Proton transfer chemical ionization mass spectrometry of fatty acid methyl esters separated by gas chromatography: quantitative aspects

The propensity of esters to accept protons in the gas phase during chemical ionization (CI) mass spectrometry (MS) has been well known for some time. This proton transfer CI almost exclusively produces the \([M+H]^+\) quasimolecular ion for most esters. While it has been suggested that this could serve as the basis for particularly sensitive quantification of fatty acid methyl esters (FAME) separated by gas chromatography (GC), the application of this approach to quantitate FAME has not been fully explored. Here, we briefly address some considerations pertinent to the application of GC with proton transfer CI-MS to quantitative analysis of FAME. Limits of detection and relative response factors (RF) for a number of FAME separated by GC and detected by CI-MS are presented and discussed. While the detection limits are comparable or superior to other detection methods, it was noted that the RF for unsaturated FAME were dramatically reduced with increasing degree of unsaturation.

Keywords: Fatty acid methyl esters, chemical ionization, mass spectrometry.

1 Introduction

The coupling of gas chromatography (GC) to mass spectrometry (MS) has provided numerous tools for the structural characterization of fatty acids (FA). While GC-MS is also generally accepted to be a reliable quantitative method, relatively few laboratories make use of GC-MS for quantitation of FAME, instead favoring the flame ionization detector (FID). Indeed, discussions of FAME quantitation by GC-MS are conspicuously absent from even relatively recent reviews on the subject of FA analysis [1–3]. Nonetheless, the sensitivity and selectivity of GC-MS make it an advantageous platform for FAME quantitation [4].

Of particular interest in this report is the application of GC-MS with chemical ionization (CI) to quantitative analysis of FAME; however, most work involving CI-MS for FA analysis has been aimed towards structural elucidation. For example, isobutane CI of trimethylsilyl FA or acetonitrile CI of FAME have proven useful for determining the position and geometry of double bonds in polyunsaturated FA [5–10]. When CI-MS is used for purposes of quantitation, the use of a CI reagent that results in a single ionized species is most useful. For example, isobutane or methane CI of FAME produces a large proportion of the \([M+H]^+\) pseudomolecular ion via proton transfer [11–16]. This has the advantage of producing a single protonated pseudomolecular ion for each analyte molecule, rather than the more complicated addition products of other CI schemes or the assortment of fragment ions that would be observed in the case of electron impact (EI) ionization. Quantitation based on the isobutane CI \([M+H]^+\) pseudomolecular ion of FAME has been reported to allow for considerable enhancement of the signal-to-noise ratio compared to quantitation based on the EI \(M^+\) molecular ion [17]. Quantitation based on the isobutane CI \([M+H]^+\) ion of FA isobutyl esters has also been reported to provide excellent detection limits [18]. These reports notwithstanding, there is relatively little information available on the quantitative applicability of GC with proton transfer CI-MS to the analysis of FAME. In this communication, we briefly report on the performance of GC-CI-MS for FAME determination.

2 Materials and methods

2.1 Standard FAME

A series of standard FAME calibration mixtures was prepared as described elsewhere, with all FAME present at equal amounts in each calibration level [4, 19]. Also, a...
commercially available standard mixture of 37 FAME (Supelco, Bellefonte, PA, USA) was used in the assessment of response factors (RF) as previously reported [4]. The original solution was diluted tenfold to give a final concentration of 1.0 mg/mL total FAME in the RF standard, with each FAME present at either 2%, 4%, or 6% of the total FAME by mass. All standards were spiked with the FAME of C21:0, used here as an internal standard, to obtain a concentration of 50.0 μg/mL. Each calibration and RF standard was analyzed in quadruplicate.

2.2 GC and MS

All analyses were carried out on a Varian CP-3800 GC equipped with a Varian Saturn 2200 ion trap MS (Walnut Creek, CA, USA). All instrumental parameters and chromatographic conditions were as described previously [4, 19]. Briefly, chromatography of FAME was performed using a 60 m × 0.25 mm DB-23 capillary column with a film thickness of 0.25 μm (Agilent Technologies, Wilmington, DE, USA). Helium was used as the carrier gas at a flow rate of 1.0 mL/min with constant flow compensation. The GC inlet was maintained at a temperature of 300 °C, and the MS transfer line was held at a temperature of 250 °C. Sample injections of 1 μL were performed without split for 30 s; thereafter, a split ratio of 10 : 1 was applied for the duration of the separation. Column oven temperature was linearly ramped from 125 °C to 240 °C at a rate of 3 °C/min, with a final hold of 1.67 min for a total analysis time of 40.00 min.

Following a 10-min solvent delay, MS acquisition was commenced over a range of 40–400 m/z at a scan rate of 2 scans/s. ACS reagent-grade isobutanol (J.T. Baker, Phillipsburg, NJ, USA) was used as the CI reagent. The ion trap instrument accommodated a vessel containing a few milliliters of the CI reagent, which was interfaced to the mass analyzer with a needle valve in-line for regulating the admission of vapor into the ion trap. The valve was adjusted such that the intensity of the isobutanol [M-H]+ ion (m/z 57) generated by EI ionization (used here for tuning the CI reagent flow only) was optimized according to manufacturer’s specifications.

3 Results

3.1 Detection limits

The limits of detection (LOD) for a variety of FAME as determined by CI-MS are given in Tab. 1. The LOD for each analyte was calculated from the corresponding calibration curve according to the linear regression slope-intercept approach [20], applied as discussed in a previous publication [4]. The tabulated LOD include those determined by monitoring the total ion counts (TIC) as well as those based on selected ion extraction (SIE). In SIE, complete mass spectra are recorded, but undesirable masses are excluded at the data processing stage for purposes of quantitation. While this involves the use of only a subset of the observed ions for quantitation, the overall result is an improvement of the signal-to-noise ratio due to elimination of most non-analyte responses from the signal. It should be emphasized that SIE is fundamentally different from selected ion monitoring (SIM). SIM involves the exclusive acquisition of a single ion for a specified dwell time; conversely, SIE involves the analysis of all ions, but the desired ion is extracted from the data. In these experiments, the [M+H]+ ion was extracted for SIE, except for FAME which yielded other ions with at least 50% abundance relative to the pseudomolecular ion; in this event, the three most abundant ions were extracted for quantitation.

For the assortment of FAME listed in Tab. 1 (i.e., ranging in carbon number from C14 to C22, and ranging in degrees of unsaturation from zero to six), most detection limits were found to be in the low picomole range. With the use of SIE, the LOD can often be reduced compared to quantitation based on the TIC, in a few cases allowing an improvement of as much as an order of magnitude. Fig. 1 provides an example of substantially reduced minimum detectable quantity attainable based on SIE of the pseudomolecular ion as opposed to monitoring the TIC. However, not all detection limits were significantly reduced by the use of SIE; in several cases, the improvement was only marginal.

<p>| Tab. 1. Limits of detection for various FAME when quantitation is performed by TIC and SIE. |</p>
<table>
<thead>
<tr>
<th>FAME</th>
<th>TIC</th>
<th>SIE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOD [μg/mL]</td>
<td>LOD [pmol]</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.33</td>
<td>1.36</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.23</td>
<td>0.85</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>0.26</td>
<td>0.97</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.37</td>
<td>1.24</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>0.43</td>
<td>1.45</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>0.34</td>
<td>1.15</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>0.26</td>
<td>0.88</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.24</td>
<td>0.82</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.64</td>
<td>1.96</td>
</tr>
<tr>
<td>C20:1n-9</td>
<td>0.62</td>
<td>1.91</td>
</tr>
<tr>
<td>C20:2n-6</td>
<td>0.33</td>
<td>1.02</td>
</tr>
<tr>
<td>C22:1n-9</td>
<td>1.04</td>
<td>2.95</td>
</tr>
<tr>
<td>C22:4n-6</td>
<td>0.20</td>
<td>0.58</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>0.19</td>
<td>0.55</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>0.37</td>
<td>1.08</td>
</tr>
</tbody>
</table>
3.2 Response factors

The average response of each analyte with respect to the internal standard was used to calculate the analyte RF according to the relationship below.

\[ RF_a = \frac{R_a C_a}{R_s C_s} \]  

(1)

The RF of an analyte \( (RF_a) \) is given in terms of the analyte response \( (R_a) \), the internal standard response \( (R_s) \), and the concentrations of the analyte and the internal standard \( (C_a \text{ and } C_s, \text{ respectively}) \). The CI-MS RF for several FAME are illustrated in Fig. 2. The RF dependence upon carbon number for CI-MS initially behaves as a direct proportion, but with increasing carbon chain length, the RF gradually becomes less dependent upon carbon number. Examination of the RF for a series of unsaturated FAME reveals an impeded response of CI-MS towards these analytes, with nearly a threefold reduction in the RF for two unsaturations as opposed to a single double bond. We have noticed that as the number of unsaturations increases, the contribution of the \([M+H]^+\) is gradually reduced, with concomitant appearance of additional fragment ions (data not shown). The exact mechanism leading to this fragmentation is presently not conclusive, or within the scope of this communication; however, this observation may serve as a partial explanation for the extensive reduction in CI-MS response to unsaturated FAME.

A non-linear CI-MS response was observed for FAME concentrations above about 25 \( \mu g/mL \) (25 ng injected). This was not unexpected, as loss of analyte ions from the ion trap can occur as a consequence of space charging when ions are present in the trap at high concentration. The non-linearity was observed for both saturated and unsaturated FAME; however, the degree to which the response deviated from linearity became exaggerated with additional degrees of unsaturation. While the LOD determined for the unsaturated FAME were in the same order as those for saturates, this was likely because the LOD calculation is based upon measurements taken at low concentrations, where the response was essentially linear for all analytes. Thus, while CI-MS of polyunsaturated FAME exhibited satisfactory performance at trace concentrations, the increasingly poor response towards polyunsaturates became far more pronounced at intermediate to high concentrations.

4 Discussion

In practice, proton transfer CI-MS for FAME quantitation is most useful for the quantitation of saturates or mono-unsaturates, as RF were drastically reduced for unsaturated FAME at concentrations of approximately 25 \( \mu g/mL \) (25 ng FAME injected). Despite this limitation, GC-CI-MS of FAME with selected extraction of the quasimolecular ion provides detection limits as low as 100 femtomoles, making it an exceptionally sensitive technique for FAME
while sensitivity is often not a concern in FAME analysis, reduced detection limits are still of interest, as they allow sample consumption to be minimized while still permitting quantitation of trace components. Recently, this laboratory published a study of GC-MS with EI ionization for FAME quantitation [4]. The detection limits presented here compare favorably to those reported for EI-MS, with CI-MS providing notably reduced LOD in several instances; however, the precision of CI-MS quantitative measurements is generally poorer than that of EI-MS, as evident when comparing the reproducibility of CI-MS and EI-MS RF.

It should be pointed out that the use of CI for some analytes does not preclude the use of other forms of ionization during the same sample analysis, since most instrumentation and accompanying software allows different analytes to be acquired in different ionization modes.
Indeed, our laboratory has successfully applied CI-SIE for estimation of trace level (i.e., undetectable by EI-MS) saturated FAME in real samples while concurrently using EI-MS for the remaining FAME via online ionization mode switching.

Importantly, MS detection methods have RF towards FAME that are more sensitive to the identity of the analyte than, e.g., FID RF. In addition, the response behavior can vary significantly among types of MS systems (e.g., in quadrupole versus ion trap MS). While this does not preclude the use of GC-MS as a powerful quantitative platform for FAME analysis, it is important that users of GC-MS for FAME quantitation address such considerations with conscientious application of rigorous quantitative calibration and quality control measures.

Acknowledgments

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References

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