exhibits considerable fronting on this particular column.

For further comparison, the mass chromatograms at *m/z* 180 and 227 from the database generated at 1 sf/s are shown in Fig. 4d. There are only three scan files in this database that contain mass spectral data at *m/z* 180. The partial pressure of the component represented at *m/z* 180 changes by 80% from scan file 123 to 124. With a scanning mass spectrometer, this would result in significant skewing of the relative ion intensities in the mass spectrum as well as making mass spectral subtraction difficult.

Mass spectra can be obtained after identification of a unique ion for each component. Ion current a *m/z* 180 is observed in scan files 1180 to 1203. Henc the data in Fig. 4c show that the mass spectrum i scan file 1220 is representative of the second compo nent with no mass spectral interferences from th first, and can be interpreted as such. The mass spec trum in scan file 1197 contains mass spectral dat from both of the components. A mass spectrun representative of the first component can be ob tained by mass spectral subtraction. By subtractin the peak intensities at *m/z* values representative o the second component found in scan file 1220 fron those in scan file 1197, a mass spectrum for the firs component will result. The base peak in scan file 1197 and 1220 is at *m/z* 147, which represents onl the second component. The ion current at *m/z* 14 in scan file 1220 is 34 384 arbitrary units (AU) an 14 704 AU in scan file 1197. Subtracting 43% [(14 704/34 384 AU) × 100] of the ion current at al of the peaks in scan file 1220 from those for corre sponding peaks in scan file 1197 will remove all o the ion current indicative of the second component leaving only ion currents indicative of the first com ponent. The mass spectrum resulting from this mas spectral subtraction is shown in Fig. 5c. This sim ple, but powerful, operation is possible only whe unskewed mass spectra, such as those obtained b TOF-MS as described here, are processed.

The first component represented in the RTIC seg ment in Fig. 4 has been identified as the TMS deriv ative of *p*-cresol. The molecular ion, $M^+\cdot$, is repre sented by the peak at *m/z* 180; the characteristic ior $[M-15]^+$, due to the loss of a methyl group fror the trimethylsilyl group, is represented by the pea at *m/z* 165. The second component has been identi fied as the bis-TMS derivative of sulfuric acid. Th molecular weight of this compound is 242 u, and th mass spectrum shows a peak at *m/z* 227 for th characteristic ion corresponding to $[M-15]^+$.

A more dramatic example of a reconstructe chromatographic peak profile, suggesting, incor rectly, the elution of a single component, is show in Fig. 6a. The peak shape and width are consister with the elution of a single component at this reter tion time. The mass spectral data in scan files 188 1890 and 1899 are shown in Fig. 7a, b and c, respec tively. The most obvious differences are the change in relative intensities at *m/z* 147, 174 and 247 in th three mass spectra, suggesting the elution of at lea

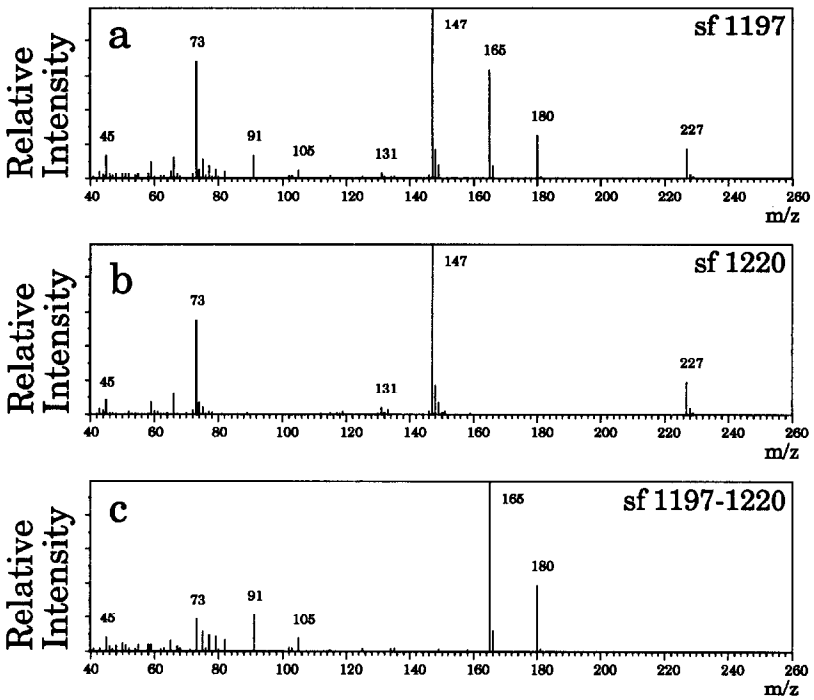

Fig. 5. (a) Mass spectrum in scan file 1197 that has mass spectral information for both of the components eluting over the peak profile shown in Fig. 4c. (b) Mass spectrum in scan file 1220. The mass spectrum shown in (c) is the result of subtracting 43% of the ion currents in scan file 1220 from the corresponding ones in scan file 1197.

two different components. Mass chromatograms at m/z 174 and 247 are shown in Fig. 6b. Again, the mass chromatograms do not overlap completely, indicating the presence of two components. By identifying a unique mass spectral peak for each of the components in the chromatographic peak profile, a mass spectrum for each component can be obtained. A comparison of the mass spectra in Fig. 7a–c shows that scan file 1883 only has ion currents indicative of one of the components. Similarly, scan file 1899 only has ion currents indicative of the other component. Selection of these scan files is clearer on examination of the overlapping mass chromatograms shown in Fig. 6b. Note that at the maximum intensities at m/z 174 and 247, the retention times, shown in Fig. 6b, only differ by 0.8 s, but one can still obtain a mass spectrum for each of the components directly from the database generated at a rate of 10 sf/s.

The two components represented in Fig. 6 have been identified as bis-TMS-succinic acid and tri-TMS-glycine, respectively. The TMS ester of succinic acid has a molecular weight of 262 u and its mass spectrum shows a peak for the characteristic

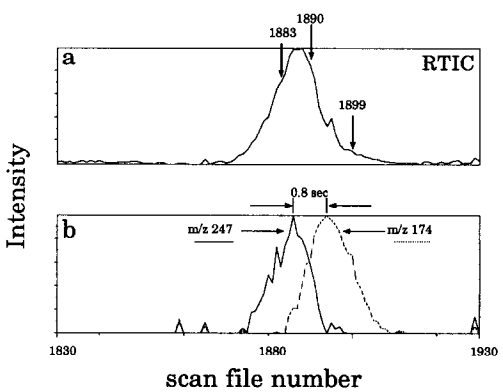

Fig. 6. (a) Region of the RTIC chromatogram generated from the database acquired at 10 sf/s. (b) Mass chromatograms at m/z 174 (broken line) and m/z 247 (solid line). The difference in the maxima of the two mass chromatograms indicates the presence of two components.

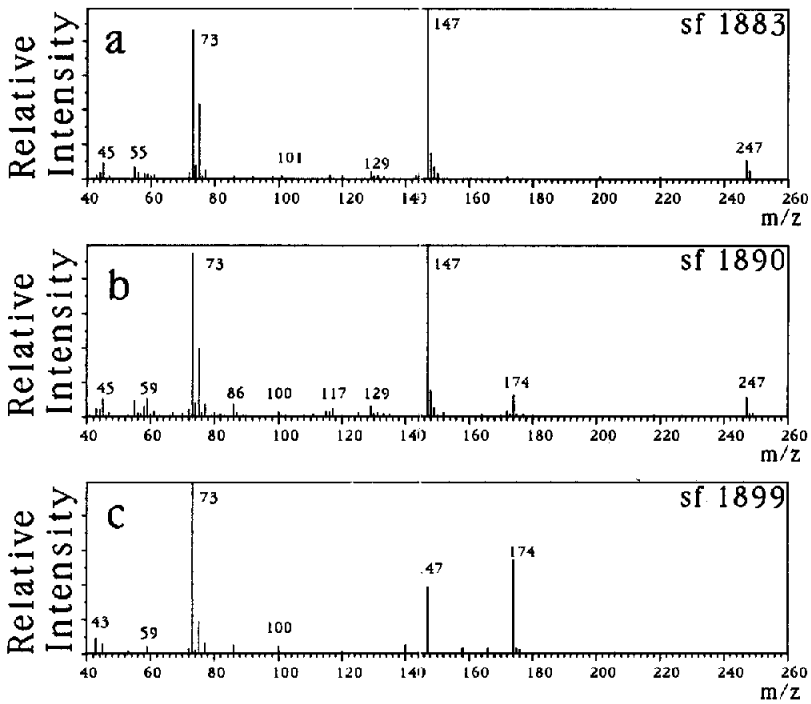

Fig. 7. Comparison of mass spectral data in scan files (a) 1883, (b) 1890 and (c) 1899 over the peak profile in Fig. 6. Comparison of the mass spectral data in each of the scan files shown, indicates that (a) (scan file 1883) represents the first component in the peak profile, (b) (scan file 1890) contains mass spectra data for both components and (c) (scan file 1899) is the mass spectrum of the second component represented by the poorly resolved doublet in Fig. 6b.

$[M-15]^+$ ion at *m/z* 247. Other peaks indicative of the TMS derivative of succinic acid are located at *m/z* 73, 129, 147, 172 and 218.

Three-dimensional data presentation

Three-dimensional (3-D) plots of mass spectra *versus* scan file number are another useful means of displaying data consisting of a large number of scan files per chromatographic peak [10]. These 3-D plots are constructed by plotting mass chromatograms for all *m/z* values in a given region of the RTIC chromatogram. This allows the temporal profiles of each mass chromatogram to be compared to indicate which chromatographic peaks result from more than one component. An example of a 3-D plot is shown in Fig. 8 for the chromatographic peak in the metabolic profile represented in Fig. 6. The 3-D plot clearly shows evidence for the two components over this region of the RTIC. The two components have peaks in common at *m/z* 73 and 147, but there are a number of peaks that are unique to one or the other components. The two major peaks that are unique to each of the components occur at *m/z* 174 and 247 (as in Fig. 6b). Chromatographic profiles for the two components can be recognized by plotting mass chromatograms at *m/z* 174 and 247. The mass spectrum of each

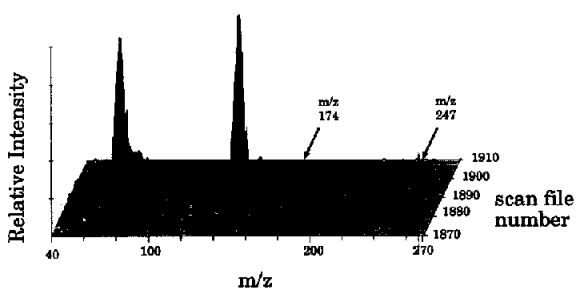

Fig. 8. Three-dimensional data presentation of the peak profile in Fig. 6. The 3-D plot shows ion current at all of the *m/z* values for direct comparison of individual mass chromatograms.

component can be obtained by examining the mass chromatograms as shown previously.

CONCLUSIONS

A GC–MS instrument based on BDTOF-MS can perform complex mixture analyses by generating complete mass spectra at a rate appropriate for specific chromatographic conditions. The scan file generation rate for an analysis is dictated by requirements to represent the chromatography accurately and provide data with good signal-to-noise ratios. The utility of this unique system has been demonstrated in the context of realistic problems encountered in the analysis of the complex mixture of organic acids extracted from human urine. The problems in such analyses frequently manifest themselves as components that are chromatographically unresolved. It is further demonstrated that long capillary columns, with high numbers of theoretical plates and peak separation, are not necessary with this technology. Rather, an analysis can be performed on a short capillary column giving the same analytical information, but with a reduced analysis time. The typical analysis time for this metabolic profile was reduced from 1 h to 15 min (this particular analysis time was limited by the rate at which the GC temperature could be increased). The success of these analyses is due to the nature of the BDTOF-MS instrument combined with the ITR. In this application, each of the ten scan files generated each second is the sum of 5000 transient mass spectra. The high scan file generation rate provides a database that can be used to represent accurately the chromatographic information, which facilitates the identification of each of the components in a complex mixture. Quantification of each of the components is facilitated owing to the greater number of points available with which to determine peak shape, width and area. The "unskewed" nature of the mass spectrum in each of the scan files facilitates the preparation of "pure" mass spectra by deconvolution of nearly co-eluting components using mass spectral subtraction. For the data presented here, compounds with retention times differing by 0.1 s can be analyzed. The three-dimensional presentation of the data allows the user to identify peaks that are representative of poorly resolved components, display the corresponding mass chromatograms and then choose scan files and background scan files for subtraction to obtain mass spectra for closely eluting components.

In closing, we note that the concepts discussed here, including the use of mass chromatograms, RTIC chromatograms and mass spectral subtraction, in the context of GC–MS are more than 20 years old. However, their utility can be a source of frustation for chromatographers because the low spectral acquisition rates of scanning mass spectrometers yield reconstructed chromatograms that have much less detail than those acquired by GC only. With the capabilities of the instrument described here, these established methods become much more useful to the chromatographer who is using a mass spectrometer as the chromatographic detector. Scan rate limitations no longer degrade chromatographic information available in databases obtained by GC–MS.

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