APPLICATION NOTE

Gas Chromatography/ Mass Spectrometry

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Analysis of FAMEs Using Cold EI GC/MS for Enhanced Molecular Ion Selectivity

Introduction

Characterization of fatty acid methyl esters (FAMEs) is used in several important fields ranging from biofuel analysis to fat content in foods and blood. They are generally derivatized from free fatty acids and mono-, di-, and triglycerides. FAMEs may be saturated, mono- or polyunsaturated, linear or branched, and of variable chain lengths.

Electron Ionization Gas Chromatography/Mass Spectrometry (EI GC/MS) is often used to characterize FAMEs, but may fail to produce a useful molecular ion ($M^{+\bullet}$) for short, unsaturated, or branched chains, making compound identification more difficult.

Contrastingly, Cold Electron Ionization GC/MS (Cold EI GC/MS) can substantially increase the $M^{+\bullet}$ intensity of these compounds, while retaining the EI fragmentation pattern for spectral library searching without modification to established GC methodologies.

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Cold EI GC/MS Ion Source

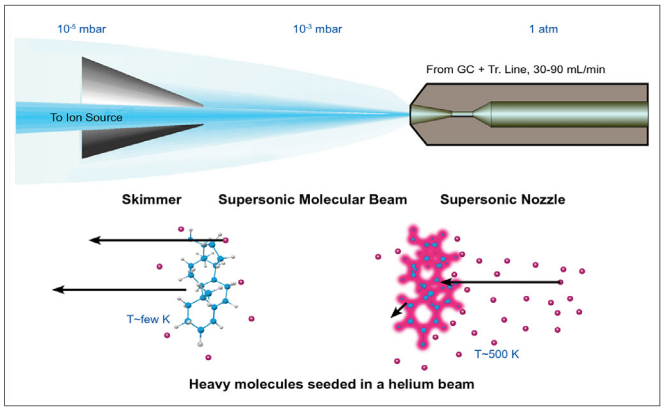

Figure 1. Cold EI GC/MS Ion Source.

- Velocity is increased (kinetic energy in the range of 1-20 eV)
- Velocity is directed along the beam (jet separation)
- Vibrational Energy is decreased (supercooling "Cold EI")
- GC column flow rate compatibility to 100 mL/min

Molecules exit the GC column and are mixed with makeup gas. Supersonic expansion through a nozzle then 'cools' the analyte molecules from about +250 °C to about -255 °C by adiabatic expansion, forming a molecular beam.

Excess carrier gas is skimmed off, and cold analyte molecules enter the ion source for ionization, molecular ion formation and subsequent mass analysis.

When used in a novel MS/MS configuration the enhanced molecular ion in Cold EI provides enhanced selectivity and valuable information on FAMEs molecular weight.

Experimental

All work was carried out on a PerkinElmer AxION iQT® GC/MS/MS system. Experimental conditions are shown in Table 1.

The enhanced Cold EI $M^{+\bullet}$ abundance allows use of this ion for more selective compound analysis, even for short-chained or polyunsaturated FAME isomers which may have unstable molecular ions with low or no intensity in EI.

Results and Discussion

A mixture of FAMEs was characterized using EI and Cold EI GC/MS. The Cold EI chromatogram is shown in Figure 2.

The compound retention times were identified using the NIST 2014 and Wiley 10th Edition mass spectral libraries. Of the 37 FAMEs in the test mix, all except 3 (coeluting at 12.21 min) were chromatographically resolved or could provide "clean" spectra through background subtraction.

Specific attention is given to those compounds which have a relative intensity percentage, RI (%), of <5% of the $M^{+\bullet}$ in NIST, as increasing their $M^{+\bullet}$ intensity would be most beneficial to identification and quantification. These are highlighted red in Figures 2, 5 and Table 2, and include a number of the shorter chain FAMEs such as Methyl hexanoate and Methyl decanoate, and polyunsaturated isomers such as *cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester and *cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester. Also shown are the Chemical Abstracts Service number (CAS No.), the Cold EI RI (%) of the $M^{+\bullet}$, the enhancement ratio of the Cold EI to EI $M^{+\bullet}$ RI (%), and the number of double bonds in the compound.

To better understand any pattern of EI $M^{+\bullet}$ relative intensities, the compounds were sorted by the number of double bonds and then molecular weight, as shown in Figure 3.

Table 1. Cold EI GC/MS Ion Source.

Gas Chromatograph:	PerkinElmer Clarus® 680
Injector Type: Injector temperature: Injection:	Programmable Split/Splitless 250 °C 0.4 µL, split 200:1
Oven Program:	50 °C, ramp to 240 °C at 20 °C/min
Analytical Column:	PerkinElmer Elite™ - 5MS 30 m x 0.25 mm ID x 0.25 µm
Carrier Gas: Sample:	1 mL/min Helium 99.999+% purity Supelco 37 Component FAME Mix

Mass spectrometer:	PerkinElmer AxION® iQT™ MS/MS
GC Transfer Line Temperature: Ion Source Temperature:	250 °C 200 °C
Acqusition Range: Acqusition Time:	*m/z* 40-400 0.20 sec
Solvent Delay:	none
Ion Source Type: Ion Source Mode: Background Noise Removal: Cold EI makeup gas: Ion Source Mode: Cold EI makeup gas:	Cold EI Cold EI On 50 mL/min Classical EI 2 mL/min
Filament:	5 µA

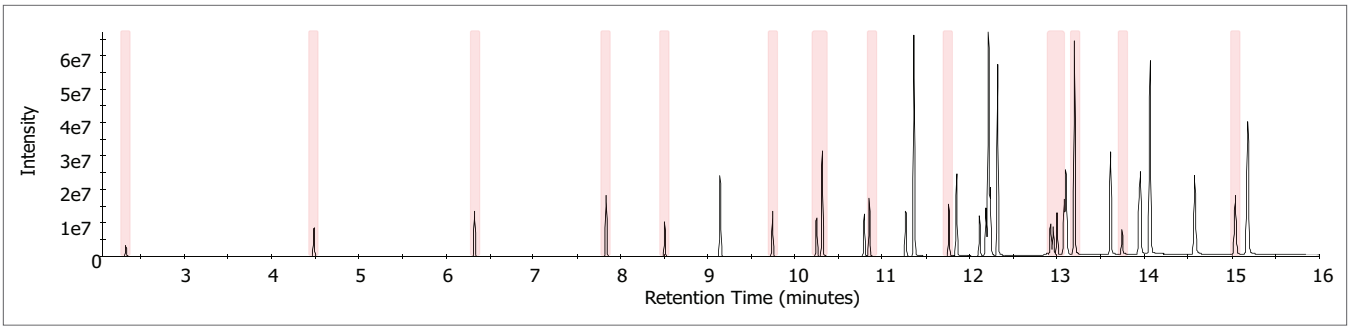

Figure 2. Cold EI chromatogram of FAMEs mixture. FAMEs with NIST EI molecular ion relative intensities below 5% are highlighted in red.

Table 2. FAMEs with NIST EI molecular ion relative intensities below 5% are highlighted in red, and compared to their Cold EI results. FAMEs in retention time order.

Ret. Time	Compound Name	CAS No.	Formula	MW	NIST EI RI %	Cold EI RI %	Cold EI Enhancement	Double Bonds
2.33	Methyl butyrate	623-42-7	C5H10O2	102.1	1.4	48	655	0
4.48	Methyl hexanoate	106-70-7	C7H14O2	130.1	0.5	4	7	0
6.33	Methyl octanoate	111-11-5	C9H18O2	158.1	3.8	16	4	0
7.83	Methyl decanoate	110-42-9	C11H22O2	186.2	1.5	50	44	0
8.50	Methyl undecanoate	1731-86-8	C12H24O2	200.2	2.0	85	57	0
9.14	Methyl laurate	111-82-0	C13H26O2	214.2	6.0	130	37	0
9.74	Methyl tridecanoate	1731-88-0	C14H28O2	228.2	4.0	196	84	0
10.24	Methyl myristoleate	56219-06-8	C15H28O2	240.2	4.0	96	358	1
10.31	Methyl myristate	124-10-7	C15H30O2	242.2	4.0	259	117	0
10.79	Methyl *cis*-10-pentadecenoate	90176-52-6	C16H30O2	254.4	4.2	102	374	1
10.84	Methyl pentadecanoate	7121-64-1	C16H32O2	256.2	6.0	405	140	0
11.26	Methyl palmitoleate	1120-25-8	C17H32O2	268.2	6.0	89	356	1
11.36	Methyl palmitate	112-39-0	C17H34O2	270.3	10.0	590	115	0
11.76	*cis*-10-Heptadecenoic acid methyl ester	77745-60-9	C18H34O2	282.3	3.5	77	808	1
11.85	Methyl heptadecanoate	1731-92-6	C18H36O2	284.3	7.9	698	216	0
12.11	Methyl γ-linolenate	16326-32-2	C19H32O2	292.2	23.0	269	22	3
12.18	Methyl linolelaidate	2566-97-4	C19H34O2	294.3	30.0	358	162	2
12.23	*cis*-9-Oleic acid methyl ester	112-62-9	C19H36O2	296.3	6.0	56	650	1
12.32	Methyl stearate	112-61-8	C19H38O2	298.3	14.0	993	172	0
12.92	*cis*-5,8,11,14-Eicosatetraenoic acid methyl ester	2566-89-4	C21H34O2	318.3	0.8	41	78	4
12.96	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester	2734-47-6	C21H32O2	316.2	0.2	4	28	5
13.00	*cis*-8,11,14-Eicosatrienoic acid methyl ester	21061-10-9	C21H36O2	320.3	3.0	340	234	3
13.07	*cis*-11,14-Eicosadienoic acid methyl ester	2463-02-7	C21H38O2	322.3	10.0	194	523	2
13.09	Methyl *cis*-11-eicosenoate	2390-09-2	C21H40O2	324.3	2.8	31	1965	1
13.11	*cis*-11,14,17-Eicosatrienoic acid methyl ester	55682-88-7	C21H36O2	320.3	8.0	242	51	3
13.20	Methyl arachidate	1120-28-1	C21H42O2	326.3	10.0	880	338	0
13.61	Methyl heneicosanoate	6064-90-0	C22H44O2	340.3	20.0	861	203	0
13.74	*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	301-01-9	C23H34O2	342.3	0.0	2	na	6
13.94	*cis*-13,16-Docosadienoic acid methyl ester	61012-47-3	C23H42O2	350.3	12.0	139	301	2
13.95	Methyl erucate	1120-34-9	C23H44O2	352.3	6.0	22	816	1
14.06	Methyl behenate	929-77-1	C23H46O2	354.3	16.0	746	260	0
14.57	Methyl tricosanoate	2433-97-8	C24H48O2	368.4	16.0	999	281	0
15.04	Methyl nervonate	2733-88-2	C25H48O2	380.4	3.4	20	985	1
15.18	Methyl lignocerate	2442-49-1	C25H50O2	382.4	14.0	110	390	0

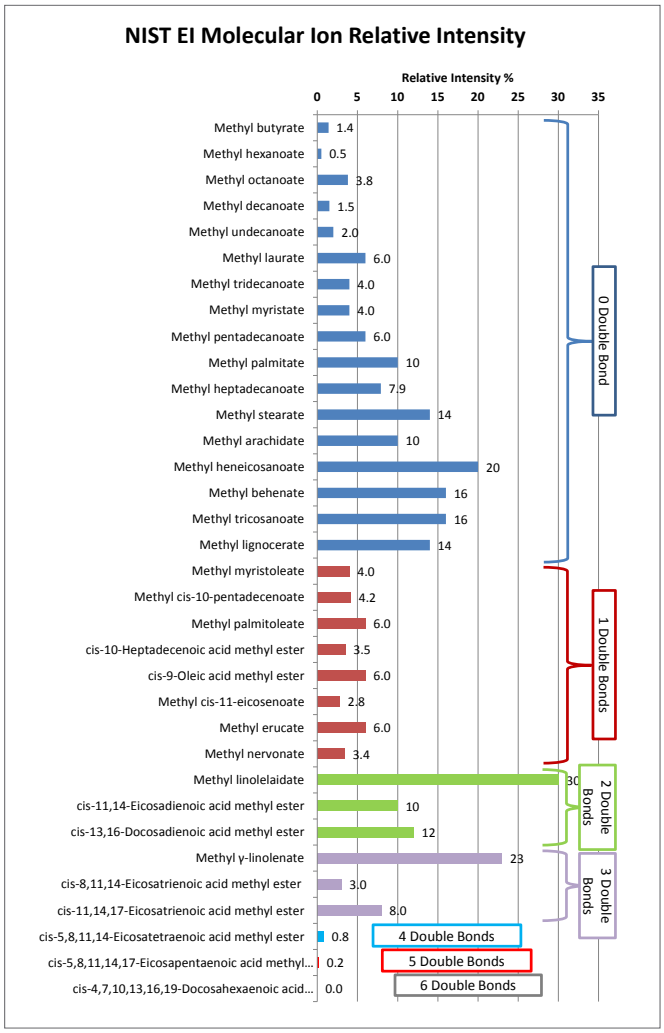

Figure 3. NIST EI molecular ion relative intensity % sorted by number of double bonds and molecular weight. Compounds are color coded by number of double bonds.

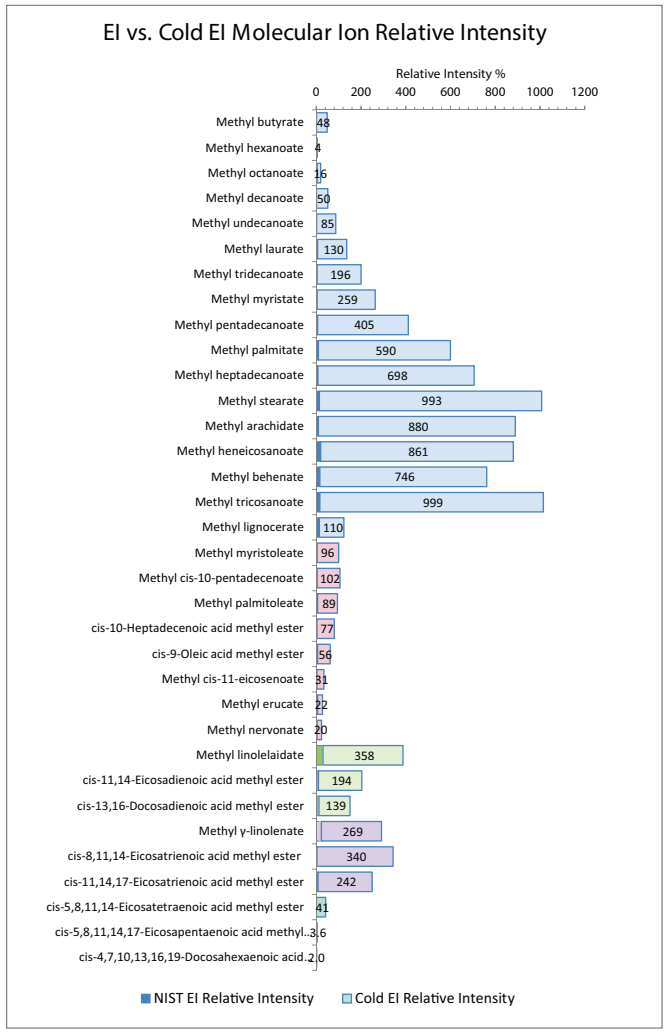

Figure 4. Much larger Cold EI (hashed pattern) molecular ion relative intensity % compared to NIST EI (solid pattern). Values >100 have the next largest peak defined as 100%.

Sorted in this manner and extrapolating with this limited data set, it appears that very short-chained and unsaturated FAMEs with 4 or more double bonds are more likely to produce small molecular ions, while longer chain saturated FAMEs and those with 2 or 3 double bonds tend to produce larger ones.

Cold EI was found to enhance the molecular ions in all FAMEs. Large $M^{+\bullet}$ intensity enhancements are noted for mid-length unsaturated linear chains. Nearly all the compounds with EI $M^{+\bullet}$ RI (%) below 5% now are above 5%. This is seen in Figure 4.

The most unsaturated compound (*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester) exhibited no $M^{+\bullet}$ at all in EI, but shows a discernable one in Cold EI. The average increase of the $M^{+\bullet}$ RI (%) was a factor of 33.

The large observed enhancements make the $M^{+\bullet}$ clearly more unique and thus selective for Cold EI than for EI. Cold EI $M^{+\bullet}$ mass chromatograms can be used for quantification with better selectivity than a lower mass fragment ion which might otherwise have to be used for EI.

This enhanced $M^{+\bullet}$ in Cold EI provides improved selectivity and less ambiguous molecular weight determination in complex mixtures. It also provides a more intense and unique ion which can enhance MS/MS selectivity and detection limits.

Cold EI spectra of some FAMEs with smaller EI $M^{+\bullet}$ relative intensities are shown in Figure 5 with their NIST library search results, and red highlighting of the molecular ion. Some of the names are synonyms.

Cold EI cooling is shown to increase the RI (%) of the $M^{+\bullet}$ and reduce fragmentation. This lower internal energy may also change the preferred fragmentation energies and kinetics, leading to the enhancement of mid-mass ions more stable at these lower energies.

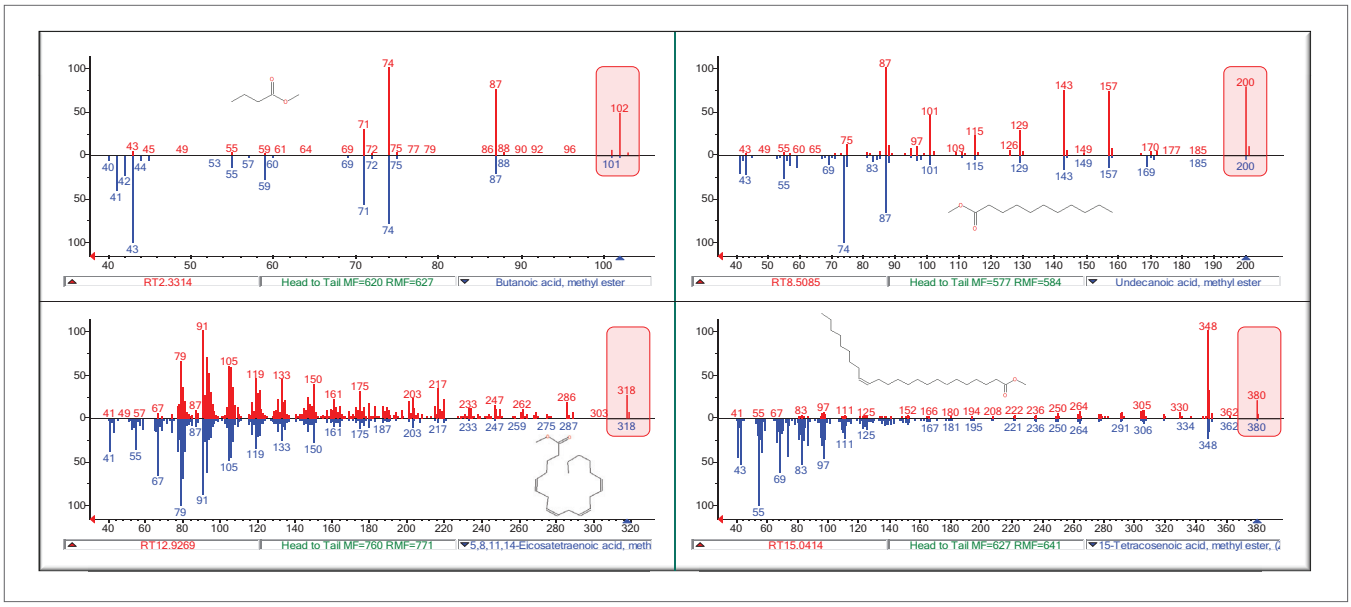

Figure 5. Comparison of Cold EI spectra (top, red) to NIST library-searched EI reference spectra (bottom, blue) showing the enhanced Cold EI molecular ion (boxes).

Conclusion

Cold EI GC/MS enhances FAMEs molecular ion intensity, improving analysis specificity and ease of molecular ion identification for compounds with low EI molecular ion relative intensity.

*Not for use in diagnostic procedures.

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