Quantification of Tentatively Identified Extractables and Leachables with Mass Spectrometry and Polyarc/FID

Application Note

Chemical Characterization for Biocompatibility

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Introduction

Extractable and Leachable (E&L) evaluations have become increasingly more vital to successful medical device product development and regulatory submissions. According to requirements and guidance from the US Food and Drug Administration (FDA), European Medicines Agency (EMA), ISO and the Product Quality Research Institute (PQRI)[1-4], E&L profiles should be established by exhaustive extraction with multiple solvents of varying polarities and reliable determination with distinctive analytical techniques.

Gas chromatography mass spectrometry (GC-MS) analysis with headspace (HS) or liquid injection detects volatile and semi volatile components in E&L studies. Due to the complexity of materials and the unpredictability of an E&L analysis, two-step analysis (initial screening and target analysis) is usually employed for qualification and quantification of compounds detected. This is a costly, time-consuming and labor-intensive process.

The PolyArc/FID system converts carbon atoms of organic molecules found in the column effluent into methane which then generates an FID response. The resulting detector response is uniform on a per carbon basis and allows the FID to have a truly universal carbon sensitivity. This eliminates the need for calibration standards for each identified compound to determine an internal or external standard's response factor. Gas chromatography using full scan MS combined with parallel PolyArc/FID detection could apply to E&L studies, integrating a two-step process into a one-step analysis.

Two XTI-5MS Columns were installed into the front inlet of a 6890 GCMS. One of the columns was connected to the 5975 Mass Spectrometer while the other was connected to the PolyArc and FID. Two groups of known extractables and leachables of concern were prepared in acetone solutions at concentrations ranging from 50 µg/mL to 100 µg/mL. These preparations were spiked with a known volume of internal standard at a concentration of 25 µg/mL before triplicate analysis on the instrument. Calculation of the relative response factors of each analyte was performed with both the Mass Spectrometer and PolyArc/FID data for comparison.

Experimental

GC conditions

Front inlet	Split/Splitless
Split Flow	5:1
Inlet temperature	280°C
Inlet pressure	12.0 psi
Column Flow	1.5 mL/min, Constant Flow
Septum purge flow	3 mL/min
Oven	40 °C (2 min), 10 °C/min to 310 °C (7 min)
Column	XTI-5 (30 m × 0.25 mm × 0.25 µm)
Syringe	10 µL
Injection volume	1 µL

FID conditions

Temperature	300 °C
H_2	1.5 mL/min
Air	350 mL/min
Makeup	20 mL/min (He)
Sampling rate	50 Hz

Polyarc reactor conditions

Setpoint	293 °C
H_2	35 mL/min
Air	2.5 mL/min

Mass Spectrometer conditions

Transfer Line Temperature	300 °C
MS Detection	EI, 70eV with 3 min solvent delay
Source Temperature	250 °C
Mass Range	33-650 amu

Results and Discussion

In this study, the relative response factor (RRF) was determined from both MS and PolyArc/FID response using the following equation:

$$\mathrm{RRF} = \frac{A_x \times C_i}{A_i \times C_x}$$

Where:

A_x = peak area of E&Ls
Ai = peak area of internal standard
Cx = concentration of E&Ls (concentration µg/mL for MS; moles of carbon for PolyArc/FID)
Ci = concentration of internal standard (concentration µg/mL for MS; moles of carbon for PolyArc/FID)

The analysis was executed in three replicates and the average RRF and %RSD is reported and compared in Tables 1 and 2 below.

Most of the E&Ls compounds of interest have a RRF above 0.7 from the PolyArc/FID detection with very low %RSD, while the RRFs from the MS range from 0.03 to 1.5 with a greater %RSD. It is common for an analyte's concentration to be semi-quantified by the use of an internal, surrogate or standard. That is, an internal standard is added to a sample at a known concentration and the E&L analyte's concentration is semi-quantified from the internal relative response. The accuracy of such a concentration estimate depends on the similarity of the response of the method for the E&L analyte and the internal standard. The more the RRF deviates from 1, the less accurate the concentration estimate is. For example bisphenol A has demonstrated to have vastly different responses on the Polyarc/FID vs MS detection. Table 1 shows a RRF of 0.9447 from the PolyArc/FID, which means the actual result is approximately 1.06 times the calculated amount injected on the system. However, through MS detection, bisphenol A's RRF is 0.2375, thus the actual result would be 4.2 times the calculated amount injected. This difference in RRFs by bisphenol A shows that if this were an unknown compound rather than one with an established RRF the MS detection would lead to a larger underestimate of concentration. An underestimation of concentration could negatively impact your risk assessment making you think the analyte is safer than it really is.

An alternate adjustment could be adopted by generating a RRF database and applying to actual analysis, which could be extremely useful for those E&L analytes like organic acids which show significant suppression in MS and PolyArc/FID. In such a situation, the %RSD can be critical. PolyArc/FID shows better accuracy even for stearic acid analysis.

Table 1: Relative Response Factors (RRF) for Group 1 E&Ls

Analytes	Rt (min)	RRF by MS	%RSD of RRF from MS	Average RRF from PolyArc	%RSD of RRF from PolyArc
2-Fluorobiphenyl	14.2	1.4897	6%	1.0162	1%
IS (n-Hexadecane-D34)	16.5	1.0000	N/A	1.0000	N/A
bisphenol A	22.9	0.2375	0%	0.9447	0%
Dibutyl phthalate	20.8	0.2609	4%	0.7629	0%
DEHP	25.9	0.4704	16%	0.7350	1%
Erucamide	27.6	0.0443	1%	0.7341	1%

Table 2: Relative Response Factors (RRF) for Group 2 E&Ls

Analytes	Rt (min)	RRF by MS	%RSD of RRF from MS	Average RRF from PolyArc	%RSD of RRF from PolyArc
Heptanoic Acid	9.8	0.1882	3%	0.8210	1%
Undecanoic Acid	15.3	0.4408	6%	0.7012	0%
IS (n-Hexadecane-D34)	16.5	1.0000	N/A	1.0000	N/A
Myristic Acid	18.7	0.0809	23%	0.5657	2%
Diisopentyl phthalate	21.9	0.4038	1%	0.8759	1%
Stearic Acid	22.6	0.0324	19%	0.4300	2%
Hexacosane	26.2	0.2749	22%	0.8585	1%
Di-n-octyl phthalate	27.3	0.3369	17%	0.7875	1%

Conclusions

Semi-quantification of tentatively identified compounds is calculated using the relative area of the tentative identification in relation to the relative area of the internal standard. Thus an idea relative response factor for each tentative identification would be as close to 1.0 as possible. The results demonstrate that the Polyarc/FID system produces relative response factors closer to ideal than the mass spectrometer across these known analytes tested. The incorporation of the Polyarc/FID system into E&L analysis would provide semi-quantification that is more accurate giving more confidence to the risk assessment for biocompatibility.

References

1. FDA's 510(K) Requirements https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/510kClearances/
2. EMA. Scientific Guidance, Guideline on Plastic Immediate Packaging Materials, 2005
3. ISO-10993-12
4. PQRI. Leachables and Extractables Working Group, Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, 2006

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