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Technical Report

Analysis of MOSH and MOAH using SPE prior to GC×GC-MS analysis

Luigi Mondello[1]

Abstract:

The present work is focused on the development/optimization of a comprehensive two-dimensional gas chromatography method, with dual detection [flame ionization (FID) and mass spectrometric], for the simultaneous identification and quantification of mineral-oil contaminants in a variety of food products. The two main classes of contaminants, namely saturated and aromatic hydrocarbons, were previously fractionated on a manually-packed silver silica solid-phase extraction (SPE) cartridge. The presence of a series of unknown compounds was investigated using the mass spectrometric data, and were tentatively-identified as esterified fatty acids, most probably derived from vegetable oil based ink.

Keywords: Food, MOSH, MOAH, GC×GC, Comprehensive GC, Quadrupole mass spectrometer

1. Introduction

Mineral oil products derive from crude petroleum, through distillation processes and various refining steps, and contain proportions of mineral oil saturated hydrocarbons (MOSH, including *n*-alkanes, isoalkanes and cycloalkanes), and mineral oil aromatic hydrocarbons (MOAH), mainly consisting of alkylated polyaromatic hydrocarbons (PAH)[1].

Mineral oil contamination in foods, deriving from a variety of sources, has been studied for quite a long time[2-4]. One of the major sources of contamination is paperboard packaging, an issue known since 1997[2], even though it has gained great attention only recently[5,6] Such a contamination derives from the printing inks applied directly to the packaging, and/or from the ink used in the newspapers, employed to produce recycled fiber. It has also been demonstrated that mineral oil migrating from paperboard usually contains a large proportion (15–20%) of MOAH[3,4], which are more of a worry from a toxicological viewpoint.

The occurrence and danger of mineral oil products in foods has been discussed widely in recent years[7-10]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA), in 2002, reported a list of admissible daily intake (ADI) values for different white mineral oils[8]; based on such data, an envisioned limit of 0.6 mg kg^{-1} was proposed for MOSH migration (up to C25) in dry foods from paperboard packaging[7]. The European Food Safety Authority (EFSA), published an opinion in June 2012[9], casting doubts on the "JECFA" list, due to the lack of sufficient toxicological information and, as a consequence, the JECFA values were recently withdrawn[10]. Furthermore, even though EFSA emphasized the potential carcinogenic risk of MOAH constituents[9], an official approved evaluation of MOAH is still lacking.

Most of the approaches reported over the last decades have been directed to the analysis of MOSH, exploited as a contamination marker, using both off- and on-line techniques. Off-line methods based on prep LC, or solid-phase extraction (SPE), have been described[11-18]. The lipid fraction can be eliminated either through saponification, followed by silica-gel column chromatography [11,12], or directly through a prep LC silica column[13,14], or an SPE cartridge[15-18]. Several techniques, based on the use of glass SPE, have been described; with regards to packing materials, a variety of solutions have been proposed, such as activated silica gel[16], non-activated silica gel[17], or silver (Ag) silica gel[18].

Considering the application of all methods, it can be affirmed without a doubt, that the most popular technique has been on-line liquid chromatography-gas chromatography (LC-GC), with a silica LC column[6,19-23]. Additionally, and in consideration of the toxicological relevance of MOAH, work has been directed to the clear pre-separation of the MOSH from the MOAH. For example, Biedermann and co-workers exploited the separation efficiency of an LC silica column, in an on-line LC-GC system, to separate the MOAH from the MOSH, and these from the lipidmatrix[20]. It must also be noted that off-line SPE methods, using a Ag silica-gel SPE cartridge, have been developed for MOSH and MOAH determination[24,25].

With regards to detectors, flame ionization (FID) systems have been widely employed for the reliable quantification of the humps of unresolved complex mixtures (UCM), generated in MOSH/MOAH applications; FIDs are useful because they provide virtually the same response *per* mass of hydrocarbons, even though the lack of structural information is certainly a major drawback[23]. In fact, the attainment of profound information on the composition of MOSH and MOAH constituents, can provide

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fundamental information on potential toxicity, and on the contamination source. Such an objective was reached by Biedermann and Grob, who used an MS detector, along with the additional information generated by a comprehensive 2D GC (GC×GC) analysis[26]. A pre-separation of the MOSH and MOAH groups was achieved through off-line LC, a process necessary to avoid the overlapping of steranes and hopanes (present in the MOSH fraction), with alkylated (two- and three-ring) aromatics. The GC×GC system was coupled alternatively with an MS system, for qualitative purposes, and with an FID system for quantification, and hence, two applications were required to obtain both information types. A GC×GC-MS method, after an off-line LC pre-separation step, has also been exploited by Mondello and co-workers, to attain a more expanded view on MOSH contamination in homogenized baby foods[27].

The present document describes a GC×GC method, characterized by dual MS/FID detection, for the qualitative and quantitative analysis of MOSH and MOAH in various foods. The pre-separation step was performed by using Ag-SPE.

2. Experimental

2-1. Samples and chemicals

CH_2Cl_2 and *n*-hexane were purchased from Sigma-Aldrich (Milan, Italy), and distilled before use. The C7–C40 standard mixture, the paraffin oil (code 18512), $AgNO_3$, and silica gel 60 (particle size 0.063–0.2 mm, 70–230 mesh) were purchased from Supelco and Sigma-Aldrich (Milan). Glass SPE cartridges (6 mL glass tubes with a frit) were purchased from Macherey-Nagel (Chromabond, Düren, Germany).

2-2. Samples and preparations

Samples of pasta, rice and icing sugar, were purchased in a supermarket. The ground samples were extracted overnight using *n*-hexane, and then purified through Ag-SPE. Briefly, a 1:2 food to solvent ratio was employed to extract MOSH and MOAH from the samples. After, an aliquot of the extract was concentrated prior to SPE clean-up, on a Ag silica gel cartridge. Silver silica gel was prepared by adding a $AgNO_3$ solution (0.75 g/mL in Milli-Q water, Millipore, Bedford, MA, USA) to previously activated (400°C overnight) silica gel, blended for about 30 min, and left to rest for 12 h; finally, the mixture was heated at 75°C overnight to eliminate the remaining water. The SPE cartridge was manually packed with 1 g of Ag silica, prior to sample loading (250 µL). First, the sample was eluted with 1 mL of *n*-hexane, which was discharged; then, the MOSH constituents were eluted with 1.5 mL of *n*-hexane, followed by 0.5 mL of *n*-hexane/dichloromethane (50:50 *v/v*); a 0.5 mL *n*-hexane/dichloromethane fraction followed, which was discharged; finally, the MOAH class was eluted with further 7 mL of *n*-hexane/dichloromethane (50:50 *v/v*).

The eluted fractions were concentrated to a final volume of 100 µL to increase sensitivity, since large volume injection (LVI) was not used.

2-3. GC×GC-MS/FID analysis

GC×GC experiments were performed on a system consisting of a GC-2010 gas chromatograph, and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan).

The primary column, an SLB-5ms 30 m × 0.25 mm ID × 0.25 µm d_f [silphenylene polymer, virtually equivalent in polarity to poly (5% diphenyl/95% methyl siloxane)], was connected to an uncoated capillary segment (1.0 m × 0.25 mm ID, used to create a double-loop), and to a 1.0 m × 0.10 mm ID × 0.10 µm d_f Supelcowax-10 (polyethylene glycol) segment (Supelco). The second column was connected through a capillary column splitter (SGE) to two uncoated capillaries, with these linked to the FID (0.5 m × 0.1 mm ID) and to the MS (0.25 m × 0.05 mm ID) systems.

2-4. Method parameters

Modulation was performed every 6000 msec, by using a loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (350°C) was 375 msec.

GC oven temperature program: 50°C to 280°C (hold 7.5 min) at 4°C/min. Carrier gas, He, was supplied at an initial pressure of 243 kPa (constant linear velocity mode). Injection temperature: 360°C. Injection mode and volume: pulsed injection (300 kPa hold for 1 min) in the split mode (1:10); 6 µL. The FID was operated as follows: H_2 flow: 40.0 mL/min; air flow: 400.0 mL/min; make up (He): 30.0 mL/min.

MS parameters: samples were analyzed in the full scan mode with a scan speed of 20,000 amu/sec and a mass range of 40–510 *m/z*; spectra generation frequency: 33 Hz; interface and ion source temperatures were 250°C and 200°C, respectively. MS ionization mode: electron ionization.

Bidimensional visualization was carried out by using the ChromSquare v. 1.5 software (Shimadzu Europe, Duisburg, Germany). The MS libraries used for spectral matching were NIST05, FFNSC, and FAME library.

3. Results and Discussion

3-1. GC×GC-MS/FID *optimization and validation*

GC×GC method optimization was achieved by using offset printing ink, which is formed mainly of MOSH (> 90%), and by a minor MOAH fraction. Apart from problems related to co-elution, if the offset ink had been injected neat then the MOSH group would have overloaded the columns and modulator, while the MOAH constituents would have been barely detected; therefore, a pre-separation on the Ag-SPE cartridge was necessary.

Flow division between the FID and MS units was a compromise among different necessities, the main one being the attainment of a satisfactory sensitivity for quantification purposes. Because the detectors employed operate under different pressure conditions, the employment of two branches with equal IDs proved to be a non-ideal choice; the reason was related to the fact that an excessively long "MS" branch was required to generate an adequate flow resistance, to divert the majority of the effluent to the FID. Such a configuration would have led to substantial differences in the second-dimension elution times, between the qualitative and quantitative experiments. A good compromise was found through the use of an MS-linked 0.25 m × 0.05 mm ID branch, and a 0.5 m × 0.1 mm ID FID one.

Such a splitting configuration produced the following flow conditions: about 84% and 16% of the effluent reaching the FID and MS at the initial analysis temperature, respectively. The split ratio changed slightly during the GC run, with about 87% and 13% of the effluent diverted to the FID and MS, at the end. Since the calibration curve was constructed under the same analytical conditions, the quantitative results were not affected.

The GC×GC dual-detection operational conditions were optimized with the aim of maintaining the same chromatography performance, compared to an MS-only system, as shown in Fig. 1. In the MS-only approach, with the same analytical columns, the head pressure (approx. 150 kPa) was selected to generate about 20 cm/sec and 210 cm/sec, in the first and second dimension, respectively. In the dual-detection approach, a 243-kPa pressure produced the same gas velocity in the first dimension (to attain the same elution temperatures), and a slightly lower one in the second (180 cm/sec).

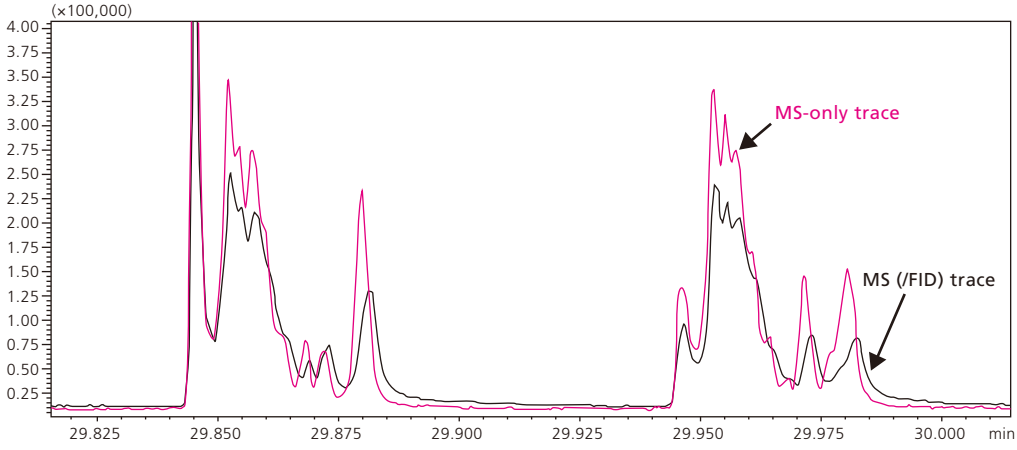

Fig. 1 Comparison of raw TIC chromatogram expansions (printing ink analyses), obtained using a GC×GC-MS and a GC×GC-MS/FID system

A six-point (each point was derived through duplicate applications) calibration curve was constructed through the FID trace, using solutions of paraffin oil in *n*-hexane, in the 0.35–24 mg/Kg range. The least squares method was exploited to estimate the regression line, while the linearity and the goodness of the curve were evaluated through the regression coefficient (0.9993), and a visual inspection of the residual plot, and were confirmed using Mandel's fitting test ($F_{calc}<F_{tab}$). The significance of the intercept ($p = 0.03$) was established running a *t*-test, at the 5% significance level.

Measurement of the limit of quantification (LoQ), in mineral oil analyses, is tightly related to the MW distribution of the contaminants, hence on the hump width. However an approximate estimation of the LoQ was made by considering the standard deviation (n=3), calculated at the lowest calibration point, multiplied by 10. The LoQ was estimated to be approximately 1.2 mg/Kg.

3-2. Food analysis

MOSH and the MOAH fractions, relative to pasta, icing sugar and rice, were quantified up to C25 (as required by the envisioned limit), using the aforementioned method; attention was paid, during integration, to eliminate the natural alkanes from the MOSH compounds, and the "unknown" peaks from the MOAH group. Specifically, for GC×GC-FID quantification, the "polygonal integration function" was applied, which enabled the definition of a polygonal area in which all the integrated peaks are automatically summed, and the data relative to each peak is saved as well. Thus, the undesired peaks can be easily selected, and subtracted from the total area. Quantification information, relative to the three foods, is listed in Table 1.

Table 1 Quantification values relative to the MOSH and the MOAH fractions, in samples of pasta, icing sugar, and rice, using Ag-SPE-GC×GC-MS/FID

Food	MOSH <C25 (mg/Kg)	MOAH< C25 (mg/Kg)
Pasta	3.5	1.6
Icing sugar	8.4	1.3
Rice	33.8	2.2

3-3. GC×GC-MS results for the MOAH fraction

The peaks present in the GC×GC chromatograms, for the three samples, were tentatively-identified on the basis of MS database similarities (≥ 80%) and in accordance with linear retention indices (LRI), contained in the same database. Since a widely-accepted procedure for the calculation of GC×GC LRI values has not been developed, such data were calculated in a one-dimensional mode; furthermore, a rather wide LRI filter window (± 25 units) was applied (to eliminate wrong matches), to compensate for the retention effects of the polar capillary. The tentatively identified compounds, along with experimental and database LRI, are listed in Table 2.

Two compounds were outside the LRI range; specifically, octyldodecanoate and octyltetradecanoate were characterized by a difference of +56 and +57 units, respectively. It noteworthy that, in these cases, the database LRI values (http://webbook.nist.gov/chemistry/), were attained using a methyl silicon capillary column [(Ultra-1) 25 m × 0.32 mm × 0.25 µm], while in the present research a 30 m × 0.25 mm ID × 0.25 µm silphenylene polymer phase was used. Since the similarity matches were satisfactory, and the analyte locations in the 2D chromatogram gave a further idea on the chemical structure, these solutes were given a name.

Figures 2, 3 and 4 show GC×GC-MS chromatograms for the pasta, icing sugar and rice samples, respectively.

Table 2 Compounds identified in the "MOAH" GC×GC–MS analysis; database-derived (database LRI) and experimental LRI (defined as LRI) values, and spectral similarities (MS%)

	compound	pasta MS%	ice sugar MS%	rice MS%	LRI	database LRI
1	Isopropyldodecanoate	94	93	95	1622	1627
2	Dioctylether	92	93	94	1667	1688
3	2-Ethylhexyl octanoate	87	85	87	1703	1715
4	Ethyltetradecanoate	-	-	82	1798	1795
5	Isopropyltetradecanoate	93	94	93	1821	1828
6	Isoamyldodecanoate	-	92	-	1846	1844
7	6,10,14-Trimethyl-2-pentadecanone	-	-	96	1846	1846
8	2-Heptadecanone	-	-	95	1896	1906
9	Methylhexadecanoate	95	91	93	1929	1925
10	Ethylhexadecanoate	92	83	94	1992	1993
11	Isopropylhexadecanoate	90	90	90	2025	2024
12	Abietatriene	84	81	83	2085	2075
13	Octyldodecanoate	86	84	83	2102	2158
14	2-Nonadecanone	-	-	92	2108	2106
15	Methyloctadecanoate	90	92	90	2130	2124
16	Dodecyloctanoate	95	91	-	2175	2177
17	n-Butylhexadecanoate	92	93	90	2198	2188
18	Octyltetradecanoate	83	85	-	2302	2359
19	Tetradecyloctanoate	84	90	-	2380	2375
20	n-Butyloctadecanoate	84	88	87	2395	2388
21	Pentadecyloctanoate	-	85	84	2477	2475
22	Octylhexadecanoate	83	84	83	2504	2505
23	Di(ethylhexyl) phthalate	95	93	94	2542	2550
24	1-Tetracosanol	-	-	92	2697	2710
25	Squalene	93	93	-	2828	2847
26	1-Hexacosanol	91	-	92	2884	2877
27	Tetradecyltetradecanoate	81	81	89	2968	2950

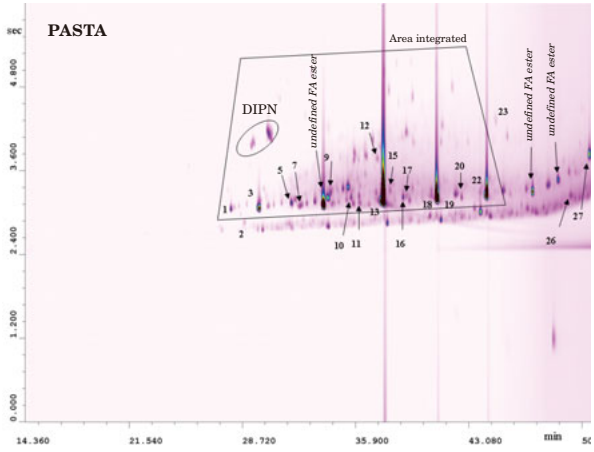

Fig. 2 GC×GC-MS chromatogram, relative to the pasta MOAH fraction. Identification as reported in Table 2. FA: fatty acid; DINP: diisopropylnaphthalenes

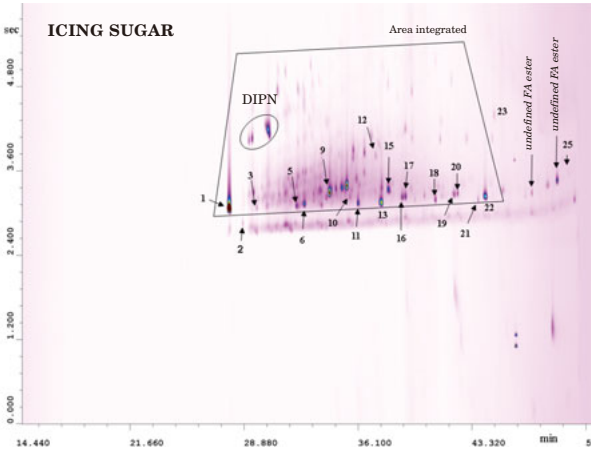

Fig. 3 GC×GC-MS chromatogram, relative to the icing sugar MOAH fraction. Identification as reported in Table 2.

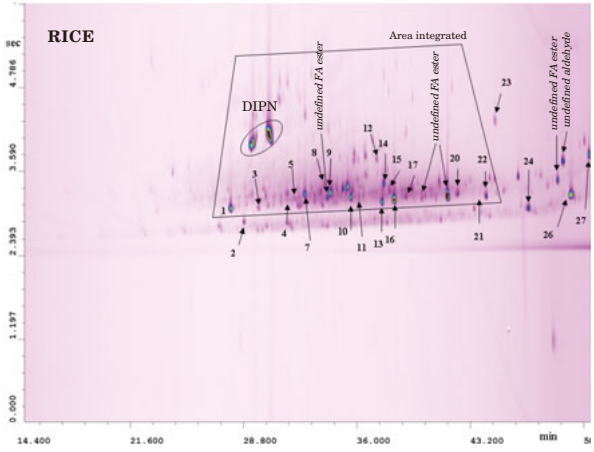

Fig. 4 GC×GC-MS chromatogram, relative to the rice MOAH fraction. Identification as reported in Table 2.

The identification of the specific aromatic compounds, present in the MOAH "cloud", was outside the scope of the investigation; however, even if desired, the identification of such constituents could not have been performed with satisfactory reliability, because of the low amounts of such constituents. However, it was possible to determine the MOAH quantities (FID trace) and patterns, which are highly important to define the contamination source.

A series of peaks, present in the MOAH fraction, were identified as esterified fatty acids. However, their presence did not affect reliable quantification because these compounds were subtracted from the total MOAH area. The esterified fatty acids derived from the paperboard packaging. In fact, in a sample of pasta analyzed prior to box packing, no sign of MOAH contamination was observed.

The possibility to use offset printing ink, based on vegetable oils, has been known for more than fifteen years, though its use has become more frequent since contamination from paperboard packaging has become an issue of worry.

A series of "unknowns" in the GC×GC-MS chromatograms were labeled as "undefined FA esters", since the relative spectra were clearly that of FA esters, although the database searches gave different possible "homologue" matches with good similarities, but not always with a correspondent LRI value. Hence, it was not possible to identify such compounds with sufficient reliability, even though they were marked in the figures, since their chemical nature was evident. It could also not be excluded that such FA esters were not contained in the MS database. For example, in the pasta sample (Fig. 2), only three out of the four main peaks were identified, namely octyldodecanoate, octyltetradecanoate, and octylhexadecanoate. However, it can be deduced from its 2D position that the "undefined FA ester" was most probably octyldecanoate, even though such a compound was not present in the MS databases used. A good "visual" similarity was observed with the spectrum reported in the NIST web site, however no LRI information was found, thus this compound remained unidentified.

It is noteworthy that practically the same compounds were found in all the samples subjected to analyses; however, different quantitative profiles were observed, probably due to a different ink-type and/or to a different contamination source. It can be hypothesized that the vegetable oil offset printing ink was directly used in the pasta packaging (highly contaminated), while it was present, in different amounts, in the recycled fiber used for the packaging of the other two food sample.

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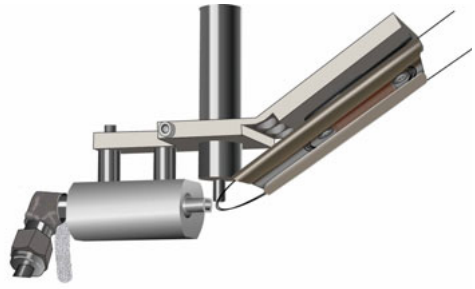
Shimadzu GC×GC-QP System

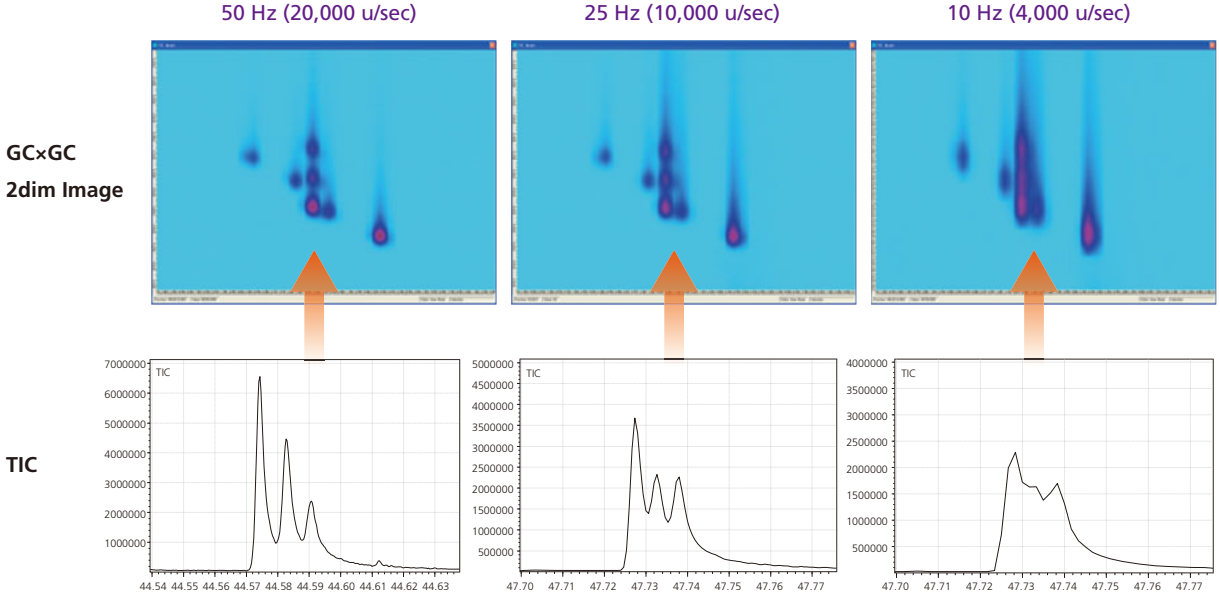


Zoex ZX1 2stage thermal modulator providing excellent modulation.

Shimadzu GCMS-QP2010 Ultra makes possible to obtain the data with high scan speed up to 20,000 u/sec.

The 2-dim chromatogram of fatty acids and scan speed

The high scan speed of GCMS-QP2010 Ultra has the potential of increasing the separation power of the second dimension, that promotes applicability of high sensitive, user friendly and economical quadrupole mass spectrometer to GC×GC-MS analysis.



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