Image Cover Sheet

CLASSIFICATION	SYSTEM NUMBER
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TITLE

ISOTOPE ENRICHMENT OF PALMITIC ACID ESTERS

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Letter to the Editors

Isotope enrichment of palmitic acid esters by EI GC/MS

Dear Editors,

The precision of ratio of (m + 1)/(m + 0) for determination of (1-^{13}C)palmitic acid greatly improves if instead of monitoring molecular ions 271 *m/z*/270 *m/z* of methyl palmitate, fragment ions of pentafluorobenzyl ester of palmitic acid are used. The ester group is eliminated in EI source, yielding positive ions suitable for the isotope enrichment determinations.

INTRODUCTION

In their recent publication, Patternson and Wolfe[1] discuss a precision determination for (m + 1)/(m + 0) of methyl palmitate, using molecular ions 271 *m/z*/270 *m/z* and conclude that it is greatly affected by the concentration of the analyte. It is well-known that protonation of the molecule (self-chemical ionization) can occur in EI ion source at high analyte concentrations, and is both concentration- and compound-dependent. It is also influenced by the construction and cleanliness of the ion source, and by the presence of other co-eluting compounds in the ion source. Thus, there are several potential sources of variability, which can limit the usefulness of molecular ions for the ion ratio determination. We report here, what we believe to be a simple solution to the problem, namely, the use of mass fragments containing the 1-^{13}C label, as suggested by Kienle and Magni.[2] In studying (1-^{13}C)palmitic acid metabolism, we have utilized pentafluorobenzyl esters (PFB) of palmitic acid and heptadecanoic acid. These yield positive ion fragments suitable for the calculations of ^{13}C enrichment.

EXPERIMENTAL

Mass spectra were measured on a Hewlett Packard MSD 5970 mass spectrometer with 5890 gas chromatograph. Standard autotune parameters were obtained by HP Pascal Chemstation. Gas chromatographic conditions were as follows: injector 260 °C, mass spectrometer interface 280 °C, column J&W DB-1, 30 m, 0.25 mm i.d., 0.25 μm stationary film thickness, head pressure of He_2 was 10 psi, splitless injector opened after 40 s. The temperature profile was: 200 °C for 1 min, then programmed at 15 °C min^{-1} to 280 °C, then held at 280 °C for 5 min. The retention time for palmitic ester was 6.2 min, for heptadecanoic ester, 7.2 min. Mass fragments 255 *m/z*, 256 *m/z*, and molecular ions 436 *m/z* and 437 *m/z* were monitored for the pentafluorobenzyl (PFB) ester of palmitic acid, 269 *m/z* and 450 *m/z* for heptadecanoic acid ester. The dwell time was set at 10 ms. The plasma samples were extracted using a published method.[3] Derivatization was performed as described earlier.[4–6] The dried acids were reacted with 10 μl pentafluorobenzyl bromide in 100 μl of 10% diisopropylethylamine in acetonitrile, for 10 min at 40 °C. The standard curves were constructed and used for concentration determinations as described by Wolfe.[3]

RESULTS AND DISCUSSION

During our first approach to the assay setup, we realized that the imprecision encountered with monitoring molecular ions of methyl palmitate (around 3% CV) is greater than one would expect, simply from the variability in integration of mass chromatographic peaks. We concluded that some self-chemical ionization was contributing to (m + 1) ion creation. The fragmentation of pentafluorobenzyl esters of carboxylic acids is known to proceed at the ester bonds. The resulting positive ions fragments retain the 1-^{13}C label when present. In case of palmitic ester, the (m + 1)/(m + 0) is determined by monitoring fragments 255 *m/z* (4.0%) and 256 *m/z*. Heptadecanoic acid, an internal standard, fragments in a similar fashion, producing 269 *m/z* fragment (3.5%) which was used for quantitation. The base peak 181 *m/z* was present in both spectra, and corresponds to the pentafluorobenzyl group. Molecular ions 436 *m/z* (1.1%) and 450 *m/z* (0.8%) respectively, were also present. The proposed fragmentation was confirmed by using (1-^{13}C)palmitic acid, resulting in a corresponding increase of 256 *m/z* and 437 *m/z* ion intensities. To test our theory of self-CI, we have looked at the molecular ion and a fragment ion of PFB ester of palmitic acid. The table shows statistics encountered in the same injection while measuring (m + 1)/(m + 0) using either molecular ions ratio (437 *m/z*/436 *m/z*) or fragments ratio (256 *m/z*/255 *m/z*). The presented data were obtained from different plasma samples over a period of two days. The table is sorted by the increasing area of the 255 *m/z* fragment and does not reflect the order of injections into GC/MS.

The improvement of coefficient of variation for the (m + 1)/(m + 0) measurements, when the fragments 256 *m/z* and 255 *m/z* were

*Area 255 *m/z*	*Area 256 *m/z*	*Area 436 *m/z*	*Area 437 *m/z*	256 *m/z*/255 *m/z*	437 *m/z*/436 *m/z*
1152.6	209.2	257.9	64.5	0.181503	0.250097
1237.5	220.1	307.5	69.3	0.177859	0.225366
5278.6	937.5	1222.0	297.3	0.177604	0.243290
8067.8	1435.3	1936.6	464.6	0.177905	0.239905
14591.3	2617.9	3722.1	897.7	0.179415	0.241181
15526.9	2786.8	3896.9	951.5	0.179482	0.244168
17469.6	3106.2	4452.0	1053.7	0.177806	0.236680
19259.3	3442.5	4849.1	1154.8	0.178745	0.238147
22597.2	4053.8	6054.5	1452.2	0.179394	0.239855
27729.7	5006.0	7892.3	1851.3	0.180528	0.234570
			mean	**0.179024**	**0.239326**
		$n = 10$	**SD**	**0.001295**	**0.006541**
			%CV	**0.72**	**2.73**

* Area × 100

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employed, convinced us that the use of PFB derivatives is an acceptable approach to amend the precision of the enrichment determinations, without having to resort to complicated mathematical analysis. The proposed mathematical approach[1] does not correct for all factors contributing to chemical ionization.

Sincerely

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