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QUANTITATIVE RESPONSES OF NORMAL ALKANES IN GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS

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SUMMARY

The quantitative response factors (RF) of fourteen long-chain normal alkanes were determined for gas chromatography–mass spectrometry with electron-impact (GC–EIMS) and chemical ionization (GC–CIMS). In GC–EIMS analysis, the compounds tested gave very similar RF/unit weight (RF/W) to the total ion current (TIC) and to the mass chromatograms of some characteristic fragment ions. In the GC–CIMS analysis, the alkanes showed similar RF/mol (RF/M) to TIC. However, an average RF/M for the quantitation of a large number of compounds using GC–CIMS could not be developed because of the considerable variations involved.

INTRODUCTION

Gas chromatography–mass spectrometry (GC–MS) has been widely accepted for quantitative analysis of individual target compounds in an organic mixture because of its sensitivity and specificity. The capability of monitoring one or several selected ions characteristic of a specific compound allows analyses which are free of interference from chromatographic co-eluates. Ideally, an isotope-labelled compound is required to serve as an internal standard for a reliable GC–MS quantitation¹. In many practical analyses, however, an external standard compound is commonly used instead, due to the expense and availability of labelled standards. In either case, the difficulty of obtaining individual standard compounds for calibration greatly restricts the utility of GC–MS for the quantitative multicomponent analysis of a complex mixture. A recent study demonstrated the use of GC with flame ionization detection (FID) for the determination of average response factors for certain organic compound classes². Within each class, the characteristic response factor can be employed to quantify a number of compounds. The use of average response factors in GC–FID analysis greatly reduces the number of available standard compounds necessary for multicomponent quantification. It would be very desirable to apply this concept to GC–MS techniques, since then both qualitative and quantitative analyses could be achieved simultaneously.

A survey of the quantitative behaviour of many compounds has to be based on a number of determinations, but reliability and reproducibility depends heavily

on the instrumental performance. In the past, performance was limited in GC-MS analysis by three major factors. First, compared with the detectors which are more commonly used with a GC instrument, a mass spectrometer is very complicated. Many operational parameters need to be controlled in a quadrupole mass spectrometer to achieve certain predefined performance criteria. These parameters include the condition of the filament, the electron beam energy, the repeller voltage, mass and sensitivity calibration by electronic parameters, and the condition of the electron multiplier. Simultaneous adjustment of so many parameters demands considerable operator skill. The daily manual tuning of the mass spectrometer resulted in poor reproducibility in GC-MS quantitative determinations. It was very common to observe a variation of more than 10% in quantitative determinations over a period of a few days. Second, the storage capability and the manipulation of voluminous raw data generated in a GC-MS run were very limited by the computer system used in the older instruments. Monitoring various selected ions for many compounds during a GC-MS run was beyond their capability. An accumulation of sufficient data for comprehensive comparisons required many GC-MS runs over a period of several days, during which significant error would be generated. Furthermore, incomplete transfer of the analytes and discrimination against certain compounds often occurred in the GC-MS interface and GC sample injector²⁻⁴. These problems in the old GC-MS instruments created difficulties in the study of the quantitative response of a number of compounds, obscuring any possible relationships.

Over the past few years, the performance of the GC-MS instrument has been dramatically improved mainly by the addition of powerful computers to the GC-MS system. A new sophisticated computer used in conjunction with the GC-MS provides automatic and accurate tuning, and also controls the operational parameters before and during a GC-MS analysis. These accomplishments result in a considerable saving of time and greatly improve the consistency in performance. The use of powerful computers dedicated to the specific task of storing and processing the extensive raw data of a GC-MS run has tremendously enlarged the utility of the run. The information which used to be drawn from many repetitive runs can now be obtained from a single GC-MS analysis, making it much more valuable. In addition many innovations have been made in the development of GC-MS hardware. A direct inlet GC-MS interface allows the direct insertion of a chromatographic column into the ion source, providing a complete transfer of GC eluate to the mass spectrometer. The discrimination against both high and low boiling point compounds is minimized by the use of a cool on-column injector in the gas chromatograph^{5,6}. Now one has a much greater chance to reveal any relationship in the response behaviour of many compounds in GC-MS analysis.

Two compounds classes were chosen for the study of quantitative response of organic compounds in GC-MS analysis. One is long-chain normal alkanes, which undergo extensive cleavage of bonds in electron-impact (EI) ionization. The second class, polycyclic aromatic hydrocarbons (PAHs), show much less fragmentation in the EI ionization process. This paper focuses on the alkanes and their response to total ion current (TIC), and to some specifically chosen ions which were determined by GC-EIMS. Since chemical ionization (CI) facilities have become available to a large number of laboratories interested in GC-MS quantitative analysis, the response behaviour of alkanes in GC-CIMS were also investigated and are discussed. The results obtained for PAHs will be discussed elsewhere⁶.

EXPERIMENTAL

Standard chemicals

The long-chain normal alkane standards used were purchased from Polyscience (Niles, IL, U.S.A.) with purities higher than 98%. The concentration of each compound in the standard solution was *ca.* 100 ng/ μ l.

Gas chromatographic-mass spectrometric analysis

All GC-MS analyses were performed on a Hewlett-Packard HP5987A gas chromatograph-mass spectrometer with an HP1000 data system (DS) and an HP7914 Winchester disk drive. A direct inlet interface coupling a Hewlett-Packard HP 5880A gas chromatograph to the mass spectrometer allows the insertion of a chromatographic fused-silica capillary column into the ion source. The chromatographic conditions of the HP5880A were: cool on-column injector at room temperature; column temperature programmed from 80°C for 1 min to 300°C at a rate of 6°C/min; and helium carrier gas flow-rate at 1 ml/min.

The HP5987A GC-MS-DS system is capable of several modes of operation including EI, positive ion CI (PICI), and negative ion CI (NICI). In the GC-EIMS analyses, the ionization voltage was operated at 70 eV and ion source temperature was 250°C. A supplier program AUTOTUNE was used to automatically adjust the mass spectrometer parameters to achieve certain performance criteria based on the reference compound perfluorotributylamine (PFTBA). The linear scan ranged from 50 to 500 a.m.u. at a scan-rate of 1000 a.m.u./s.

The reagent gas in GC-PICIMS was ultra-high purity methane (Linde Specialty Gas, Union Carbide, Canada). Before tuning for GC-PICIMS, the mass spectrometer is first automatically tuned against the reference compound PFTBA. Methane was then introduced into the mass spectrometer and the source pressure was adjusted to give maximum abundance of the CH_3^+ , C_2H_5^+ , and C_3H_7^+ ions in the methane gas mass spectrum. The electron energy was set at 230 eV and the ion source pressure and temperature at 1.6 torr and 200°C, respectively. The mass spectrometer was then tuned using the reference compounds PFTBA and benzophenone.

All the response factor determinations of the alkanes were carried out with identical operating conditions in GC-EIMS and GC-PICIMS analyses, respectively.

The HP1000 data system allows reconstruction of a mass chromatogram at any desired mass, and manual or automatic integration of peak areas.

Response factor calculation

Two kinds of response factors, RF/unit weight and RF/mol, were calculated as follows:

$$\text{RF/W} = \frac{\text{peak area counts of a compound}}{\text{weight of a compound injected (ng)}}$$

$$\text{RF/M} = \frac{\text{peak area counts of a compound}}{\text{number of moles of a compound injected (nmol)}}$$

RESULTS AND DISCUSSION

Response factors of n-alkanes in GC-EIMS

For the quantitation of a given compound by GC-MS analysis, the mass chromatogram for a particular ion of this compound was usually selected, but the possibility of utilizing the peak of this compound on the TIC has been greatly increased by the use of a high resolution GC column. It is easy to test whether a peak of the TIC corresponds to a single component by inspection of the mass spectrum of the peak.

The TIC responses of fourteen alkane standards in GC-EIMS analysis were determined and are listed in Table I. The values of both RF/M and RF/W are given in this table. Those compounds tested showed an increase in RF/M values as their molecular size increased, however they have very similar RF/W. Slightly lower values in the response to high boiling point compounds are usually observed, possibly due to the error in the integration of a broad peak. An average RF/W of 2030 area counts/ng with a relative standard deviation (R.S.D.) of 2.7% was obtained for the fourteen alkanes studied.

The response behaviour observed is quite similar to that in the study of GC-FID response of alkanes². In a GC-FID mechanism, every hydrocarbon is broken down to the same distribution of single-carbon species despite molecular size^{7,8}. The FID signal is proportional to the amount of carbon atoms introduced, therefore all alkanes have a similar RF/W. The ionization mechanism proposed for alkanes in GC-FID can be used as a basis for the explanation of the mechanism involved in GC-EIMS.

The long-chain normal alkanes are completely broken down under EI ionization at 70 eV. During the initial electron-molecule interaction, the alkane molecule

TABLE I

RESPONSE FACTORS OF *n*-ALKANES TO TOTAL ION CURRENT IN GC-EIMS

<i>Compound</i>	<i>Area counts/nmol</i>	<i>Area counts/ng</i>	<i>R.D.M. (%)</i> *
C ₁₄ H ₃₀	400 100	2021	13.0
C ₁₅ H ₃₂	436 478	2059	12.6
C ₁₆ H ₃₄	468 915	2075	15.1
C ₁₇ H ₃₆	493 461	2056	15.0
C ₁₈ H ₃₈	519 252	2044	16.4
C ₁₉ H ₄₀	556 445	2076	15.8
C ₂₀ H ₄₂	590 775	2095	14.3
C ₂₁ H ₄₄	619 378	2092	15.4
C ₂₂ H ₄₆	615 976	1987	15.9
C ₂₃ H ₄₈	655 754	2024	12.1
C ₂₄ H ₅₀	668 557	1978	11.7
C ₂₅ H ₅₂	711 789	2022	7.3
C ₂₆ H ₅₄	731 056	1997	4.0
C ₂₈ H ₅₈	744 733	1890	1.3

Average RF/W = 2030 area counts/ng
R.S.D.** = 2.7%

* Relative deviation from mean (R.D.M.) was based on two determinations.

** Relative standard deviation was based on the determinations of fourteen alkanes.

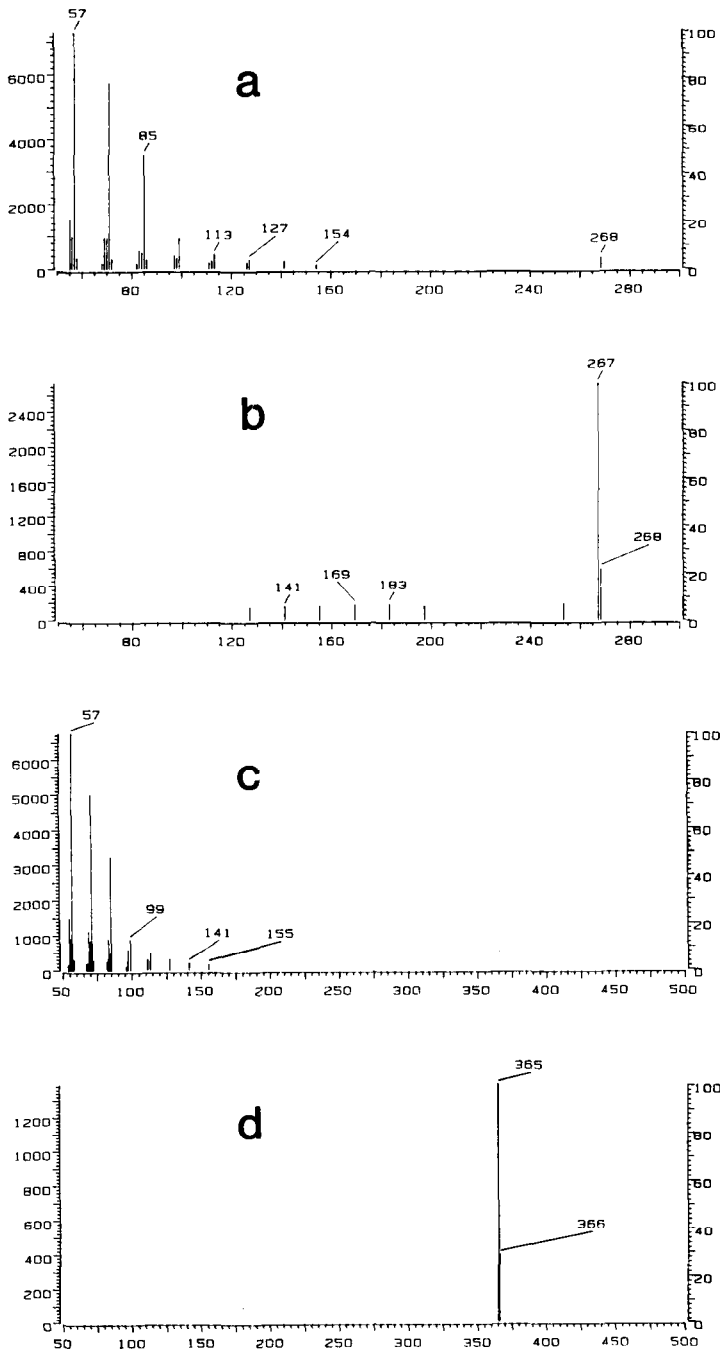


Fig. 1. (a) EI and (b) CI mass spectra of $n\text{-C}_{19}\text{H}_{40}$, and (c) EI and (d) CI mass spectra of $n\text{-C}_{26}\text{H}_{54}$. For EIMS and CIMS conditions, see the Experimental section.

receives a considerable amount of energy above its ionization energy. The most easily cleaved bonds are nearly equivalent along the carbon-carbon frame regardless of the difference in the molecular weight. This results in an *n*-alkane fragmentation pattern highly characterized by a cluster of peaks separated by 14 a.m.u. The EI mass spectra of two typical long-chain alkanes are illustrated in Fig. 1a and c. The *n*-alkanes containing more than eight carbon atoms show very similar mass spectra and can only be distinguished based on their low intensity molecular ions⁹. The most intense fragments are at C₃, C₄ and C₅⁹⁻¹¹. The responses of those fragment ions predominate the TIC peak of an *n*-alkane in GC-EIMS. Therefore, all long-chain alkanes exhibit very similar RF/W values.

One advantage in the use of GC-MS for quantitation is that one can monitor one or several ions during the analysis. These analytical ions are selected on the basis of both intensity and freedom from interference of other chromatographic co-eluates. According to these criteria, three major fragment ions of *n*-alkanes, C₄H₉⁺, C₅H₁₁⁺ and C₆H₁₃⁺, were chosen for this response study. In our work which deals with the analysis of complex mixtures, the mass range lower than 50 a.m.u. is not usually scanned in order to minimize the interference of abundant small fragments. Therefore, another intensive ion, C₃H₇⁺, was not involved in this study.

The determined values of RF/W for the three ions selected for the *n*-alkanes are listed in Table II. Preliminarily, all long-chain alkanes have similar RF/W values for the C₄H₉⁺, C₅H₁₁⁺, and C₆H₁₃⁺ ions. This observation is in agreement with the explanation mentioned above. Among these three ions, the average RF of C₄H₉⁺ gives a slightly higher sensitivity, but with a larger R.S.D. in the quantitative analysis. Better consistency in the responses of the different *n*-alkanes was found using the C₅H₁₁⁺ and C₆H₁₃⁺ ions.

TABLE II
RESPONSE FACTORS OF *n*-ALKANES TO SELECTED FRAGMENT IONS IN GC-EIMS

Compound	RF at <i>m/z</i> 57 area counts/ng (R.D.M. %)*	RF at <i>m/z</i> 71 area counts/ng (R.D.M. %)*	RF at <i>m/z</i> 85 area counts/ng (R.D.M. %)*
C ₁₄ H ₃₀	642 (7.0)	426 (0.5)	255 (1.7)
C ₁₅ H ₃₂	633 (7.5)	452 (10.0)	271 (13.8)
C ₁₆ H ₃₄	613 (2.8)	447 (6.8)	281 (13.6)
C ₁₇ H ₃₆	615 (7.7)	450 (11.6)	283 (13.9)
C ₁₈ H ₃₈	570 (0.1)	428 (3.8)	274 (7.5)
C ₁₉ H ₄₀	588 (3.7)	442 (7.5)	281 (11.2)
C ₂₀ H ₄₂	594 (5.3)	448 (6.5)	288 (10.3)
C ₂₁ H ₄₄	599 (6.6)	435 (1.8)	285 (6.5)
C ₂₂ H ₄₆	565 (7.4)	429 (11.0)	282 (13.3)
C ₂₃ H ₄₈	571 (5.0)	432 (7.4)	286 (10.7)
C ₂₄ H ₅₀	553 (3.4)	422 (6.8)	282 (6.4)
C ₂₅ H ₅₂	552 (0.9)	422 (3.2)	282 (4.2)
C ₂₆ H ₅₄	564 (2.9)	422 (0.1)	275 (0.7)
C ₂₈ H ₅₈	538 (7.2)	399 (5.1)	260 (2.2)
Average RF/W	586 (5.4)**	432 (3.4)**	278 (3.5)**

* R.D.M. was based on two determinations.

** R.S.D. was based on fourteen alkanes.

The extensive fragmentation observed in the EI mass spectra of long-chain *n*-alkanes produced very weak signals for the parent ions, although those parent ions showed high selectivities. In practice, it is not suitable to use the molecular ions of *n*-alkanes for GC-EIMS quantitation work due to the errors involved in the measurements of small signals. To utilize the selectivities of ions at high mass, a soft ionization technique can be employed in GC-MS quantitative analysis.

Response factors of n-alkanes in GC-PICIMS

The most common alternative ionization technique is PICI, which uses the combination of electron-impact ionization and ion-molecule collision ionization. In this gentle ionization process the carbon-carbon cleavage reactions are greatly minimized¹¹. Abundant product ions are usually formed from the sample molecule by gain or loss of hydrogen according to the proton affinity of the sample molecule. These ionic species, $[M + 1]^+$ and $[M - 1]^+$, are called quasimolecular ions. Alkanes have low proton affinities and generally produce $[M - 1]^+$ ions in GC-PICIMS. The amount of energy transferred to the quasimolecular ion during an ion-molecule collision is much lower than that transferred in EI ionization. Furthermore, since those quasimolecular ions are even-electron species, they are inherently more stable than the $[M]^+$ generated in the EI process¹⁰. Consequently, the fragmentation of the sample molecule is greatly reduced and the quasimolecular ion is normally the most intense ion in a CI mass spectrum. The typical PICI mass spectra of two long-chain alkanes are shown in Fig. 1b and d. One would predict that *n*-alkanes would exhibit different response behaviour with GC-PICIMS than with GC-EIMS, since the few high mass ions observed predominate their PICI mass spectra.

The *n*-alkane responses determined in GC-PICIMS are listed in Table III. No longer do all the alkanes have similar RF/W based on TIC. Their responses apparently decrease as the molecular weights increase. Instead, they show similar RF/M values on TIC, but with considerable deviations. The compounds tested could be sorted into two groups. One contains the compounds ranging from C₁₄ to C₂₀, and the other includes the remaining *n*-alkanes, with the exception of C₂₁H₄₄. Two average RF/M, 57 782 area counts/mol with an R.S.D. of 4.9%, and 39 018 area counts/mol with an R.S.D. of 5.7%, can be obtained for those two compound groups, respectively.

During the ion-molecule reaction processes in chemical ionization, the sample molecules introduced received an energy just above that needed for ionization. The ionization efficiency, *i.e.* the number of quasimolecular ions generated, is very dependent on the reagent gas and ion source temperature¹². In addition, the molecular structure of the sample has some effect on the ionization efficiency¹³. To achieve maximum sensitivity, CIMS conditions should be optimized. However, in the analysis of a multicomponent mixture, the optimum conditions for some compounds may not necessarily be the best conditions for other compounds. Consequently, under given GC-CIMS operation conditions, the yield of quasimolecular ions varies slightly from compound to compound. It is feasible to use an average RV/M on TIC for the quantitation of a small number of compounds by GC-PCIMS. However, applying the average response factor to a large number of compounds will lead to considerable error in quantitative analysis.

Table III also lists the quantitative responses of $[M - 1]^+$ for *n*-alkanes de-

TABLE III
RESPONSE FACTORS OF *n*-ALKANES DETERMINED IN GC-PICIMS

<i>Compound</i>	<i>RF at TIC</i> <i>area counts/nmol</i> <i>(R.D.M. %)*</i>	<i>RF at TIC</i> <i>area counts/ng</i>	<i>RF at [M - 1]⁺</i> <i>area counts/nmol</i> <i>(R.D.M. %)*</i>
C ₁₄ H ₃₀	52 610 (4.4)	266	27 568 (3.2)
C ₁₅ H ₃₂	56 422 (2.1)	266	32 177 (1.9)
C ₁₆ H ₃₄	58 675 (5.3)	260	36 392 (1.8)
C ₁₇ H ₃₆	57 861 (5.1)	241	38 658 (2.6)
C ₁₈ H ₃₈	60 503 (1.3)	238	40 446 (0.3)
C ₁₉ H ₄₀	61 206 (1.4)	229	42 508 (0.3)
C ₂₀ H ₄₂	57 198 (3.3)	203	41 015 (0.0)
C ₂₁ H ₄₄	49 881 (1.2)	169	35 887 (1.3)
C ₂₂ H ₄₆	39 786 (3.6)	128	30 586 (3.8)
C ₂₃ H ₄₈	37 659 (8.1)	116	29 202 (10.3)
C ₂₄ H ₅₀	35 283 (2.4)	104	27 828 (1.1)
C ₂₅ H ₅₂	39 419 (2.3)	112	30 236 (2.9)
C ₂₆ H ₅₄	40 724 (4.3)	111	31 837 (5.2)
C ₂₈ H ₅₈	41 235 (16.4)	105	32 679 (6.3)

* R.D.M. based on two determinations.

terminated in GC-CIMS analysis. Considerable variations are noted in the RF/M values for $[M - 1]^+$ among those *n*-alkanes. The possible reasons for these variations have been discussed above. Obviously, it is not recommended to employ a single average RF of $[M - 1]^+$ for the quantitative analysis of a large number of *n*-alkanes using GC-CIMS.

In both GC-EIMS and GC-CIMS, the abundances of ions produced in MS are very dependent upon the source temperature, ionization voltage and other MS operation parameters. Therefore, real samples and standards must be run under the same GC-MS conditions when external standards are used for quantitation purposes.

The response behavior of organic compounds on GC-MS varies among compound classes. The results of this study does not imply that the observation of alkane response can be generalized to other organic compound classes. This will be further discussed elsewhere⁶.

CONCLUSIONS

Due to a complete cleavage of the carbon-carbon bonds in GC-EIMS analysis, long-chain normal alkanes have very similar mass spectra in which a few characteristic fragment ions are predominant. This complete cleavage results in the *n*-alkanes having similar RF in terms of response per unit weight of compound introduced. The uses of an average RF/W in GC-EIMS analysis can be applied to the responses of *n*-alkanes to TIC and to the fragmentation ions C₄H₉⁺, C₅H₁₁⁺ and C₆H₁₃⁺.

In GC-CIMS analysis, small groups of *n*-alkanes show similar RF per mol to TIC, owing to the abundant quasimolecular ions and lack of fragmentation observed in their mass spectra. In practice this consistency in RF/M values is sufficient to allow one to use an average RF for the quantitation of a smaller group of *n*-alkanes.

However, the quasimolecular ion abundances are very dependent on the ionization conditions used. This dependency varies slightly from one compound to another, which results in a variation in the RF/M observed.

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