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Review

Tandem mass spectrometry —an additional dimension of chromatography for the determination of biomarkers in fossil fuels

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ABSTRACT

A triple-stage quadrupole mass spectrometry (MS) system used in the MS–MS mode with both gas chromatography (GC) and direct-insertion probe as inlet systems, can provide methods alternative to GC–MS for biomarker analyses. Specific parent/daughter ion relationships can be utilized to monitor and resolve classes of biomarkers or individual components in a complex mixture. Furthermore, deuterated analogues of naturally occurring biomarkers can be utilized for quantitation purposes since the parent/daughter relationship for the standard will differ, depending on the number of deuterium atoms present, even though the relative retention times are identical.

The utilization of GC–MS–MS and the direct insertion probe MS–MS to determine biomarker distributions in crude oils both qualitatively and quantitatively are discussed in this paper. The results from the study show that it is possible to use the probe data to correlate oils on the basis of their source materials and if necessary select samples for more detailed analysis by GC–MS–MS. A major advantage of the direct insertion probe method is speed of analysis although some component resolution is lost. In an effort to minimize this problem and still maintain the rapid analysis time, the use of short columns in GC is also described. This approach permits relatively rapid analyses with limited chromatographic resolution but at relatively high levels of sensitivity.

CONTENTS

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1. INTRODUCTION

Advances in the areas or organic, petroleum, and environmental geochemistry have been rapid in the past two decades. Examination of the literature reveals two main reasons for the developments in these areas. The first of these is advances in various aspects of chromatography, particularly those in gas chromatography (GC) and the evolution of columns from the large-diameter packed columns, 2 or 3 feet in length, to the very-high-resolution and high-temperature fused-silica and aluminum-coated capillary columns available today. The second major advance is the coupling of liquid and gas chromatography to mass spectrometry (MS) that permits identification as well as separation of individual components in complex mixtures.

More recent developments in the past two or three years have provided the opportunity for further innovative advances in the above-mentioned areas. Such developments include supercritical fluid chromatography and its coupling with mass spectrometry, tandem mass spectrometry (MS–MS) and finally the combination of GC with isotope ratio MS. The complex nature of samples typically analyzed in geochemical studies make them ideal candidates for investigating and exploiting many of these new chromatographic and spectrometric techniques. Space does not permit a review of all the developments that have occurred in these areas and how they have influenced various aspects of geochemistry. The area that will be discussed in detail will be the combination of gas chromatography and tandem mass spectrometry (GC–MS–MS) for the determination of biomarker distributions in fossil fuels.

A discussion of MS–MS in a volume devoted to chromatography may seem a little out of context. However, as will be demonstrated below, the MS–MS approach provides an additional dimension of separation for extremely complex mixtures containing large numbers of co-eluting components, both isomers and homologues. In many cases even chromatographic columns with the highest levels of resolution will not totally resolve all components.

There are many designs of commercially available tandem mass spectrometers, but since this paper is more concerned with applications than instrument design it is not proposed to describe such systems in detail. For the most part all of the results described in this study have been obtained through utilization of a triple-stage quadrupole tandem mass spectrometer, where the middle quadrupole is a collision cell and operates in an R_F (radio frequency) mode only. A brief description will be given on how this instrument is used under optimum conditions for analyzing the mixtures typically encountered in various types of geochemical samples following a short review of developments in the field of tandem mass spectrometry.

MS–MS has gained rapid acceptance in the analytical community due largely to its ability to provide sensitive and selective rapid analyses of complex mixtures with minimal sample clean-up [1]. Yost and Enke [2] developed the triple-stage quadrupole mass spectrometer that made mixture analysis by MS–MS practical. MS–MS with

the direct-insertion probe (DIP) method emulates GC–MS by replacing the chromatograph with a mass separator. Whereas GC–MS is limited by the time required for chromatographic separation, the second mass separator requires only an additional ion transit time of 10^{-5}–10^{-3} s [3]. Another advantage of MS–MS with DIP is its capability of analyzing components which cannot be volatilized sufficiently in standard injection systems. The combination of GC with MS–MS further enhances the separation potential of the approach and provides a means of separating components both chromatographically and spectrometrically.

The number of papers in the literature describing the utilization of MS–MS for the analysis of geochemical samples has steadily increased. Gallegos [4] was among the first to report the measurement of appropriate metastable ion transitions in a magnetic sector mass spectrometer to study the distribution of sterane and triterpane homologues from an organic-rich sediment. Measurement of metastable ion transitions during GC–MS analysis was subsequently used to observe sterane isomer distributions without interference from unresolved sterane homologues [5] and to identify extended tricyclic terpanes up to C_{45} in crude oils [6]. The technique also permitted ready detection of the ubiquitous trace component, C_{30} desmethylsteranes, which were subsequently proposed as indicators of marine organic source material [7]. Steen [8] used high-resolution selected-metastable-ion monitoring to determine the distribution of various biomarkers present in oils and source rocks.

Ciupek *et al.* [9] presented an overview of the MS–MS capabilities and its applications to fuel-related materials, *e.g.* coal-derived liquids and diesel particulate samples, using high and low collision energy MS–MS data obtained on reversed-geometry and triple-stage quadrupole spectrometers. Wood and co-workers [10] confirmed the presence of a series of long-chain alkyl aromatic and heteroaromatic hydrocarbons in a Utah boghead coal, using a triple-stage quadrupole mass spectrometer. Summons and co-workers [11,12] have used GC–MS with multiple reaction monitoring in a number of applications to discriminate isomers of branched alkanes from ancient and modern sediments. Snowdon *et al.* [13] demonstrated the value of tandem mass spectrometry in petroleum exploration. Their study showed that GC–MS–MS provided sufficient chemical compositional detail of oils and potential source rocks to solve correlation problems.

Daughter spectra of selected molecular ions are also useful for compound identification. Chou and Wood [14] tentatively identified an alkylaromatic hydrocarbon in shale extracts with tandem mass spectrometry. Hunt and Shabanowitz [15] described the use of a triple-stage quadrupole mass spectrometer operating in the neutral-loss mode for the analysis of organosulfur compounds in crude petroleum distillates. The total analysis time per sample was under 20 min, and qualitative differences in the spectra of several crude oil samples were apparent. Tandem mass spectrometry has also been used for determination of porphyrins [16] and [17]. Complex mixtures of porphyrins present problems for GC–MS analysis due to their extreme involatility. However, with the advent of tandem mass spectrometry this obstacle can be overcome. The added specificity of MS–MS enables the individual porphyrins to be characterized directly from complex fuel matrices.

In recent studies the utilization of GC–MS–MS for the analysis of complex biomarker mixtures has been described [18] and [19]. Operation of the system in the MS–MS parent mode permits spectrometric resolution of many unresolved compo-

nents in these mixtures, greatly aiding in the utilization of the biomarker distributions for evaluation of source, maturity, migration, and characterization of depositional environments.

As mentioned above, one of the major advantages of MS–MS is its ability to resolve components spectrometrically on the basis of specific parent/daughter ion relationships. Hence, this paper describes results of studies designed to develop screening techniques for the rapid determination of biomarker distributions in crude oils, both qualitatively and quantitatively, using the DIP method. Although this does provide a rapid method for screening and significant resolution, there are still a number of components that will not be separated. For instance, hopane and gammacerane have parent ions at m/z 412 and intense daughter ions at m/z 191, and DIP-MS–MS will not resolve these components. An alternative approach for screening is to use short (3 m) GC columns to provide some chromatographic resolution, while at the same time maintaining a rapid analysis time [20]. Several examples describing the application of these techniques to the characterization of crude oils and, in particular, the utility of the approach in differentiating oils from different source rocks will be discussed. In addition to illustrating the use of DIP and short GC columns in conjunction with MS–MS, some more conventional examples of utilizing GC–MS–MS fused-silica columns will also be described. The purpose of this paper is to illustrate the application of MS–MS to various geochemical problems and the type of data obtainable from such applications.

2. EXPERIMENTAL

Samples used in this study were either unfractioned crude oil samples or oils that were fractionated by standard thin-layer chromatography techniques to provide saturate, aromatic, and polar fractions. Samples from the following areas were used in this study: Offshore Taiwan, Williston Basin, New Mexico, Gulf of Suez, Dubai, Norway, Chaidamu and Shanganning Basins, China and Monterey, California. Whole oils or selected fractions were analyzed with the Finnigan MAT TSQ70 mass spectrometer. The chromatographic column used for most of the routine analyses was a 25 m × 0.25 m I.D. aluminum-coated fused-silica capillary column (Scientific Glass Engineering), coated with a 0.1-μm film of the HT-1 phase. The column was temperature-programmed from 40 to 330°C at 2°C/min with an injector temperature of 280°C. The transfer-line temperature was set at 280°C and the ion-source temperature was 200°C. The ion source was operated in the electron impact mode at an electron energy of 70 eV. The collision gas was argon at 1 mTorr, and the collision energy was generally −10 eV. Additional experiments were also performed with short columns (3 m) for GC to show that despite the loss of GC resolution, MS–MS techniques can actually resolve many components rapidly.

For the DIP inlet system, the sample was loaded in the crucible, the solvent was evaporated and the sample was mounted on the tip of the DIP system, which was then inserted into the ion source. The DIP temperature was programmed from 50°C to 300°C in 2 min to permit distillation of the sample directly into the ion source.

The TSQ mass spectrometer was operated either as a single-stage mass spectrometer (*i.e.*, Q1MS or Q3MS) or tandem mass spectrometer in the parent mode. Selected-ion monitoring (SIM) and selected-reaction monitoring (SRM) techniques

were used for routine biomarker analysis. In the SRM techniques of tandem mass spectrometry, the parent/daughter ion pairs monitored consisted of molecular ions (parents) and characteristic base peak fragments (daughters) commonly observed in the electron-impact (EI) spectra. Each specific parent/daughter ion pair was monitored for 0.02 s before switching to the next ion pair, thus permitting a large number of experiments to be undertaken during each analysis. Introduction of deuterated standards into the sample and subsequent monitoring of the appropriate deuterated parent/daughter ion relationships, regardless of whether the undeuterated standard analogue exists in the original sample, permits absolute quantitations to be undertaken [21].

3. RESULTS AND DISCUSSION

A convenient way in which to introduce the MS–MS concept is to use the separation of steranes and diasteranes in crude oils. It is well documented that several

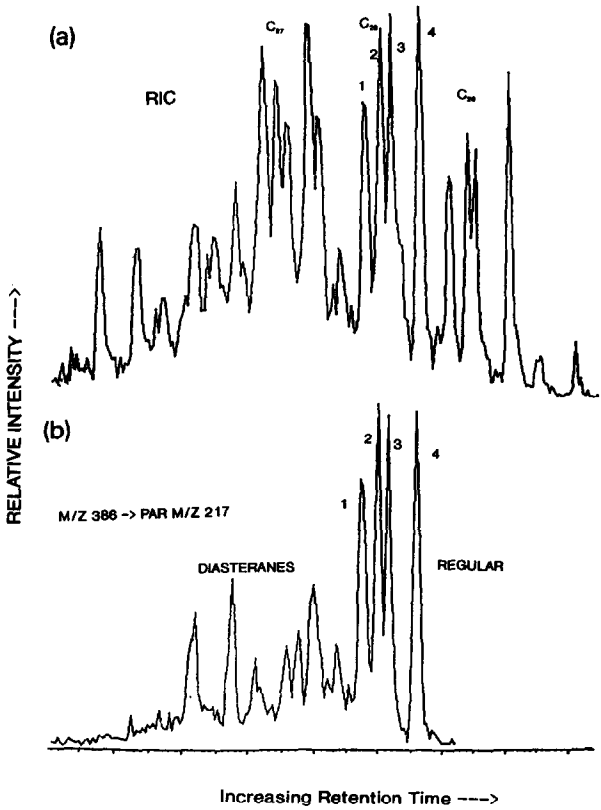

Fig. 1. (a) Reconstructed ion chromatogram (RIC) for the four parent/daughter ion reactions monitored for the C_{27}–C_{30} steranes, namely *m/z* 372/217, 386/217, 400/217 and 414/217. (b) Deconvolution of the data shown in (a) permits one to obtain distributions for the individual sterane homologues as illustrated here with the distribution of the C_{28} steranes. (In both chromatograms peaks 1 and 4 are 14α,17α-20*S*- and 20*R*-C_{28} steranes, respectively and peaks 2 and 3 are the 14β,17β-20*R*- and 20*S*-C_{29} steranes, respectively).

isomers and homologues are eluted in the C_{27}–C_{30} sterane region of a crude oil chromatogram [22]. To obtain an accurate assessment of the relative proportions of the various homologues it is desirable to resolve them completely. For most geochemical samples, such a separation is not achievable with even the best capillary columns available today. To separate the various steranes using MS–MS and GC–MS–MS it is possible to monitor the 4 parent/daughter ion pairs for the C_{27}–C_{30} steranes, namely *m/z* 372/217, *m/z* 386/217, *m/z* 400/217, and *m/z* 414/217, respectively. The result of monitoring these four parent/daughter combinations, are shown in Fig. 1a. Individual homologue distributions can now be resolved from the total sterane distributions as shown by using the C_{28} steranes (Fig. 1b). Subsequent integration of the peak areas can provide an accurate assessment of the relative proportions of the individual steranes and enable various ratios to be calculated to provide source information. Furthermore, such complete separation permits accurate calculation of commonly used maturity parameters, based on individual homologues. Each pseudo-homologue distribution consists of diasteranes and regular steranes, but if necessary, diasteranes and regular steranes can be separated by monitoring the parent ions of both *m/z* 189 and 217, respectively, as shown in Fig. 2. This combination of GC and MS–MS permits complete separation of virtually all homologues and isomers of the diasteranes and steranes and can provide an accurate assessment of any maturity, source, or depositional environment, parameter based on these values. Another important application of MS–MS to sterane analyses is the ability to distinguish C_{30}-4-desmethyl and 4-methyl-steranes. The former have an intense daughter ion at *m/z* 217 and the later at *m/z* 231, and monitoring the *m/z* 414 → 217 and *m/z* 414 → 231 parent/daughter pairs provides a rapid means of distinguishing between the two types of steranes.

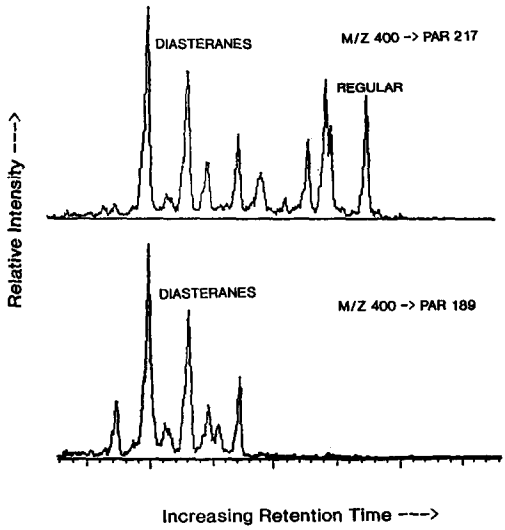

Fig. 2. Diasteranes can be resolved from the sterane–diasterane mixture by monitoring the *m/z* 400 → 189 relationship, and the *m/z* 400 → 217 relationship using the C_{29} steranes as an example.

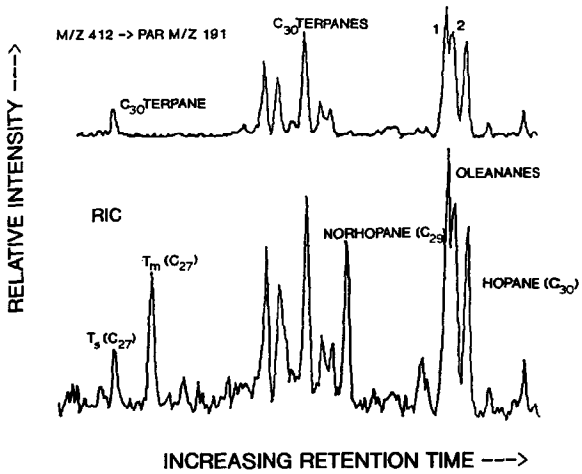

Fig. 3. Monitoring the m/z 412 → 191 parent/daughter ion relationship for an Indonesian oil sample revealed the presence of a number of C_{30} terpanes including two isomers of oleanane in this chromatogram, namely 18α(H)- and 18β(H)- oleanane (peaks 1 and 2, respectively).

3.1. Terpanes

There are many potential applications for MS–MS in the separation and characterization of the complex mixtures of terpanes found in geochemical samples. One example of this is the separation, spectrometrically, of the tricyclic terpanes from tetracyclic and pentacyclic terpanes. Although all of these compounds have an intense daughter ion at m/z 191, the parent ions for each series of terpanes differ by 2 mass units and the spectrometric separation of these three classes of terpanes is therefore possible. This is particularly useful in the region of the chromatogram where the C_{27} to C_{35} hopanes are eluted, since in this region there is also a large degree of overlap between the tricyclic and pentacyclic terpanes.

The MS–MS approach can also provide additional information on unidentified terpanes in crude oil samples, or on those incorrectly identified. An example is the identification of a C_{30}-terpane which is eluted between the two C_{27} trisnorhopanes, namely T_s and T_m (Fig. 3). Although this C_{30}-pentacyclic terpane has yet to be unequivocally identified, its relative concentration appears to be significantly higher in samples formed from higher plant material. In addition to this C_{30} terpane, there is a component which is eluted after T_m and has often been assigned as 17β(H)-trisnorhopane according to early work and simply on the basis of m/z 191 chromatograms [23]. However, repeated analyses of a variety of crude oil samples have led us to suggest that in many examples this component was not 17β(H)-trisnorhopane but rather a C_{28} pentacyclic terpane with a parent ion at m/z 384 (Fig. 4). This component shows an intense m/z 384 → 191 parent/daughter relationship and not the intense m/z 370 → 191 parent/daughter relationship shown by the T_s and T_m components which elute in the same region. In support of our tentative identification, more recent work has now established that this compound is the 17α(H),21β(H)-29,30-bisnorhopane in many oils and source rock extracts, particularly those derived from carbonate sources. T_s is the first member of this homologous series.

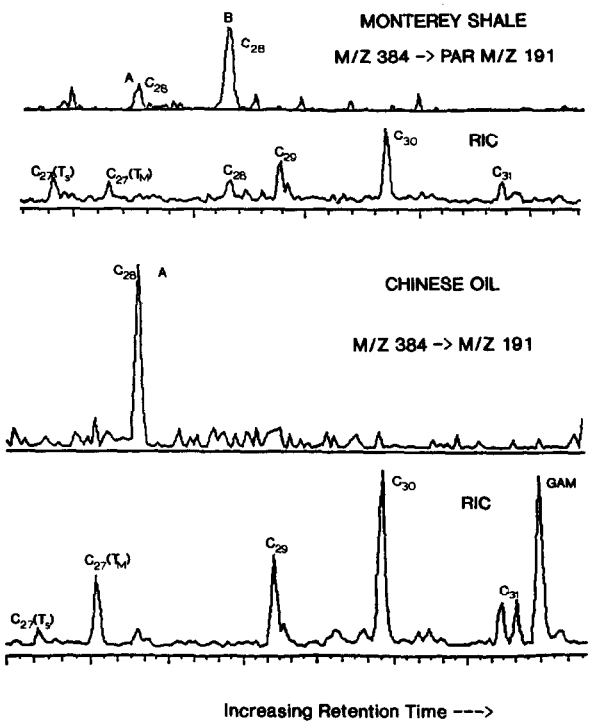

Fig. 4. The upper two chromatograms in this figure are derived from the analysis of an oil from the Monterey Formation, CA. Note the characteristically high concentrations of the 28,30-bisnorhopane (B) with the longer retention time than the 29,30-bisnorhopane (A). The bottom chromatograms are derived from a Chinese oil and unlike the Monterey sample clearly show the predominance of the early eluting 29,30-bisnorhopane (A) which elutes much closer to the C_{27}-trisnorhopanes. Other hopanes are identified by carbon number on the chromatograms along with gammacerane which is labelled GAM.

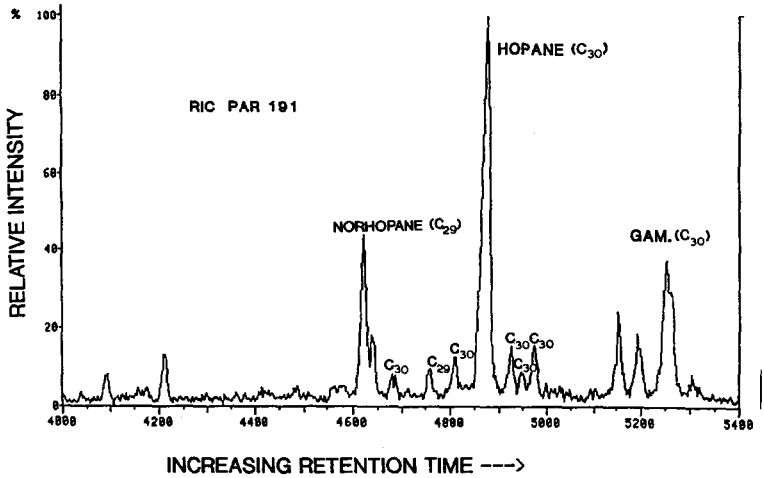

Fig. 5. Monitoring of the m/z 412 → 191 parent/daughter relationship for this Chinese oil sample provides good evidence to illustrate the presence of several C_{30}-terpanes in addition to 17α(H), 21β(H)-hopane and gammacerane (GAM).

The presence of additional terpanes, albeit in trace amounts, is relatively easy to discern in various regions of the chromatogram by use of the TSQ mass spectrometer operating in the parent mode. An example of this can be seen in Fig. 5, where a Chinese crude oil was analyzed in the parent mode with particular emphasis on the search for C_{30} terpanes, using the m/z 412 → 191 parent/daughter relationship. The presence of a significant number of C_{30}-terpanes was observed in this region of the chromatogram in addition to the C_{30} compounds hopane and gammacerane. Although their identities have still not been unambiguously established, their presence permits them to be used as correlation parameters, if necessary.

Another use of the MS–MS approach is to separate components of different molecular weights having the same daughter ion. Rinaldi *et al.* [24] commented on the presence of hexacyclic-C_{31}-hopanoids in certain oil samples which have a tendency to be inseparable from gammacerane on the chromatographic columns with particular liquid phases. Fortunately, the hexacyclic terpanes have a parent ion at m/z 424 compared to m/z 412 for gammacerane. Hence analysis of samples and simultaneous monitoring of the m/z 412/191 and m/z 424/191 parent/daughter relationships can be used to demonstrate the presence of both classes of compounds in crude oils (Fig. 6). The C_{31}-22(*S* and *R*)-homohopanes are also shown in Fig. 6 and have a parent ion at m/z 426 and daughter ion at m/z 191 but have slightly shorter retention times than the other two components mentioned above.

The preceding examples show that it is possible to obtain more additional information by the combined use of GC and MS–MS in the analysis of complex mixtures than by GC–MS and SIM. Many of the additional components observed in the mixtures may not always be immediately identifiable. This does not necessarily constitute a problem, since these components can be incorporated into maturity, source, and depositional environment parameters and their identities can be estab-

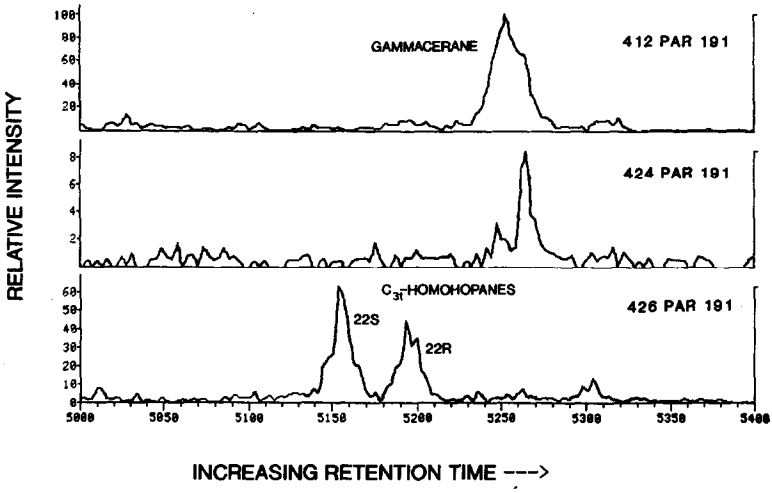

Fig. 6. Co-eluting components are readily resolved using the MS–MS approach. For example it was anticipated that this Chinese oil sample contained a C_{31}-hexacyclic hopane which co-eluted with gammacerane. Monitoring of the m/z 412 → 191 and m/z 424 → 191 parent/daughter relationship completely resolved these two components.

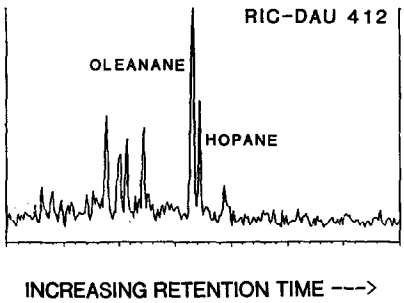

Fig. 7. In addition to obtaining parent data using the MS–MS approach it is possible to obtain daughter data on various components simultaneously. In this diagram all of the daughter spectral data for the ion at *m/z* 412 have been deconvoluted from the parent data and summed to produce this total ion current chromatogram of *m/z* 412 daughter data. The peaks labelled C_{30} are known to be pentacyclic terpanes but their precise identity remains to be established.

lished at a later date. One approach to establishing the identity of the unknown components partially is to operate the MS–MS system alternately in the parent and daughter modes. In this mode of operation, one complete set of parent experiments is performed and then a daughter experiment is performed, and this process is continually repeated throughout the analysis. The daughter experiments are performed on parent ions of components known to be eluted in various regions of the chromatogram. The sample shown in Fig. 7 was known to contain a number of previously unidentified C_{30} pentacyclic terpanes. Daughter spectra of the parent ion at *m/z* 412 were continually collected and at the end of the experiment, the parent and daughter data were deconvoluted, and complete daughter spectra for each component were obtained. Collision activated decomposition (CAD) spectra for the parent ions of two of the major components in the daughter ion chromatogram, namely 18α(H)-oleanane and hopane, are shown in Fig. 8. The collision spectra of these two components show many similarities to previously published electron-impact spectra for 18α(H)-oleanane and hopane. Similar daughter experiments could also be performed, using other parent ions throughout the GC analysis to provide a complete collection of daughter spectra. A major advantage of daughter spectra or CAD spectra over EI spectra is that the need to undertake background subtractions is virtually eliminated. The fact that only the parent ion of interest is permitted to enter the collision cell virtually eliminates all background ions from interfering components. Thus, for C_{30} pentacyclic terpanes, only the parent ions at *m/z* 412 enter the collision cell, and the daughter spectra subsequently obtained will be from that particular parent ion. This is a major improvement over EI spectra obtained with a single stage analyzer system. In such a situation it is necessary to remove interfering ions from the spectrum by carefully selecting background spectra. Poor selection can often lead to erroneous spectra being obtained.

3.2. Biomarker quantitation by tandem mass spectrometric techniques

A common feature of oils derived predominantly from the organic matter of higher plants is the relatively low proportion of steranes relative to triterpanes, as determined from the ion intensities in the *m/z* 191 and *m/z* 217 mass fragmentograms,

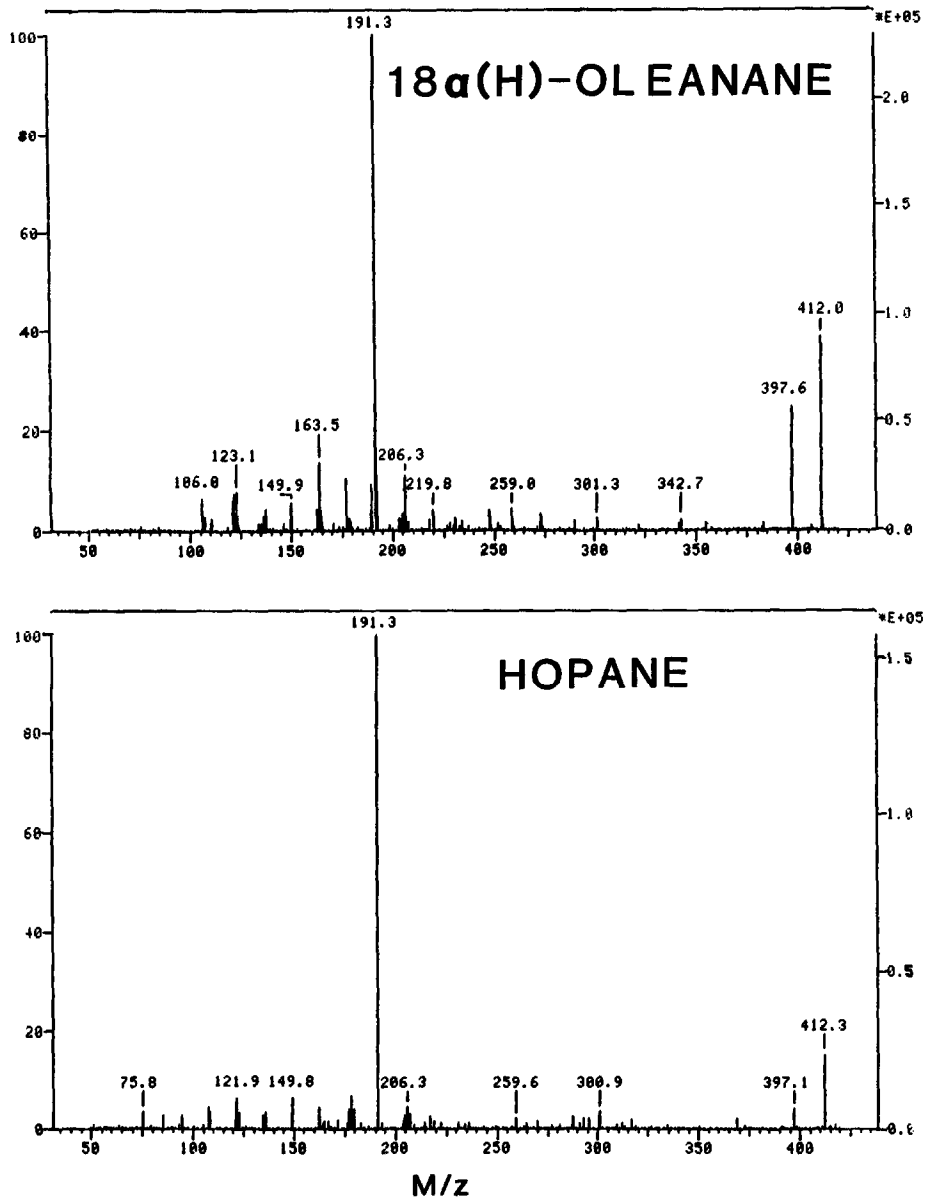

Fig. 8. Collision activated decomposition spectra of the *m/z* 412 parent ion for 18α(H)-oleanane and hopane.

respectively. For example, triterpanes in the Gippsland Basin oils, Australia, are present in approximately 3–4 times the concentration of the steranes [25], and in oils from the Mahakam Delta, Indonesia, approximately 8–10 times the concentrations of steranes [26]. For Taranaki oils from New Zealand, the ratios of hopanes to steranes were within the range 1.0–2.5. Oils from Taiwan had a ratio of triterpanes to steranes

TABLE I

QUANTITATION DATA OBTAINED FROM DIP-MS–MS ANALYSIS OF OILS DERIVED FROM TERRESTRIAL AND MARINE SOURCE MATERIALS

Concentrations of various biomarkers expressed in ng/μl using ^{2}H-cholestane as internal standard. 372, 386, 400 and 414 correspond to the C_{27}, C_{28}, C_{29} and C_{30} steranes; 398, 412 to the C_{29} and C_{30}-pentacyclic terpanes; 318-C_{23} tricyclic terpane and 330 to the C_{24}-tetracyclic terpane.

Sample location	372	386	400	414	398	412	318	330	372+386+400	372/400[a]	398/412[a]	217/191[a]	318/330[a]
Terrestrial sourced samples													
Offshore Taiwan	8.87	8.21	26.28	1.60	29.20	47.30	17.20	51.40	43.46	0.34	76.50	0.57	0.33
	12.56	8.76	37.17	3.49	24.05	47.55	11.20	103.90	58.48	0.34	71.60	0.82	0.11
	8.09	8.71	33.81	1.46	36.65	89.65	8.80	59.05	50.61	0.24	126.30	0.40	0.15
	9.27	8.35	22.00	1.43	9.25	22.05	8.60	18.85	39.62	0.42	31.30	1.27	0.46
Average	8.09	7.83	25.33	1.89	19.93	36.00	14.31	43.95	41.25	0.33	55.93	0.80	0.36
Marine sourced samples													
Williston Basin	31.28	22.03	29.51	11.38	22.29	17.26	37.89	18.49	82.82	1.06	39.55	2.09	2.05
New Mexico	66.40	31.40	42.74	8.67	11.27	19.55	29.57	25.60	140.54	1.55	30.82	4.56	1.16
Gulf of Suez	34.98	29.65	30.32	12.11	19.04	15.75	17.65	8.34	94.95	1.15	34.79	2.73	2.12
Dubai	33.13	27.67	29.16	15.18	20.13	17.28	18.96	7.56	89.96	1.14	37.41	2.40	2.51
Norway	51.35	43.00	42.00	19.48	12.38	18.38	19.45	12.53	136.35	1.22	31.23	4.37	1.55
Average	43.43	30.75	34.75	13.36	17.02	17.74	24.70	14.50	108.92	1.23	34.76	3.23	1.88

[a] These values represent ratios of the components with the various ions indicated.

ranging from 6 to 8. All of these oils are thought, or known to be produced from source rocks containing a predominance of organic matter derived from higher plants. Though the ratio of triterpanes to steranes can be derived from the GC–MS data, MS–MS provides a means to rapidly determine the absolute concentrations of terpanes and steranes in oils and differentiate between those oils derived from marine organic matter *versus* those from higher plant organic matter.

To illustrate how the MS–MS approach can be used for quantitation purposes, five oils from Taiwan produced from source rocks containing a predominance of higher plant material and five oils from other basins worldwide derived from marine source materials, were examined and characterized by MS–MS (Table I). Samples were analyzed either as whole oils or as saturate fractions. In either case, known amounts of a deuterated cholestane standard {[$^2H_2$]$C_{27}\alpha\alpha\alpha$(20R) cholestane, with a similar mode of fragmentation to $C_{27}\alpha\alpha\alpha$(20R) cholestane but a molecular weight of 374 a.m.u. and a characteristic daughter ion at *m/z* 219}, was added as an internal standard to a known amount of sample prior to fractionation. The samples containing known concentrations of the internal standard were subsequently analyzed by direct-insertion probe tandem mass spectrometry (DIP-MS–MS) operating in the parent mode. In all of these experiments the parent ions of daughter ions at *m/z* 123, 191, 217 and 219 were monitored to determine sesquiterpanes, diterpanes, tricyclic terpanes, tetracyclic terpanes, pentacyclic terpanes, steranes and the deuterated cholestane respectively.

The intensities of parent ions detected during the DIP-MS–MS analysis of these oils were summed to produce a composite spectrum showing the distribution of the various biomarkers under examination. The composite DIP-MS–MS spectra obtained from two oils analyzed in this study, one produced from higher plant source material from the Gulf of Suez, are shown in Fig. 9. For comparison, distributions of the triterpanes and steranes obtained by conventional SIM of the ions at *m/z* 191 and 217 for the oil produced from higher plant material are shown in Fig. 10. Based on the concentration of [$^2H_2$]$C_{27}\alpha\alpha\alpha$ cholestane in the original sample, absolute amounts of C_{27}, C_{28}, C_{29} and C_{30} steranes can be determined from these spectra and amounts of the terpanes relative to the internal sterane standard can also be determined from data shown in Fig. 9. The quantitative data derived from all ten oils examined in this study are summarized in Table I.

It is not possible to make a direct comparison between the biomarker distributions obtained from the DIP-MS–MS data (*e.g.* Fig. 9) *versus* the multiple ion detection (MID) data (*e.g.* Fig. 10). The DIP-MS–MS data reflect the intensity of various parent ions in the original sample, and hence each parent ion will contain a contribution from several components. However, MID data only reflect variations in intensity of the major fragments of these different compound classes and for the most part, one peak in the chromatogram represents one component. For example, the peak at *m/z* 330 in the DIP-MS–MS data is not one component but a composite of all the terpane compounds having a parent ion at m/z 330. Likewise the peak at *m/z* 372 corresponds to all the C_{27} regular and rearranged steranes. Despite the inability to resolve individual isomers, the DIP-MS–MS approach is still extremely valuable for rapid and quantitative screening of oils and source rock extracts.

Despite certain limitations still being evaluated, the data presented in Table I clearly show that oils from marine *vs.* terrestrial sources can be distinguished by using

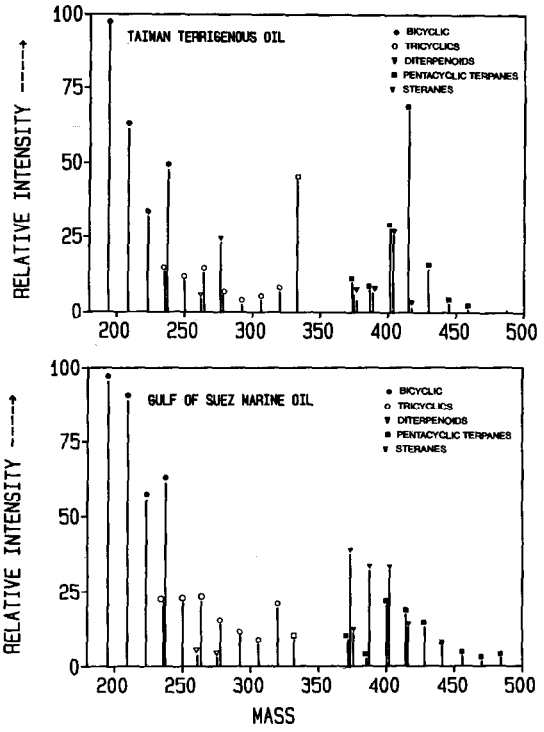

Fig. 9. Parent spectra obtained by DIP-MS–MS in parent mode. (a) A terrigenous oil from offshore Taiwan. (b) A marine oil from Gulf of Suez.

this DIP quantitation approach. Oils derived from terrestrial sources are dominated by the C_{29} steranes, with relatively low C_{30} sterane concentrations. The terrestrial oils also have high concentrations of C_{30} terpanes and C_{24} tetracyclic terpanes. In some cases, the C_{30} terpane content is relatively high, due to the presence of 18α(H)-oleanane as well as hopane in many of these samples. These two C_{30} terpanes, *m/z* 412 with daughter ions at *m/z* 191, cannot be separated by DIP-MS–MS. The marine oils generally have a higher concentration of steranes and C_{23} tricyclic terpanes than the oils from terrigenous sources. Various ratios, *e.g.* C_{27} steranes to C_{29} steranes (shown as 372/400 in Table I), steranes to terpanes (shown as 217/191 in Table I) and tricyclic terpanes to tetracyclic terpanes (shown as 318/330 in Table I) based on these quantitative data are also very different in terrigenous oils compared to marine oils and are therefore also useful for differentiating oils from different types of source materials.

3.3. GC–MS–MS analyses using short GC columns

In the preceding section, characterization of biomarker distributions with the DIP-MS–MS and deuterated cholestane used as an internal standard has been described. A major limitation of this approach is the fact that certain isomers and homologues cannot be resolved. For example, all of the C_{27} steranes with parent ions at *m/z* 372 and daughter ions at *m/z* 217 will be present as one peak in the cumulative

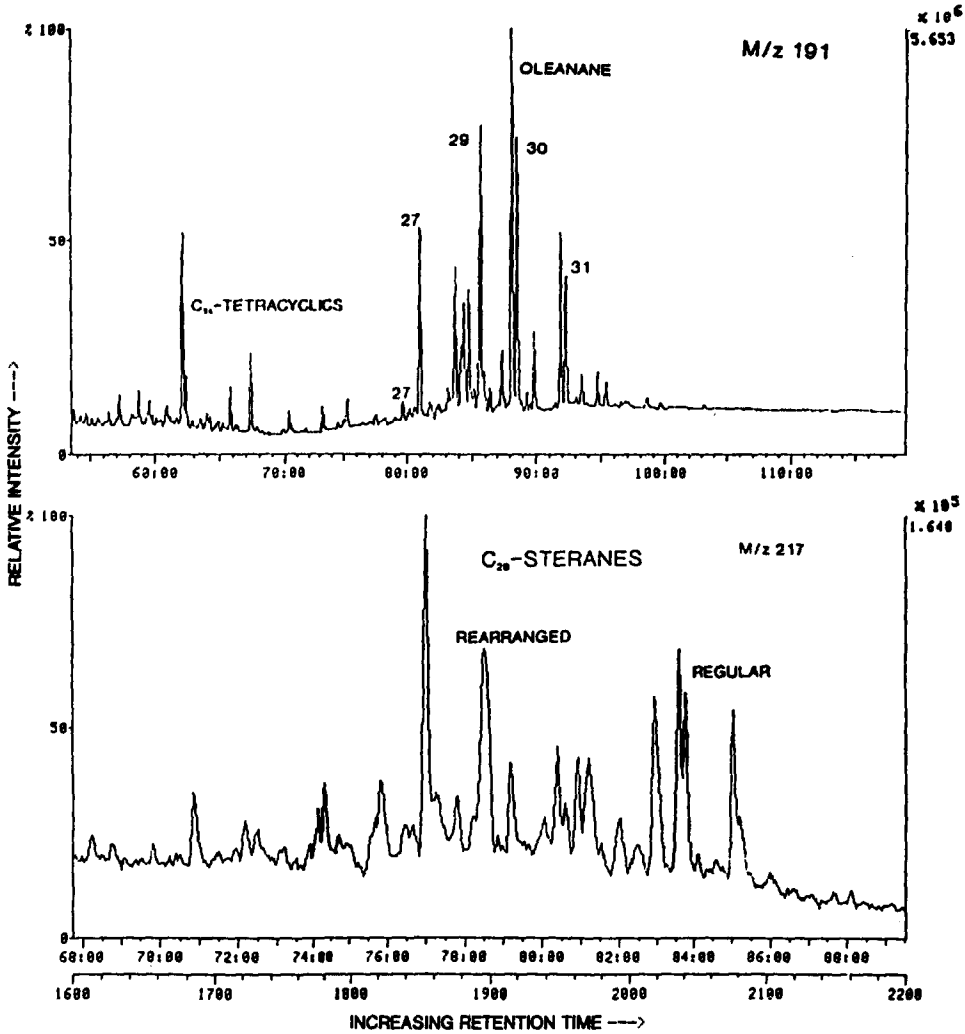

Fig. 10. The *m/z* 191 and *m/z* 217 mass fragmentograms of a terrigenous oil sample from offshore Taiwan.

spectrum. To maintain a relatively rapid analysis time whilst improving the resolution it is possible to use short chromatographic columns, 3 m in length, in conjunction with the MS–MS system [20]. This provides some separation of components with similar molecular weights, *i.e.* hopane and gammacerane with a relatively rapid analysis time and provides a better response (signal-to-noise) than the DIP method, due to better sample transfer into the ion source.

Thus, Fig. 11 shows a comparison between the direct insertion probe for 100 pg cholestane and the GC analysis of the same quantity of cholestane, demonstrating the far better signal-to-noise ratio obtained in the short-column GC analyses. Similarly, in Fig. 12 the distribution of the pentacyclic parent ions of *m/z* 191, obtained by both

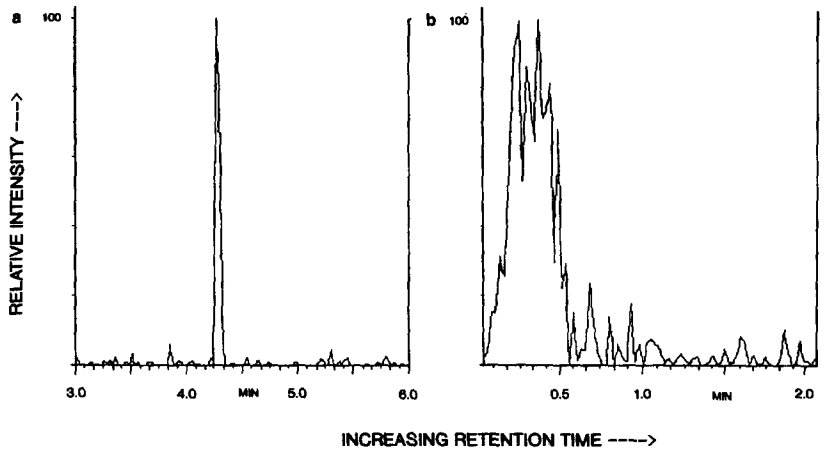

Fig. 11. A comparison between the analysis of 100 pg of 5α-cholestane in (a) GC–MS–MS mode and (b) the DIP-MS–MS mode.

techniques, are compared. Naturally the DIP method offers no resolution, but the short 7-min GC analysis time separates the various hopane homologues and starts to resolve the hopane diastereomers at C_{32}. The 22*S*- and 22*R*-C_{31}-homohopanes are not resolved on such a short column. However, when the data from these two chromatograms are used to sum the spectrum of the pentacyclic parents of *m*/*z* 191 from both techniques, the resultant terpane distributions, shown in Fig. 13, are virtually identical.

Another example demonstrating an advantage of the short column approach

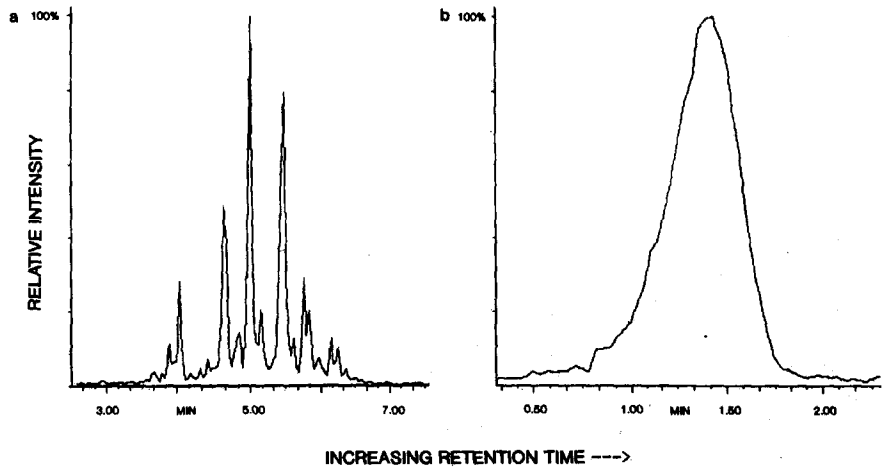

Fig. 12. Analysis of a crude oil to determine the pentacyclic terpane parent ions of *m*/*z* 191 in (a) the GC–MS–MS mode and (b) DIP-MS–MS mode.

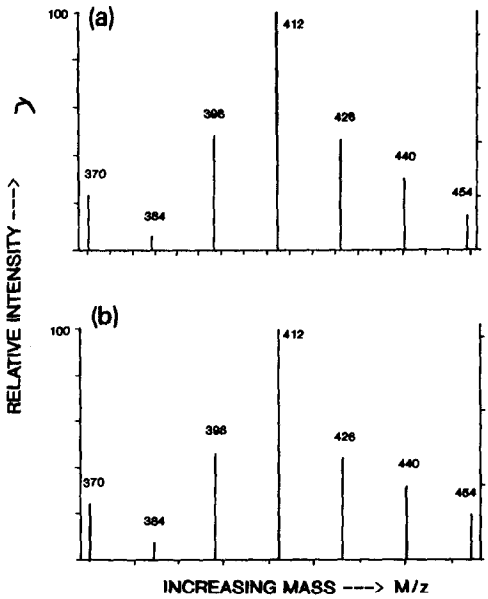

Fig. 13. Composite spectra obtained from the two data sets of Fig. 12, (a) GC–MS–MS and (b) DIP-MS–MS, showing the parent ion distribution for *m/z* 191.

over the direct insertion probe is shown in Fig. 14 and revolves around the ability to separate hopane and gammacerane both of which have molecular weights at *m/z* 412 and daughter ions at *m/z* 191. As mentioned above, similarities in the fragment ions produced from the *m/z* 412 parents of both compounds facilitate the separation of

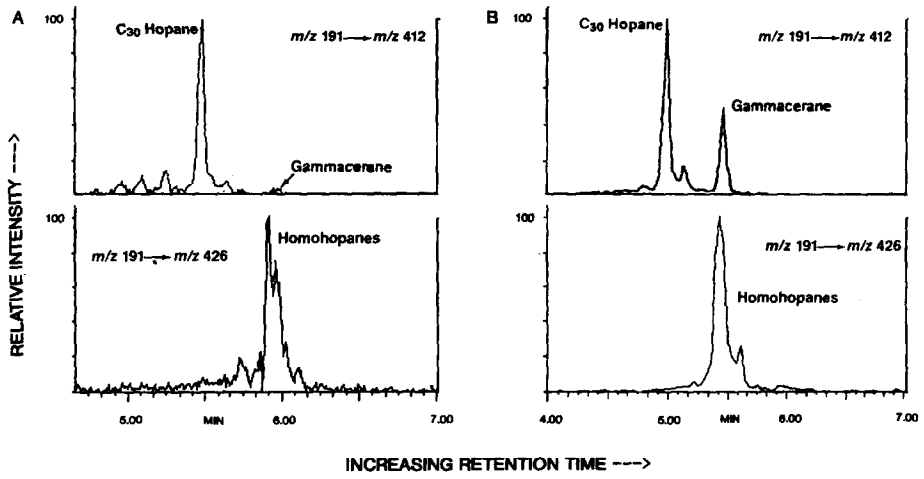

Fig. 14. Short column GC–MS–MS analysis of two oils to illustrate the rapid separation of hopanes and gammacerane resulting from the use of two different parent/daughter ion relationships. The oil for the data shown in the left hand chromatograms (A) was from the fresh-water Shanganning Basin and (B) from the moderately-saline Chaidamu Basin.

compounds with unique parent/daughter ions. Hence, although hopane and gammacerane cannot be resolved spectrometrically, the use of a short GC column will permit separation of these compounds, providing the relative gammacerane concentration in various samples rapidly. The hopane/gammacerane ratio can be used to provide us with an indication of the relative salinity of the original depositional environment [7].

4. CONCLUSIONS

This paper provides examples in which the MS–MS approach has been utilized to analyse complex geochemical samples with the system operating in either the parent or daughter mode. The resulting data may be in the form of identification of previously unidentified or tentatively identified components or it may provide information on the distribution of novel biomarkers. It is also an extremely useful technique for separating components which may have different molecular weights but are not resolved chromatographically. Operation in the daughter mode provides a novel method for obtaining collision spectra of specific parent ions which may be used to identify unknown components. Introduction of a complex mixture, *e.g.*, whole oil or hydrocarbon fractions, via the direct-insertion probe into a tandem mass spectrometer operating in the parent mode or daughter mode, or both, permits biomarker distributions to be obtained from these samples in a few minutes. Though the total separation of various isomers by the DIP-MS–MS approach is not possible, and the amount of information obtained from this technique is not as detailed as that obtained from the GC–MS–MS technique, the fingerprints derived are very valuable for the purposes of sample characterization and correlation.

DIP-MS–MS operating in the parent mode can be used for quantitative analyses of biomarkers by use of a deuterated internal standard. This technique allows the rapid quantitative determination of various biomarker classes as well as oil/oil correlations and source determinations. The present study has shown that differences in the biomarker concentrations between terrigenous oils and marine oils can be revealed by this quantitation technique.

The lack of homologues resolution by the DIP method can be overcome to some extent by the use of short GC columns. This technique partially resolved isomers and homologues that cannot be separated spectrometrically while keeping the analysis time short. Both of these approaches are therefore extremely valuable for rapid screening of a larger number of samples in order to obtain a preliminary idea of their biomarker distributions.

It should be clear from this study that tandem mass spectrometry provides a powerful alternative approach for biomarker analysis with many advantages over the conventional GC–MS approach. With the increasing availability of commercial tandem mass spectrometers, *e.g.*, triple-stage quadrupole mass spectrometers, these novel mass spectrometry techniques will be more and more important in the field of biomarker study. MS–MS provides an alternative and additional method of separating compounds which are not always separable by conventional chromatographic processes.

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