Application Note 10398

Pharma Materials Study: GC-MS Identification of Extractables and Leachables from Elastomer Material

Bénédicte Gauriat[1], Isabelle Froger[2], Damien Chevaillier[2], Hans-Joachim Huebschmann[3]
[1]Thermo Fisher Scientific, Les Ulis, France; [2]Septodont, Dental Pharmaceutical Company, Saint-Maur-des-Fossés, France; [3]Thermo Fisher Scientific, Singapore

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Introduction

From manufacturing to administration, pharmaceutical products come in contact with multiple packaging systems made of different materials. Detailed compatibility studies on these materials may be required to ensure that product quality remains acceptable and that no safety concern is raised due to product/material incompatibility, especially when the administration method associated with a particular dosage or form of the product might maximize the risk of exposure and interaction [1,2].

Among these studies, extractables and leachables represent a huge portion of the work the analyst does to characterize the substances potentially leaching into the product. The terms "extractables" and "leachables" are well defined by industrial working groups [3,4], mixed working groups [5] (such as the Product Quality Research Institute, the Leachables and Extractables Working Group, including pharmaceutical development scientists representing industry, health agencies, and academia) and subject experts [6].

Often extended to process materials, these definitions are consistent with the one proposed by the United States Pharmacopeia, which has added specific chapters on extractables and leachables studies [7,8]:

- *Extractables* are substances such as "organic or inorganic chemical entities that can be released from a pharmaceutical packaging/delivery system, packaging component, or packaging construction material under laboratory, conditions. Depending on the specific purpose of the extraction study […] these laboratory conditions (e.g. solvent, temperature, stoichiometry, etc.) may accelerate or exaggerate the normal storage/use conditions for a packaged form. Extractables themselves, or substances derived from extractables, have the potential to leach into a drug product under normal conditions of storage and use."

- *Leachables* are "organic or inorganic chemical entities that migrate from a packaging/delivery system, packaging component, or packaging construction material into an associated drug product under normal conditions of storage and use or during drug product stability studies. Leachables are typically a subset of extractables or are derived from extractables."

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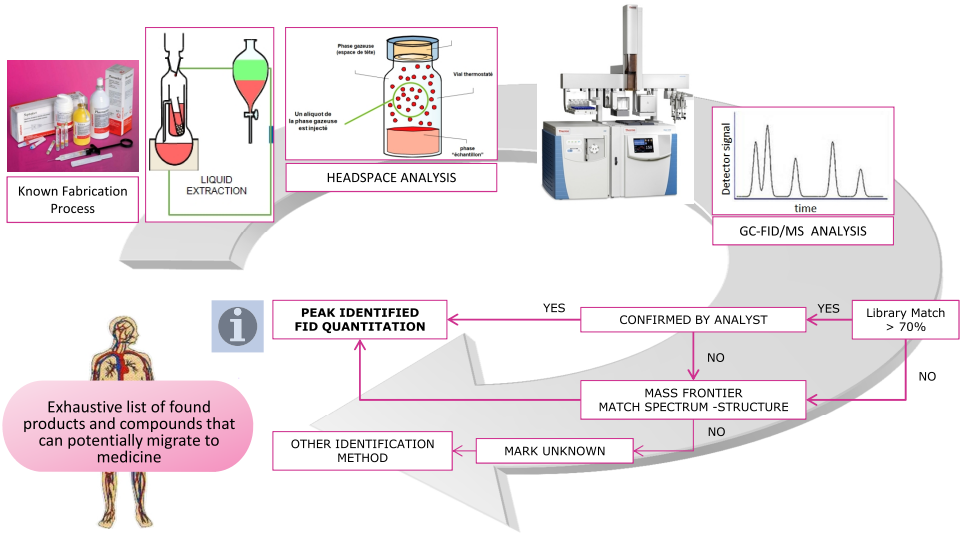

Fig.1 Workflow for extractables analysis

The identification of potential leachables through a preliminary extractable study and the attribution to the contact component from which they originate are important. Such species may react with the drug product or formulation ingredients, compromise the efficacy of the drug product or interfere with dosage consistency, and finally, may cause a negative health effect.

Studies for the determination of extractables and leachables are typically carried out using different analytical approaches e.g. by inductively coupled plasma (ICP) for elemental composition, or by liquid chromatography mass spectrometry (LC-MS) for non-volatiles. Gas chromatography mass spectrometry (GC-MS) is mostly applied for volatile components using direct headspace (HS) analysis, or liquid injection after a solvent extraction step for semi-volatile compounds.

This application note describes a part of an extractable analysis of an elastomeric plunger considered for potential use in a dental injectable cartridge using different extraction techniques, derivatization and HS analysis by single quadrupole GC-MS. A parallel classical flame ionization detection (FID) channel was configured for use in a future routine method, if required. While the composition of the plunger is known from the manufacturer, the drug product manufacturer has very little information about its composition and consequently about the substances that might migrate into the applied medicine.

Sample Preparation

The elastomeric plunger material was examined in several ways. The volatiles were determined via direct HS analysis. For the headspace injection, 10 plungers were placed in a 20 mL HS vial.

The extractables of the sample were studied by preparing three different liquid extracts for injection using three extraction procedures:

(1) Aqueous extraction of the plunger material followed by a dichloromethane (DCM) extraction of the aqueous phase, no derivatization

(2) The above DCM extract was derivatized using BSTFA (10 x 1mL ampule pack p/n TS-38830)

(3) Isopropanol (IPA) extraction

These extractions were selected for the purpose of this application note and were a part of a global extraction study including additional methods and extraction techniques.

Figure 2. ISQ GC-MS system with TriPlus RSH autosampler

Experimental Conditions

The analyses were performed using a Thermo Scientific™ TRACE™ 1310 GC with parallel FID and MS detection with the Thermo Scientific™ ISQ™ single quadrupole MS as shown in Figure 1. The parallel FID detection was accomplished by using a Silflow™ connection, which also allowed a no-vent option for easy column change without the time-consuming venting of the ISQ mass spectrometer.

The TRACE 1310 GC was equipped with a Thermo Scientific™ TriPlus™ RSH™ autosampler for both liquid and HS injections.

Table 1. GC conditions

TRACE GC 1310	HEADSPACE
Injector:	Split/splitless, 320 °C
	split 20 mL/min
Injection Volume:	1 mL headspace
Inlet Liner:	Splitless liner with glass wool, 4 mm ID (p/n 453A1925)
Oven Program:	30 °C, 3 min, 8 °C /min to 280 °C 280 °C, 10 min

TRACE GC 1310	LIQUID INJECTION
Injector:	Split/splitless, 320 °C
	1 min splitless time for liquid extracts
Injection Volume:	1 µL of liquid extract
Inlet Liner:	Splitless liner with glass wool, 4 mm ID (p/n 453A1925)
Oven Program:	40 °C , 1 min, 8 °C /min to 325 °C 325 °C , 14 min

TRACE GC 1310	LIQUID AND HEADPACE INJECTION
Carrier Gas:	He, constant pressure 125 kPa
Column Type:	TraceGOLD™-5MS, 30 m × 0.25 mm ID × 0.25 µm film thickness (p/n 26098-1420)
Transfer Capillaries:	0.2 m × 0.2 µm to FID and 2 m × 0.15 mm to MS
FID:	300 °C Air 350 mL/min Hydrogen 35 mL/min Nitrogen 40 mL/min
Transfer Line:	300 °C

Table 2. MS conditions

ISQ MASS SPECTROMETER	
Ion Source Temp.:	220 °C
Ionization:	EI, 70 eV
Emission Current:	50 µA
Full Scan:	25-700 Da, 4 scans/s (250 ms/scan)

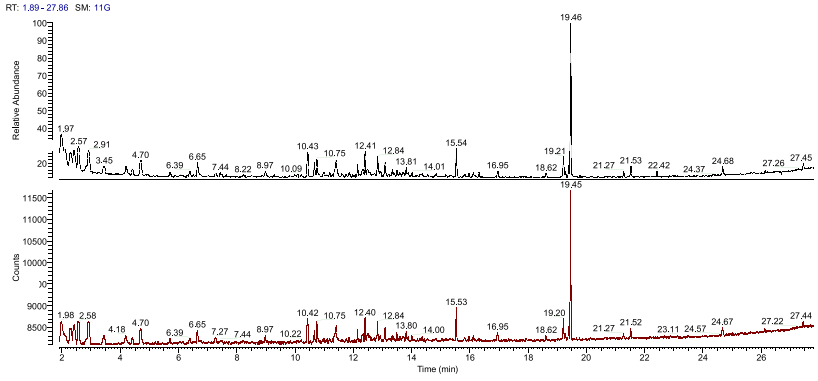

Figure 3. Chromatogram of the piston headspace analysis (top MS TIC, bottom FID)

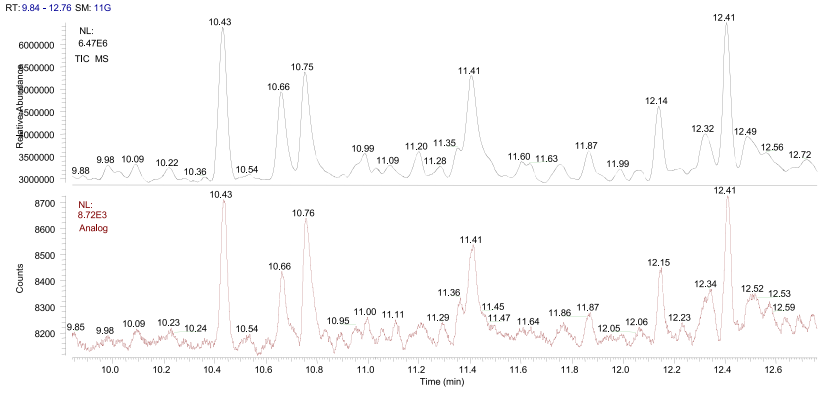

Figure 4. Zoom display - MS and FID RT match perfectly (top MS TIC, bottom FID)

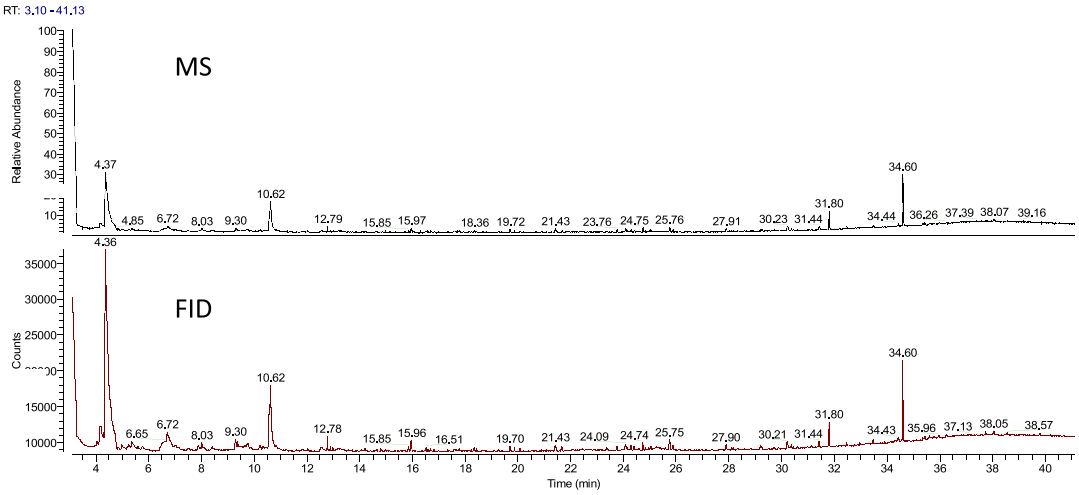

Figure 5. Chromatogram of the aqueous extract concentrated in DCM (top MS TIC, bottom FID)

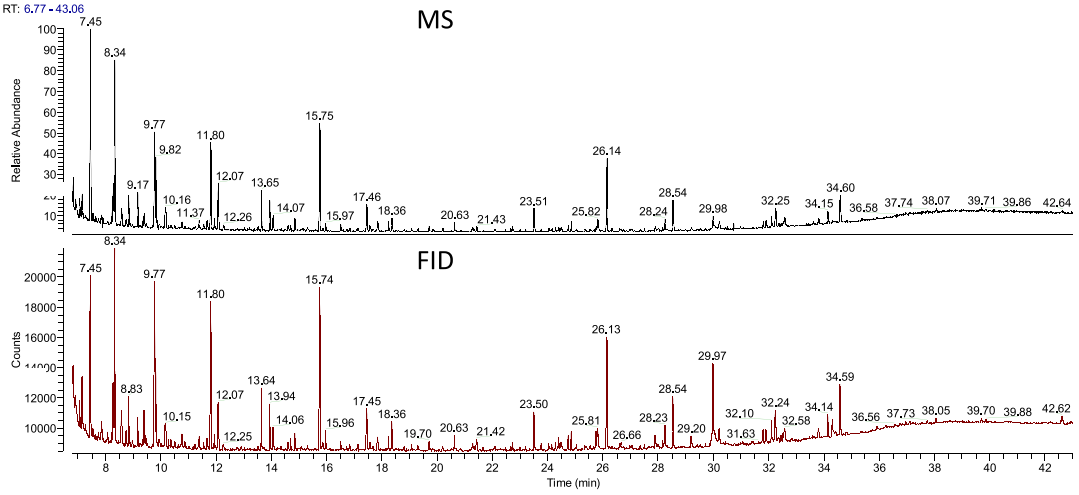

Figure 6. Chromatogram of the liquid DCM extract, derivatized with BSTFA (top MS TIC, bottom FID)

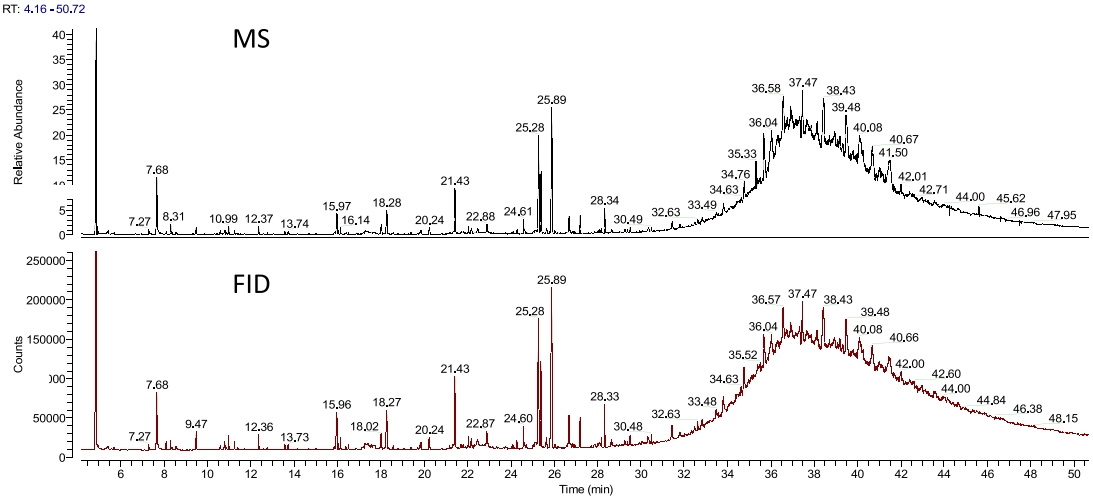

Figure 7. Chromatogram of the liquid IPA extract (top MS TIC, bottom FID)

Data Processing and Results

The ISQ mass spectrometer in full scan mode was used for identification of the unknown compounds with a parallel FID detection.

The chromatograms of the HS analysis are shown in Figures 3 and 4. The retention times for compound identification match perfectly between the MS and the FID detection. The analyses of the different liquid extraction and derivatization procedures follow in Figures 5—7, all of them with the total ion chromatogram (TIC) and FID traces. All chromatograms demonstrate that the parallel FID plus MS detection are in very good agreement of the eluted compound pattern.

AMDIS, the Automated Mass spectral Deconvolution and Identification System was used for a deconvolution of the complex chromatograms extracting the "clean" single compound mass spectra. For search and spectral comparison the National Institute of Standards and Technology (NIST) library was used. AMDIS associates the found retention time with the mass spectrum for an improved identification. All results can optionally be transferred to Microsoft® Excel for further investigation.

Mass spectra that could not be identified by library search were analyzed in the fragmentation pattern using Thermo Scientific™ Mass Frontier™ software resulting in realistic structural proposals.

AMDIS Chromatogram Deconvolution

The AMDIS deconvolution program works in three steps [9]:

Step 1: AMDIS analyzes the chromatogram. It counts the number of eluted compounds based on a minimum number of ions that show a common retention time maximum (maximizing masses peak finder). The corresponding mass spectrum is extracted and cleared from a potential contribution to baseline and coeluting compound mass intensities.

Step 2: AMDIS checks if target compounds from a user library are present by simultaneously matching retention time (or retention index, if available) and mass spectrum.

Step 3: All detected compound spectra are compared with the reference spectra of the linked libraries allowing a filter with different criteria:
- Only match those that are better than a chosen value,
- Only the top 'T' most abundant compounds in terms of their peak area,
- Only those 'S' compounds with a minimum % area over a given value, or
- All compounds.

Results

The following Tables 3 to 6 show the results from the same sample material using different extraction methods, and filter candidates that reach at least a 75% match.

The list of identified compounds of the HS analysis from Figure 1 is presented in Table 1 with compound name and CAS numbers, as well as peak quality parameters, retention time, the measured peak width and tailing information. The result of the mass spectrum library comparison is given in the two farthest right columns. The 'Reverse' fit column tells the match quality in % of the proposed library entry with the unknown spectrum.

Table 3. Compounds identified by HS analysis with a library search match >75% (of a total of 53 peaks detected)

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
>Pentane, 2-methyl-	2.28	85	1917	4.68	368	2.36
>Pentane, 3-methyl-	2.40	87	2308	5.63	442	2.84
>Cyclopentane, methyl-	2.93	79	2847	6.95	515	3.31
>Cyclohexane, methyl-	4.72	82	1333	3.25	346	2.22
>2,3-Hexanedione	6.40	83	238	0.58	106	0.68
>Hexanal	6.65	82	825	2.01	300	1.92
>Propanal, 2,2-dimethyl-	10.43	88	950	2.32	538	3.46
>Octane, 2,2,6-trimethyl-	10.66	88	445	1.09	259	1.67
>Benzaldehyde	10.75	92	1031	2.52	461	2.96
>Octane, 2,6,6-trimethyl-	13.34	79	164	0.40	115	0.74
>2-Propenoic acid, [···] Ageflex/Sipomer IBOA	19.46	95	6465	15.77	3438	22.08
>Ethanone, 2,2-dimetho5 [...] DMPA, Photocure 51	27.44	82	217	0.53	125	0.8

Table 4. Compounds identified in the IPA extract, library search match >75% (of a total of 118 peaks detected). (*** see Mass Frontier Fig. 6)

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
>Isopropanol P117	4.85	78	1737928	31.0	626333	28.9
>Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile	8.52	78	11506	0.2	5183	0.2
>Benzyl Alcohol	9.48	94	43713	0.8	23090	1.1
>Benzene, (bromomethyl)-	10.80	90	23050	0.4	10571	0.5
>Benzyl isopentyl ether	10.99	89	31562	0.6	18724	0.9
>Benzyl isocyanate	11.25	94	21095	0.4	10942	0.5
>Heptanoic acid, propyl ester	11.41	86	3680	0.1	2310	0.1
>DECANONE-2	12.60	79	3716	0.1	2230	0.1
>DODECANE	12.77	92	5686	0.1	3521	0.2
>Butane, 1,2,2-tribromo-	13.12	79	1611	0.0	1096	0.1
>Tridecane	14.69	81	3736	0.1	2055	0.1
>Benzene, (isothiocyanatomethyl)-	15.97	91	114760	2.1	47845	2.2
>2-Dodecanone	16.40	84	7773	0.1	3757	0.2
>Tetradecane	16.52	94	11436	0.2	5989	0.3
1-Bromo-3-(2-bromoethyl)heptane	18.02	65	57416	1.0	19643	0.9
1-Bromo-3-(2-bromoethyl)heptane ***	18.31	66	143599	2.6	50332	2.3
>Pentadecane, 3-methyl-	19.39	88	5468	0.1	2763	0.1
>2-Tetradecanone	19.82	89	16697	0.3	7514	0.4
>Hexadecane	19.87	90	18647	0.3	8612	0.4
>Impurity P116	21.43	93	245631	4.4	91711	4.2
>N-Benzylidenebenzylamine	22.32	94	17660	0.3	5632	0.3
>Tetradecanenitrile	24.29	81	17783	0.3	8962	0.4
Hexadecanoic acid, methyl ester	24.61	97	61160	1.1	28869	1.3
>Phthalic acid, butyl cyclobutyl ester	25.06	88	10308	0.2	4055	0.2
Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-	25.29	72	403320	7.2	163977	7.6
>Isopropyl Palmitate	25.89	76	572407	10.2	204258	9.4
>Oleanitrile	26.72	80	90096	1.6	41750	1.9
>Heptadecanenitrile	26.97	76	8891	0.2	4527	0.2
>Octadecanoic acid, methyl ester	27.19	92	73043	1.3	38750	1.8
>Isopropyl stearate	28.34	90	99362	1.8	53712	2.5
>Diisooctylphthalate @ P1828	31.81	82	22826	0.4	6778	0.3

Table 5. Compounds identified in the DCM extract, library search match >75% (of a total of 88 peaks detected).

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
>Toluene	4.17	96	17526	3.7	2914	3.2
>Benzene, 1-fluoro-4-methyl-	4.30	88	6223	1.3	2039	2.2

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
>Benzene, 1-fluoro-2-methyl-	4.37	93	244339	51.0	27516	29.7
>Benzaldehyde	7.89	90	800	0.2	307	0.3
>Benzylalcohol	9.38	88	3632	0.8	1081	1.2
>Cyclopropyl carbinol	10.63	77	55086	11.5	9086	9.8
>Decanal	12.79	88	4070	0.9	2030	2.2
>Diethylphthalate	19.72	92	1195	0.3	682	0.7
>Dibutyl phthalate	25.05	90	1253	0.3	559	0.6
>Ethyl hexyl phtalate	31.81	93	6673	1.4	3399	3.7

Table 6. Compounds identified in the BSTFA derivatized DCM extract, library search match >75% (of a total of 88 peaks detected).

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
>Disiloxane, hexamethyl-	6.83	91	1426	0.5	1588	1.0
>Dimethyl sulfone	7.14	92	5078	1.7	3467	2.1
>Trifluoromethyl-bis-(trimethylsilyl)methyl ketone	7.45	94	14274	4.8	10694	6.6
>Octane, 4-ethyl-	7.85	94	1932	0.6	1158	0.7
>1,2-Bis(trimethylsiloxy)ethane	8.34	93	20903	7.0	12757	7.8
>Cyclopropane, 1-heptyl-2-methyl-	8.58	87	3614	1.2	2127	1.3
>Silane, (cyclohexyloxy)trimethyl-	8.83	89	4655	1.6	3102	1.9
>Tetrasiloxane, decamethyl-	9.34	86	3844	1.3	2285	1.4
>Silane, (1-cyclohexen-1-yloxy)trimethyl-	9.77	90	18016	6.0	10881	6.7
>Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	9.82	95	8888	3.0	4868	3.0
>ACIDE GLYCOLIQUE	10.17	88	3744	1.3	1516	0.9
>Silane, trimethyl(phenylmethoxy)-	11.80	94	16044	5.3	9758	6.0
>3,6,9-Trioxa-2-silaundecane, 2,2-dimethyl-	11.93	89	1501	0.5	974	0.6
>Benzoic acid trimethylsilyl ester	13.65	94	7195	2.4	4126	2.5
>Octanoic acid, trimethylsilyl ester	13.95	87	5175	1.7	3037	1.9
>Octane, 2,4,6-trimethyl-	14.70	85	1338	0.5	790	0.5
>Butanedioic acid, bis(trimethylsilyl) ester	14.86	89	1936	0.6	1089	0.7
>Nonanoic acid, trimethylsilyl ester	15.75	91	19185	6.4	10747	6.6
>Benzene, (isothiocyanatomethyl)-	15.97	86	2663	0.9	1295	0.8
>ACIDE DECANOIQUE	17.46	90	5016	1.7	2737	1.7
>Lauric acid TMS	20.63	91	1867	0.6	1006	0.6
>Tetradecanoic acid, trimethylsilyl ester	23.51	86	4824	1.6	2576	1.6
>Phthalic acid, butyl cyclobutyl ester	25.05	89	655	0.2	348	0.2
>Hexadecanoic acid, trimethylsilyl ester	26.14	89	14483	4.8	7421	4.6
>Octadecanoic acid, trimethylsilyl ester	28.55	86	6429	2.1	3375	2.1
>ETHYL-HEXYL-PHTHALATE	31.81	89	1724	0.6	869	0.5
>4-Methyl-2,4-bis(4'-trimethylsilyloxyphenyl)pentene-1	33.43	91	652	0.2	380	0.2

Mass Frontier Spectrum Interpretation

While some of the acquired spectra are not included in commercial libraries, some matches show structural similarities. The Mass Frontier software analyzes the unknown mass spectrum and associates fragmentation pathways and ion structures to the unknown spectral pattern calculated from the included knowledge base of known fragmentation rules [10]. Two examples of spectrum interpretation of unknown compound spectra are given in Figure 5 for the DCM extract and in Figure 6 for the IPA extract. The expert Mass Frontier software system generated plausible proposals to explain the mass spectrum pattern.

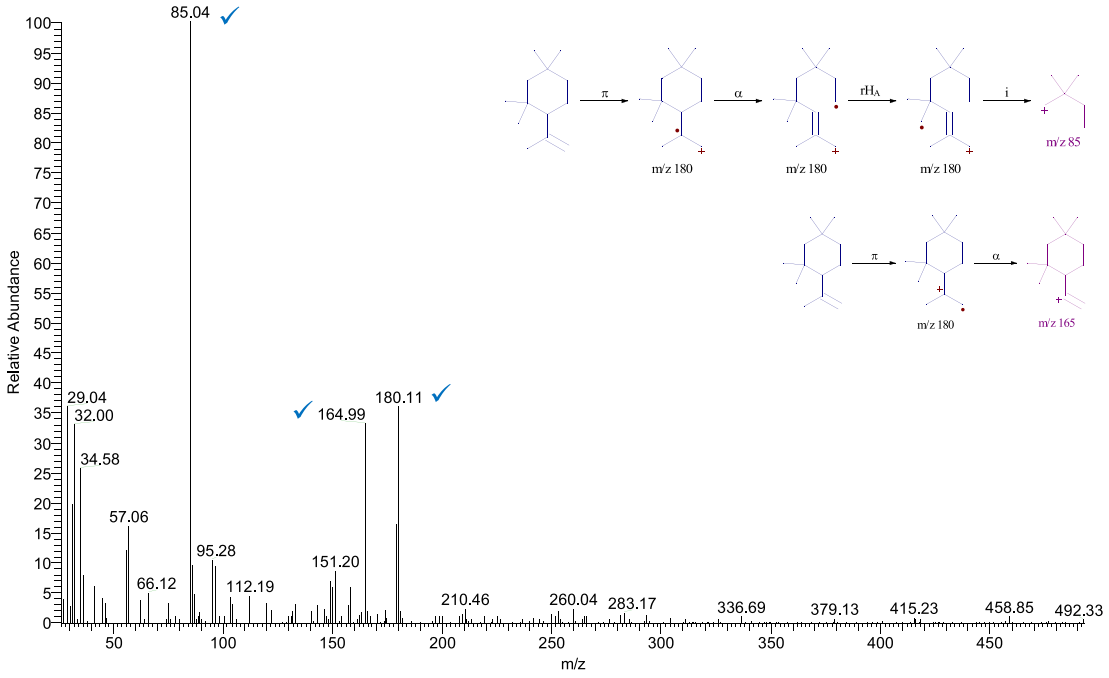

Fig. 5. DCM extract, unknown peak at RT 12.68 min

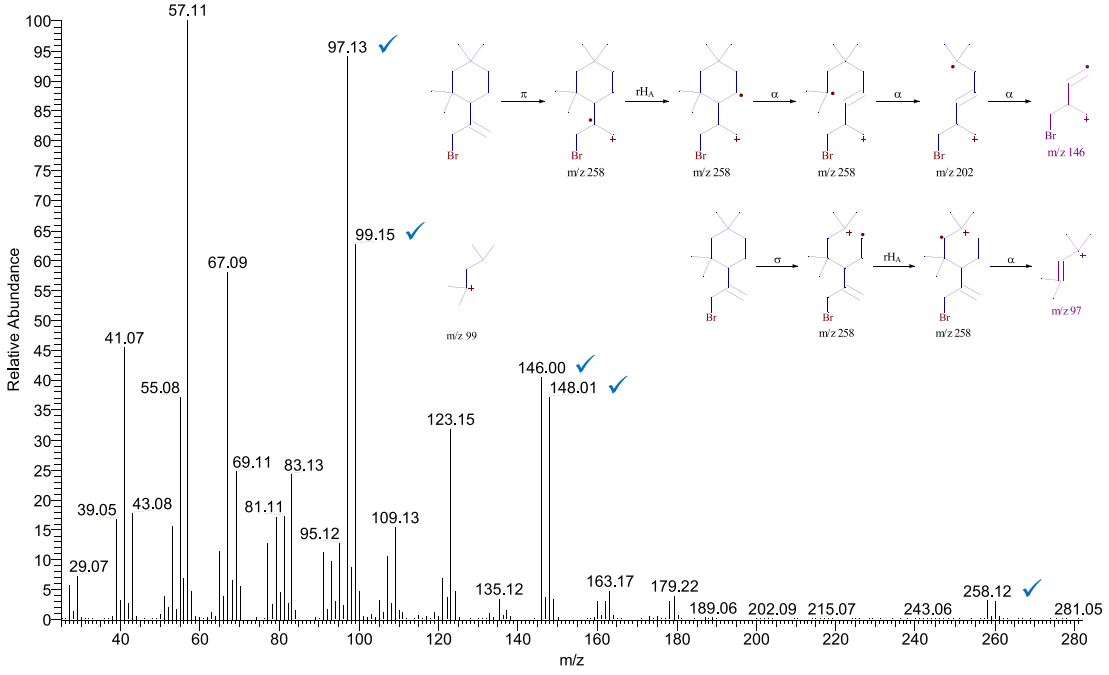

Fig. 6. IPA extract, unknown peak at RT 18.31 min

Conclusions

Parallel detection using full scan MS and FID show very good compliance in the detected compound pattern. After identification of typical major components using the mass spectrometer, routine analysis for such compounds can be run reliably using FID.

Deconvolution using AMDIS software allows you to obtain a precise isolation of the mass spectra even from co-eluting compounds. The possibility of using an individual library of target compounds and combining retention time with mass spectrum makes it a powerful tool for analytical control. Moreover, the Thermo Scientific™ Xcalibur™ mass spectrometry software allows the transfer of mass spectral libraries already available in the laboratory.

For unknown mass spectra, Mass Frontier software is a unique tool for interpretation. Structure proposals and fragmentation pathways are provided for mass spectra allowing a deeper sample and unknown elucidation.

The complete analytical system using the ISQ as a single quadrupole MS with the parallel FID on the TRACE 1310 GC, associated with acquisition and processing software is a powerful and easy-to-use solution for the identification of unknowns, routine screening, and if required, also compound quantitation for product safety control and similar quality control applications.

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