Quantitative Determination of Compounds in Mixtures by B^2E = Constant Linked Scans

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The B^2E = constant linked scan is shown to be an excellent tool for quantifications with reversed geometry mass spectrometers. Deuterium-labelled analogues may be used as internal standards, thus providing very simple clean-up procedures. The principle is demonstrated with the example of chloro-substituted benzoic acid methyl esters. Possible interferences arising from isotope peaks are discussed. The method is applied to the quantification of caffeine in beverages.

INTRODUCTION

The recently developed B^2E = constant linked scan technique[1,2] allows the search for all precursor ions generating a common preselected fragment ion in the second field free region (2nd FFR) of a reversed geometry instrument. The resulting spectrum shows narrow peaks, mainly due to the resolving power of the magnet (better than unit mass resolution). Other precursor ion scans like the accelerating voltage scan[3] and the B^2E = constant linked scan[4] yield spectra with much broader signals. The advantage of the narrow peaks in the B^2E spectra gave rise to the application of this scan technique to quantitative mass spectrometry. It allows the use of a deuterium-labelled analogue as internal standard. Even a d_1-labelled analogue is clearly separated from the non-deuterated compound.

The labelled standard and the compound of interest behave very similarly in evaporation, ionization and fragmentation. The goal of this work was to test simple applications and to detect the limitations of the B^2E constant linked scan in quantitative mass spectrometry.

RESULTS AND DISCUSSION

Fundamentals

Benzoic acid methyl ester **1** and two of its chloro-substituted homologues **2** and **3** have been chosen to demonstrate the power of the B^2E constant linked scan for quantifications.

The loss of $^{\cdot}OCH_3$ is the main fragmentation of the molecular ion of methyl esters. This fragmentation also takes place in the 2nd FFR, and may be strongly enhanced by collision induced dissociation (CID).[5]

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The search for precursors of the fragment ion at m/z 105 of **1** by a CID B^2/E and a CID B^2E constant linked scan shows the molecular ion to be the only precursor. As expected, the peak in the CID B^2/E spectrum is much broader than in the CID B^2E spectrum. In the latter, a weak signal at m/z 137 adjacent to the main peak at m/z 136 probably corresponds to the elimination of $^{\cdot}O^{13}CH_3$ from the $[M+1]^{+\cdot}$ ion. Surprisingly, in the corresponding B^2E spectra of the mono-(**2**) and di-chloro(**3**) analogues an additional $[M+2]^{+\cdot}$ signal appears (Fig. 1). In the case of **3**, this signal is even more intense than the $[M+1]^{+\cdot}$ peak. As the signal at $[M+2]^{+\cdot}$ corresponds theoretically to a loss of 33 u, it cannot be generated by the lightest isotope analogue. This is corroborated by the fact that the intensity of the $[M+2]^{+\cdot}$ signal in the CID B^2E spectrum increases in parallel with the relative abundance of the $[M+2]^{+\cdot}$ ions in the conventional mass spectrum with respect to the degree of chlorination. Thus, this $[M+2]^{+\cdot}$ signal in the CID B^2E spectrum seems to be due to an interfering fragmentation, obviously that of the analogous heavy chlorine isotope compound. This may best be seen in the fragmentation maps for **3** shown in Fig. 2(a) and (b). This mapping is a combination of a series of magnet scans at various ESA settings. The B^2E = constant line, has also been drawn for the $[M]^{+\cdot} \rightarrow [M - {}^{\cdot}OCH_3]^+$ fragmentation. Therefore, the intensities of the signals at the intersection points of that function line with the map values are equivalent to those in the B^2E = constant spectrum. Due to the CID conditions used in this experiment (Fig. 2(b)), the peaks are broadened and have distinct ridges beside them arising from fragmentations within the ESA.[6] The ridges are not present if unimolecular dissociations only are recorded (Fig. 2(a)). Following the calculated B^2E = constant line on both maps, corresponding to the fragmentation m/z 204 → m/z 173, the true origin of the unexpected $[M+2]^{+\cdot}$ peak in the B^2E spectrum under CID conditions becomes obvious. Contrary to the case of the unimolecular decomposition, where only the m/z 204 → m/z 173 fragmentation appears, under CID conditions the B^2E line cuts out a section of the broadened peak

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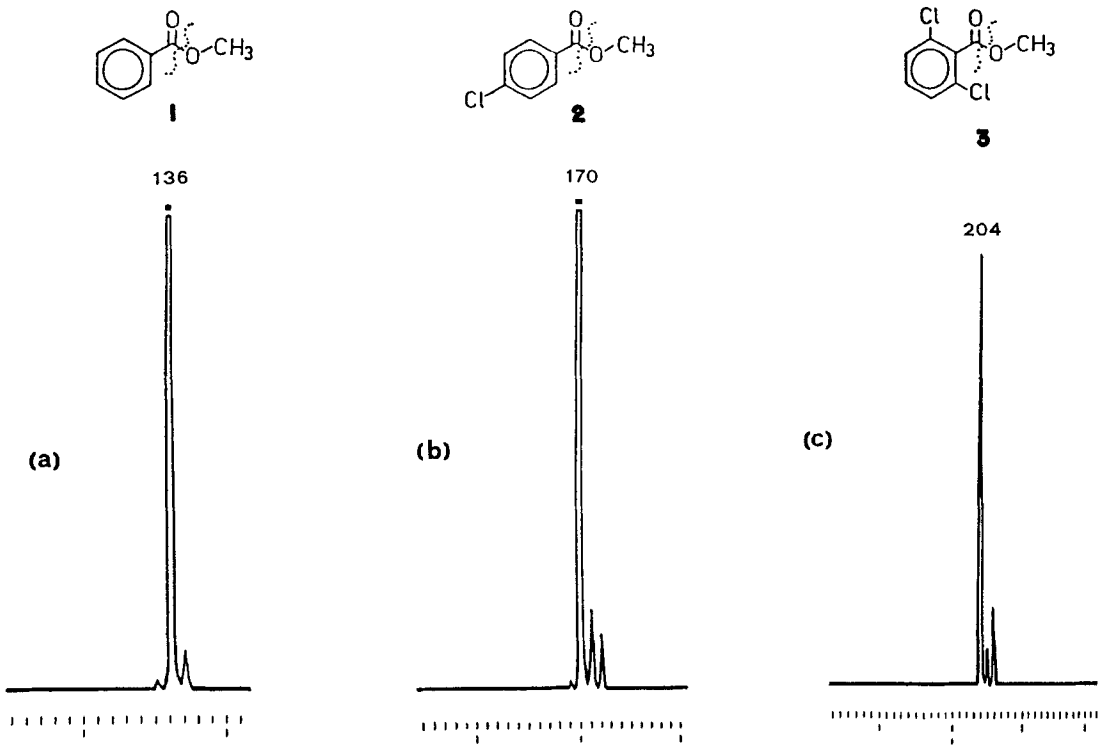

Figure 1. Partial CID B^2E spectra of the following ions: (a) m/z 105 from **1**, (b) m/z 139 from **2**, (c) m/z 173 from **3**.

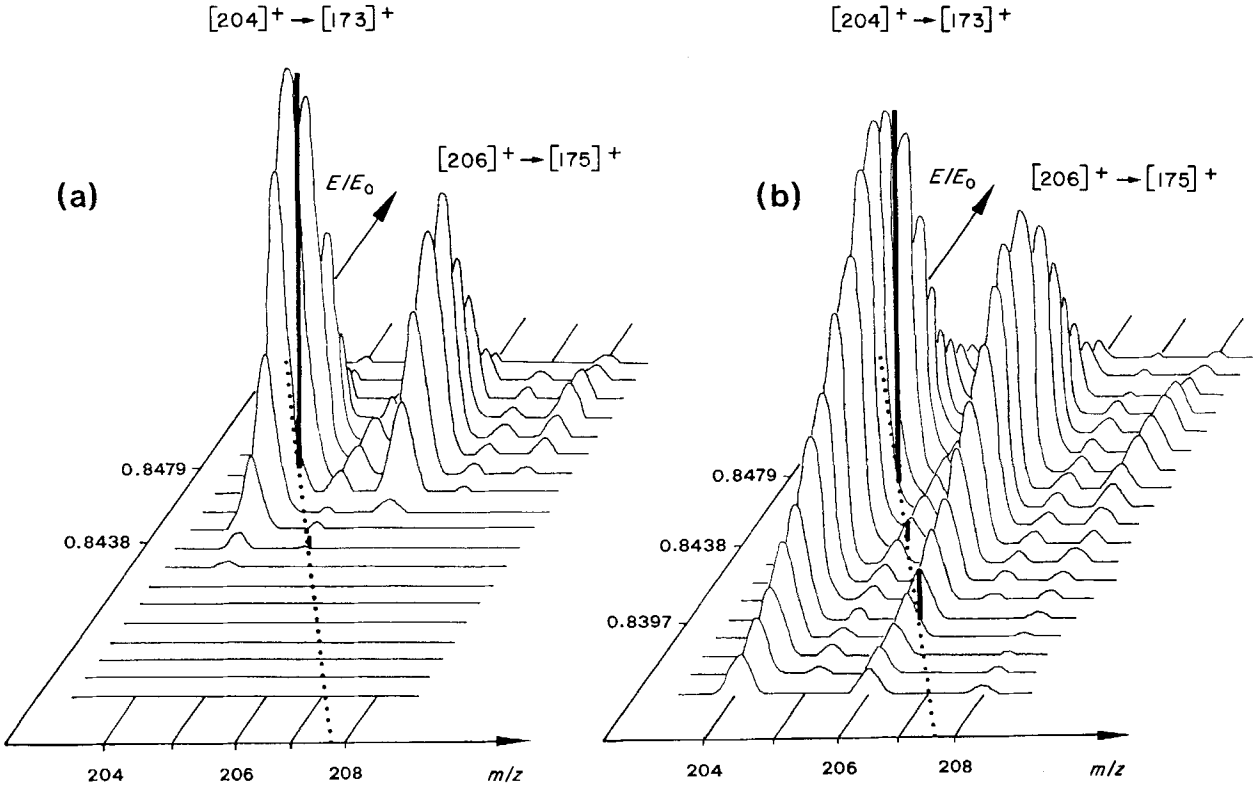

Figure 2. Mapping of the fragmentation of the molecular ion of **3** in the 2nd FFR: (a) unimolecular dissociation (loss of $^{\cdot}OCH_3$), (b) collision induced dissociation (loss of $^{\cdot}OCH_3$). Note the intensities of the fragment peaks at the intersection points with the dotted line of the B^2E = constant function for the parent ion going to the ion m/z 173.

due to fragmentations of the ion m/z 206, thus giving rise to the interference signal at $[M+2]^{+\cdot}$ in the B^2E spectrum. Taking into account this fact, it is concluded that for quantitative determinations an internal standard has to be at least 3 u heavier than the compound of interest, in order to avoid possible interferences—especially in the case of compounds containing heteroatoms analysed under CID conditions.

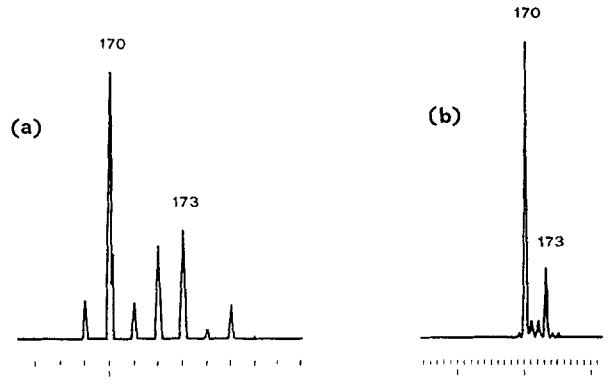

Figure 3. Partial spectra of a mixture of **2** and **4** in the ratio 1:4: (a) conventional mass spectrum (molecular ion region), (b) CID B^2E spectrum of the ion m/z 139.

Quantitative applications

The narrow signals in the B^2E = constant linked scans can be used for quantitative determinations. A compound of interest may be quantified by using the deuterium-labelled analogue; both of them will generate a common fragment ion. When considering only the narrow peaks, a d_1-labelling seems to be sufficient. With regard to the inference peaks previously discussed, a d_3-labelling is to be preferred. As an example we have chosen a mixture of **2** and its d_3-analogue (**4**), labelled in the alcohol portion of the ester, in a ratio of 4:1. In the CID B^2E spectrum of the m/z 139 ion the ratio of the two compounds is directly represented in the intensity ratios, whereas the ratios in the conventional mass spectrum are falsified by the isotope peaks (Fig. 3).

The method has also been applied to the quantification of caffeine(**5**) in beverages. The metastable molecular ion of **5** generates a fragment at m/z 109, which is suitable for a precursor ion search. Under CID conditions this fragmentation is strongly enhanced. The CID B^2E spectrum of the fragment ion m/z 109 shows two major signals at m/z 137 and m/z 194 (molecular ion). The first one is generated by a loss of $OC{=}NCH_3$ from the molecular ion. For the quantification, caffeine-d_3(**6**) is used as internal standard, which also fragments to the ion m/z 109. In the CID B^2E spectrum of that ion caffeine-d_3 gives a signal at m/z 197. In an actual sample with the internal standard, the caffeine content is calculated from the ratio of the signal intensities at m/z 194 and m/z 197 (Fig. 4). The caffeine content of five different Cola soft drinks (manufactured in Switzerland), an espresso coffee and a black tea have been determined by this method. The results are summarized in Table 1 and a spectrum of a soft drink sample is shown in Fig. 4.

The method is generally applicable to a compound, if (i) there is a precursor ion which is stable enough to give an intense signal in the conventional spectrum, and (ii) the same ion generates under CID conditions mainly one major fragment ion which does not retain the label. The method is also suitable for a profiling if there is a common fragment ion of a series of compounds. The reproducibility was found to be better than 5% in the example of caffeine.

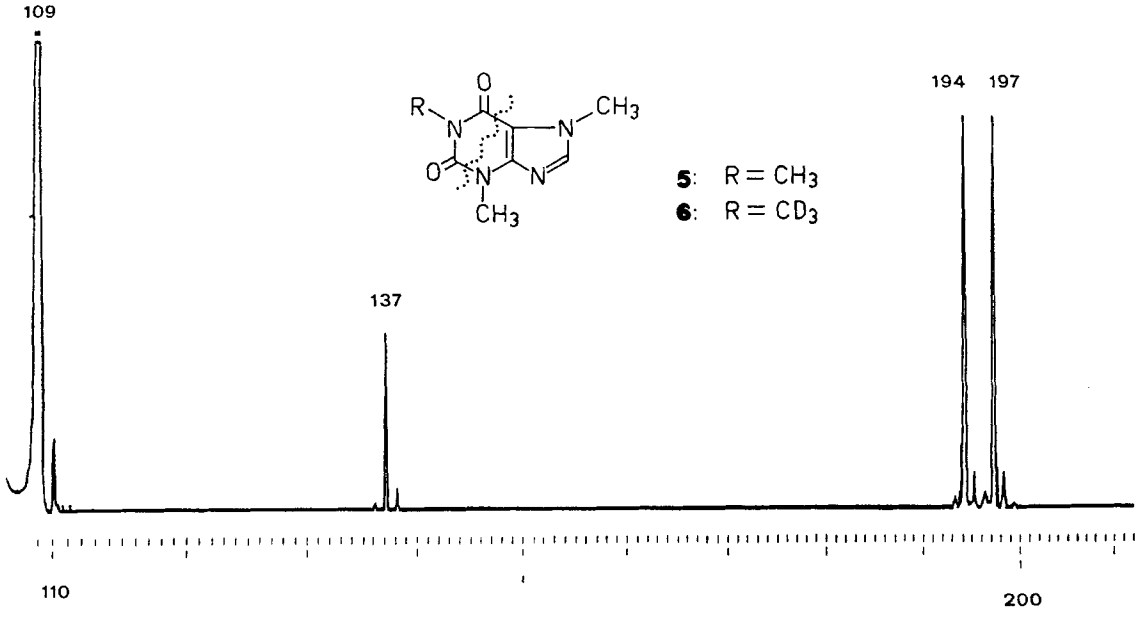

Figure 4. CID B^2E spectrum of the ion m/z 109 of a Coca Cola sample with the internal standard as caffeine-d_3 (**6**).

Table 1. Quantitative determination of caffeine in beverages

Sample/brand	mg/l
Coffee/Espresso Denner	900
Coffee/Hag, decaffeinated	44
Black tea Assam/London Tea Company	510
Coca Cola Soft Drinks[a]	73–158

[a] Five different soft drinks manufactured in Switzerland.

EXPERIMENTAL

All spectra were recorded on a modified Varian MAT CH5-DF mass spectrometer. The instrument was equipped with a small collision chamber in the second field free region.[7] Helium was used as collision gas at a pressure of 2×10^{-6} Torr, as recorded by an ionization gauge unit located in its normal position in the analyser. Linked scans were performed with a high precision digital linked scan unit MSP 8103 (Mass Spectrometer Products, CH-3098 Köniz, Switzerland). The ion source was operated in the EI mode with an electron energy of 70 eV and an accelerating voltage of 3 kV. Samples were introduced by a direct inlet system.

Benzoic acid methyl ester and *p*-chlorobenzoic acid methyl ester were prepared from the corresponding acid chlorides and methanol. Methanol-d_3 was used for the synthesis of the deuterium analogues. The methyl ester of 2,6-dichlorobenzoic acid was synthesized by the reaction of the acid chloride with sodium methanolate. For the deuterium analogue sodium methanolate-d_3 was used. Caffeine and caffeine-d_3 were prepared by methylation of theobromine (Siegfried, Zofingen) with methyl iodide and methyl iodide-d_3, respectively. Beverage samples were prepared by adding 0.2 ml sodium carbonate solution (4% in water) and 100 μg caffeine-d_3 to 1 ml of the beverage, followed by an extraction with 0.3 ml of dichloromethane. An aliquote of this extract was directly analysed at a probe temperature of 55 °C. Coffee was prepared in an espresso machine from 10 g coffee powder and 150 ml water/73 °C. Tea was prepared from 3 g loose tea in 130 ml water/80 °C for 3 minutes.

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