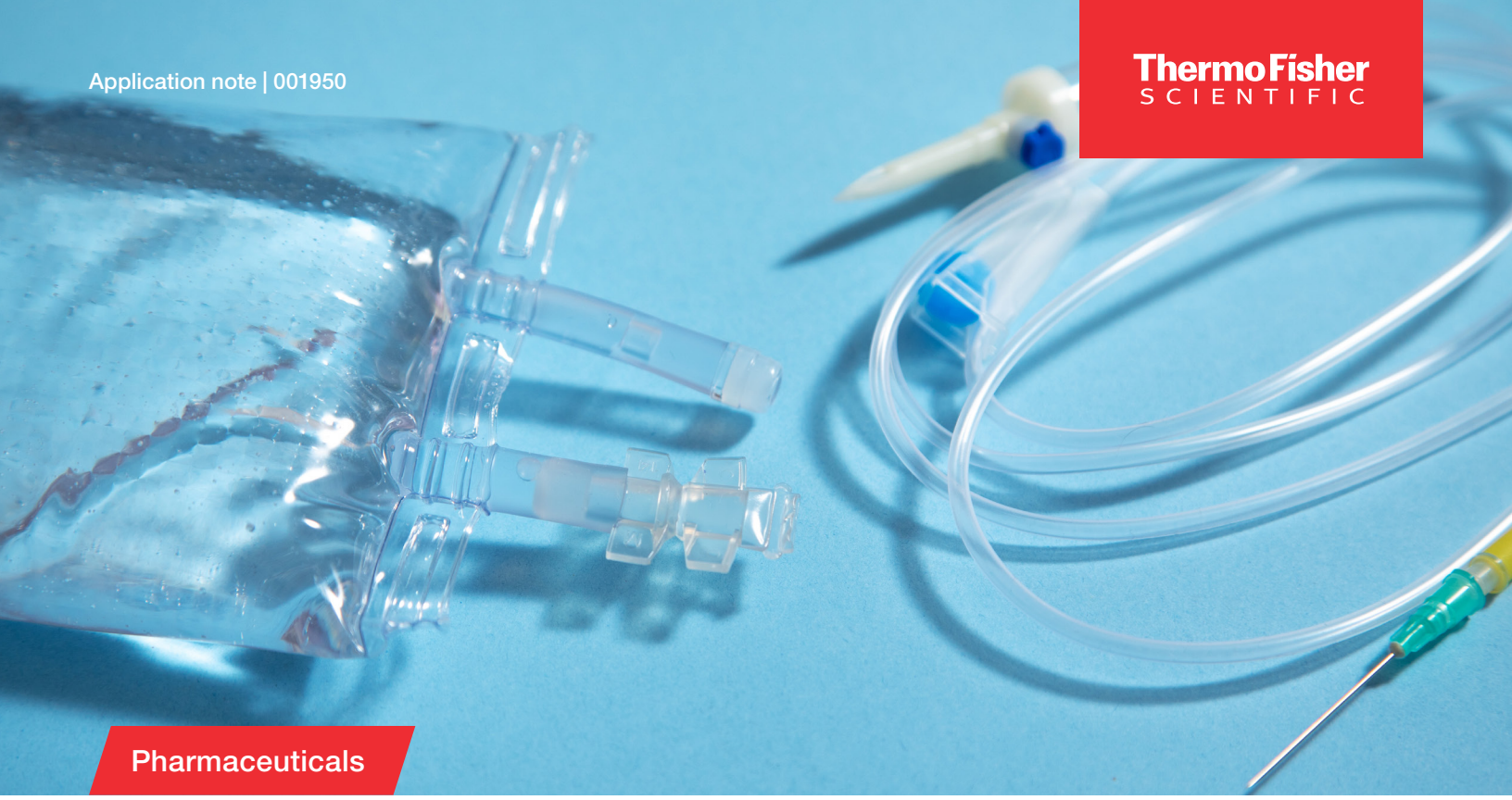
Application note | 001950



Pharmaceuticals

Confident extractable testing of medical device components using a new automated parallel extraction and evaporation sample preparation system and the multidetector approach

Authors

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Keywords

Extractables testing, pressurized fluid extraction, E&L methods pharma, Accelerated Solvent Extraction (ASE), Orbitrap Exploris 120 mass spectrometer, high-resolution accurate mass (HRAM), Vanquish Duo UHPLC system, charged aerosol detection (CAD), multi-detector

Application benefits

- Fully automated parallel extraction and evaporation of medical device components requiring minimal user intervention for a sample-to-vial workflow
- Highly reproducible and fast extraction for accelerated extractables profiling
- Efficient extraction leading to less oxidation of extracted antioxidants than reflux extraction or soaking at elevated temperatures
- Use of a spiked-in internal standard allows confident estimated concentration determination of unknown extractable compounds above AET with the multidetector LC/UV/CAD/HRAM MS analysis
- Confident annotation of extractables against comprehensive HRAM spectral libraries with Thermo Scientific™ Compound Discoverer™ software

Goal

To demonstrate the benefit of the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor system for automated extraction and on-line pre-concentration for extractables profiling of a representative medical device component

Introduction

Extractables and leachables testing is a critical part of the pharmaceutical development process, as it serves to evaluate the risk of potentially harmful substances originating from drug product packaging or medical device materials. Additionally, in the case of single-use systems employed in biopharmaceutical manufacturing, there is a risk that leaching substances may impact the production of the API.[1]

As the first step of extractables and leachables testing, controlled extraction studies serve to predict worst-case leachables profiles for the investigated materials and are often carried out on individual components using a variety of solvents and multiple extraction methods to provide relevant boundary parameters.[2] Among the recommended and commonly employed extraction techniques are sonication and reflux or Soxhlet extraction, as well as pressurized solvent extraction.[2,3] One benefit of the latter is that elevated temperature and pressure enable higher capacity of the extraction solvent to dissolve the target analytes and improve the rate of mass transport. This can result in reduced solvent volumes and shorter extraction durations, as previously demonstrated.[4] As such, accelerated solvent extraction (ASE) is employed widely for the extraction of plastic materials.[5,6]

Here, we present the application of a new accelerated solvent extraction system based on gas-assisted continuous solvent delivery—the EXTREVA ASE Accelerated Solvent Extractor system, which is capable of parallel solvent extraction of multiple samples and automated evaporation—to the extractable testing of a medical device component. The system was used to extract a polypropylene twist-off port, which was analyzed with the multi-detector platform described in a previous application note to allow semi-quantitation of the unknown extractables.[7]

Experimental

Sample preparation and extraction

The extraction of the polypropylene twist-off ports was performed using the EXTREVA ASE Accelerated Solvent Extractor system (P/N 22184-60101) using the extraction cells, collection vials and consumables listed in Table 1.

Two pieces of the medical device component were cut into smaller pieces using clean scissors to increase surface area and extraction efficiency and to allow them to be placed in the 10 mL stainless steel extraction cells. Extraction was performed with 50:50 water:isopropanol in triplicate on the EXTREVA ASE system using the parameters shown in Table 2. An empty cell was also extracted in parallel to serve as a matrix blank.

Table 1. List of reagents and consumables used

Name	Part number
Water, UHPLC grade, 1 L, Thermo Scientific™	W8-1
Acetonitrile, UHPLC grade, 1 L, Thermo Scientific™	A956-1
Isopropanol, Optima™ LC/MS grade, Fisher Chemical™	A461-1
Ammonium acetate, Optima™ LC/MS grade, Fisher Chemical™	A114-50
Thermo Scientific™ SureSTART™ Screw Glass Vial, 2 mL, Level 3	6PSV9-1PSS
Thermo Scientific™ SureSTART™ 9 mm Screw Caps, Level 3	6PSC9TST
Thermo Scientific™ Dionex™ ASE™ Stainless Steel Extraction Cell, 10 mL	22184-62225
Thermo Scientific™ Dionex™ Extraction Cell Filters for 10 mL cells	068093
Concentration Flask Assembly, 60 mL, set of 4	22184-62234

Table 2. EXTREVA ASE extraction and evaporation conditions

Parameter	Value
Extraction solvent	50:50 water:isopropanol
Extraction temperature	110 °C
Extraction pressure	200 psi
Rinsing volume (Pre-Run)	10 mL
Purge time	90 s
Gas-assisted mode	On, 10 mL/min flowrate
Cell fill volume	60%
Solvent flow rate	0.2 mL/min
Extraction time	35 min
Extraction solvent total volume	~ 28 mL
Evaporation mode	Fixed Volume
Evaporation vacuum	50 torr (1 psi)
Evaporation temperature	70 °C
Evaporation gas flow rate	50 L/min nitrogen per channel
Evaporation pre-rinse	10 mL, acetonitrile
Evaporation rinse	4 mL, acetonitrile
Evaporation final volume	1.0 mL

The solutions were then transferred into Eppendorf vials for centrifugation at 10,000 g ×10 min to remove any precipitate, and the supernatant was transferred back to clean autosampler vials and placed in the autosampler for subsequent LC/MS analysis.

To allow comparison to conventional extraction techniques, parallel extraction was performed by soaking a second set of samples in 50:50 water:isopropanol at 110 °C overnight in closed glass containers, with the extracts then evaporated to 1 mL using the EXTREVA ASE system and subsequent treatment, as above.

Sample analysis

The extracted samples were analyzed to detect non-volatile to semi-volatile extractables using a multidetector system described in more detail in a previous application note.[7]

Briefly, the LC separation was performed using a Thermo Scientific™ Vanquish™ Duo UHPLC system for inverse gradient, consisting of:

- Vanquish System Base (P/N VF-S01-A-02)
- Vanquish Dual Pump F (P/N VF-P32-A-01)
- Vanquish Split Sampler FT (P/N VF-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-03)
- Vanquish Diode Array Detector HL (P/N VH-D10-A) with Thermo Scientific™ Vanquish™ LightPipe™ flow cell, 60 mm (P/N 6083.0200B)
- Vanquish Charged Aerosol Detector H (P/N VH-D20-A)

This was connected to a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer (P/N BRE725531). The LC/UV/CAD/MS/MS analysis was carried out using the conditions listed in Tables 3 and 4.

Table 3. UHPLC experimental conditions

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™ Vanquish C18, 1.9 µm, 2.1 × 100 mm (P/N 25002-102130-V)
Mobile phase	A: 10 mM ammonium acetate in water B: acetonitrile
Flow rate	0.4 mL/min
Column temperature	45 °C (still air mode)
Autosampler temperature	6 °C
Injection volume	5 µL
Needle wash solvent	25% acetonitrile
Mixer volume	300 µL (250 µL static + 50 µL capillary mixer)
Flow split ratio	1:10 (MS:CAD)
Divert valve timing	Flow to waste from 0–0.9 min and 25.1–30 min
DAD settings	Wavelength 200–680 nm, 10 Hz acquisition speed
CAD settings	5 Hz acquisition speed, 35 °C Evaporator temperature, 1.00 Power function

Table 4. UHPLC gradient conditions

Time (min)	Analytical gradient pump–mobile phase B %	Inverse gradient pump–mobile phase B %
0.0	5	99
1.0	5	
1.755		99
18.0	99	
18.755		5
25.0	99	
25.755		5
25.1	5	
25.855		99
30.0	5	

Mass spectrometry analysis was carried out on an Orbitrap Exploris 120 mass spectrometer equipped with a Thermo Scientific™ OptaMax™ NG HESI ion source. Untargeted screening experiments on the representative extract were carried out using polarity switching data-dependent MS^2 ($ddMS^2$) experiments. The MS source conditions for both methods and important MS experiment parameters are detailed in Tables 5 and 6.

Table 5. MS source conditions

Parameter	Value
Spray voltage	+ 3,250 V / – 3,000 V
Sprayer position	1.2, M/H, center
Vaporizer temperature	75 °C
Ion transfer tube temperature	325 °C
Sheath gas	25 a.u.
Aux gas	5 a.u.
Sweep gas	0 a.u.

Table 6. MS experiment parameters for the polarity switching $ddMS^2$ Top3 method

Parameter	Value
Full Scan	
AGC Target	Standard [1e6]
Full Scan Resolution	60,000 @ *m/z* 200
Full Scan Mass Range	*m/z* 120–1200
Lock Mass Correction	EASY-IC™ Scan-to-Scan, Full Scan only
RF Level (%)	70
$ddMS^2$	
Isolation Window (*m/z*)	1.5
HCD Collision Energies (Normalized, %)	30, 50, 80
MS^2 Resolution	15,000 @ *m/z* 200
Maximum Injection Time Mode	Auto
Intensity Threshold	1.0e5
Dynamic Exclusion	5 s, Exclude Isotopes

Data processing software

The Thermo Scientific™ Xcalibur™ 4.5 software was used for data acquisition and Thermo Scientific™ Freestyle™ 1.8 SP2 software for initial data review. Quantitative analysis of the CAD and UV trace data was performed using Thermo Scientific™ Chromeleon™ CDS 7.2.10. For qualitative MS data processing and differential analysis, data were imported into Thermo Scientific™ Compound Discoverer™ 3.3 SP1 software for spectral deconvolution and compound identification using the workflow template "E and L Unknown ID with Online and Local Database Searches" with modifications to create additional analog traces and addition of the 'Fill Gaps' node.

Results and discussion

The EXTREVA ASE Accelerated Solvent Extractor (Figure 1) combines extraction and evaporation capabilities and can use six different extraction solvents channels to extract up to four samples in parallel at adjustable temperatures and flow rates. In contrast to the static extraction used in previous ASE devices, the EXTREVA ASE system uses 200 psi of nitrogen gas to pressurize the extraction cells and provide a continuous flow of the extraction solvent through the extraction cell.[8] After extraction, the EXTREVA ASE system can concentrate the collected extracts either to dryness or to a defined volume using combined application of a vacuum, nitrogen gas flow, and heating of the collection flask. For this, the instrument employs a liquid level sensor based on visual detection and artificial intelligence to allow precise endpoint detection for the concentration of extracts in autosampler vials. Critically, this step is controlled individually for each channel to account for variations in evaporation rates between vials. For extractable testing, preconcentration of the extracts allows the method to be adapted for otherwise challenging analytical evaluation thresholds without requiring additional manual liquid transfer.

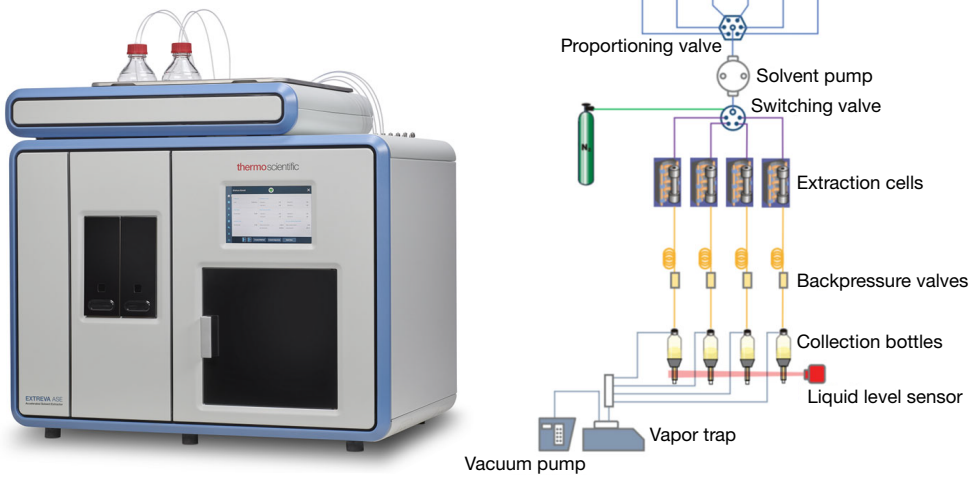

Figure 1. The EXTREVA ASE system and a schematic diagram of its flowpath

Extraction method development

In developing an optimal extraction method for the twist-off plug component, two different flow rates of 0.2 mL/min and 0.35 mL/min were compared for extraction times of 35 min and 20 min, respectively, to keep the total volume constant. As seen in the volcano plot in Figure 2, the lower flow rate resulted in higher analyte concentrations for the majority of compounds detected in the mass spectral data. Additionally, an evaluation of different extraction temperatures showed 110 °C to be most optimal (data not shown).

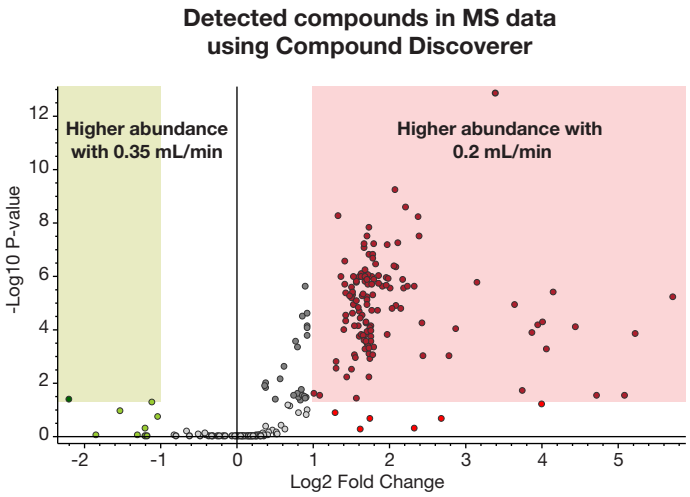

Figure 2. Volcano plot showing the comparison of areas for detected compounds in the twist-off port extracts by plotting their -Log10 P-values against the Log2 Fold Change of the Ratio of 0.2 mL/min over 0.35 mL/min. Compounds that are significantly more or less abundant at the lower extraction flow rate are displayed in the red and green shaded areas, respectively. Compounds with a fold change less between -1 and 1 are shown in gray.

To allow better detection of the extractables from the twist-off plug material using the multidetector platform, the evaporation function of the EXTREVA ASE system was used to concentrate the extract from a volume of approximately 28 mL down to 1 mL in an autosampler vial. During the evaporation step, the instrument was set to rinse the evaporation flask using acetonitrile. This feature, allowing use of different solvents in the rinsing step, is particularly useful to perform automated solvent exchange in cases where non-HPLC-compatible solvents are used in the extraction step. In testing the accuracy of the concentration step, the final volume was found to typically vary by less than 10% (Data from ten replicate evaporations of hexane to a final volume setting of 1 mL gave a gravimetrically determined average volume of 1.049 mL, with an RSD of 9.38%).

In addition to the ease of use provided by the automated endpoint detection of the concentration step, this was also beneficial in that it resulted in fewer compounds being lost than when evaporating the extract to dryness with subsequent reconstitution in an equal amount of solvent in the absence of a robust endpoint detection. Figure 3 compares the number of compounds detected in the MS data with peak areas above a threshold of 1e6.

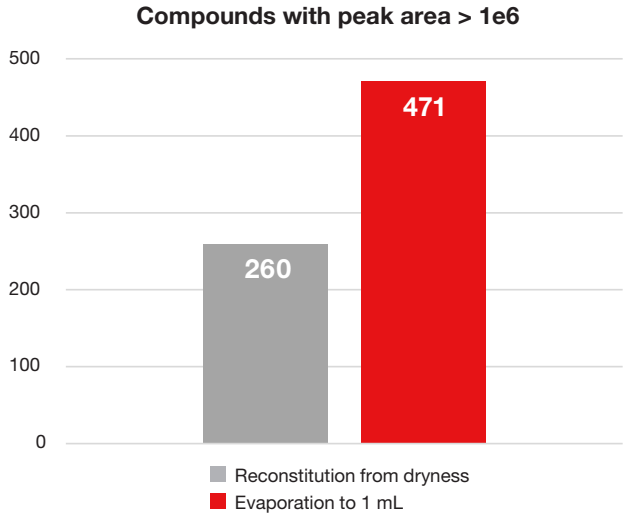

Figure 3. Comparison of number of compounds detected in the MS data above a peak area threshold of 1e6 from extractions evaporated to 1 mL or evaporating to dryness with the EXTREVA ASE system and then reconstituted to 1 mL, respectively, showing the benefit of not evaporating to dryness

Reproducibility of extraction

To evaluate the reproducibility of the optimized extraction method, three replicate extractions of the twist-off port sample were performed and analyzed in triplicate using the multidetector platform to obtain UV, CAD and MS (+/-) data. Additionally, the extraction performance was compared to a static extraction performed with an equal amount of extraction solvent at 110 °C in a closed vessel, which was subsequently transferred to an evaporation flask and concentrated to 1 mL of extract using the evaporation function of the EXTREVA ASE system.

Figure 4 shows the overlay of the of the UV chromatogram traces from three replicate extractions, indicating the excellent reproducibility. This is further supported by the principal component analysis shown in Figure 5, generated from the MS data of the replicates from the extraction with the EXTREVA ASE system and overnight soak, indicating the higher reproducibility with the former.

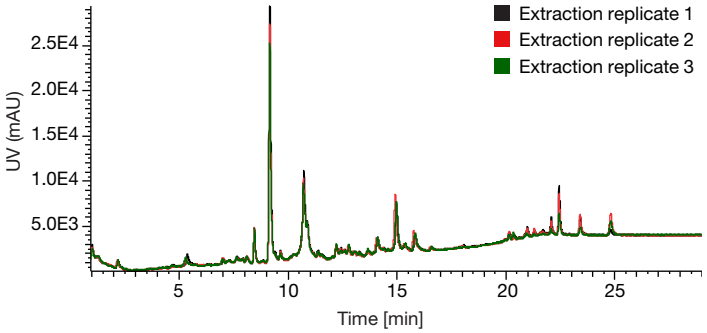

Figure 4. Overlay of the UV chromatogram traces for the three extraction replicates from the extractions carried out with the EXTREVA ASE system

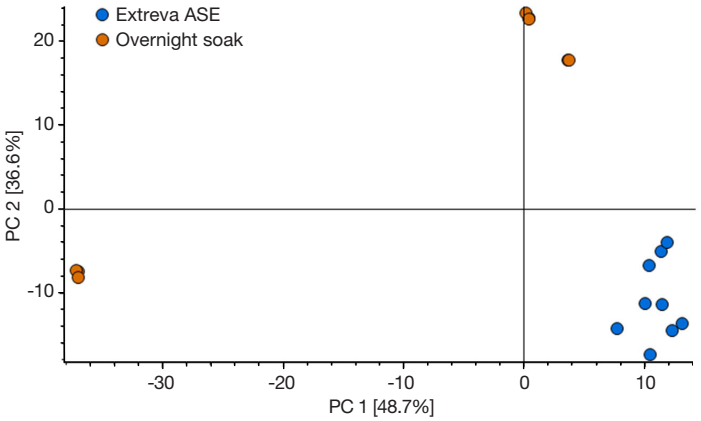

Figure 5. PCA plot of the EXTREVA ASE system (blue) and overnight soak (orange) extracts obtained from three replicate extraction and injections, respectively, with the close clustering of the EXTREVA ASE system extract replicates indicating their superior reproducibility

Of note, in comparing the relative abundance of analytes detected from the two different extraction methods, especially compounds with higher molecular weight were more efficiently extracted using the EXTREVA ASE system, as highlighted in Figure 6.

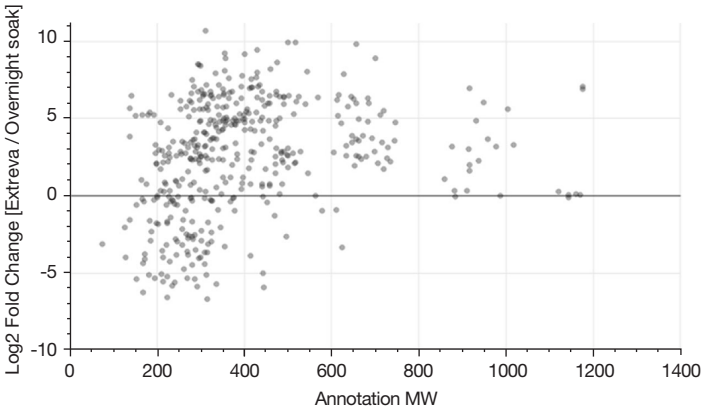

Figure 6. Plot of Log2 Fold Change for the ratio of peak areas for EXTREVA ASE system extraction over overnight soak against the annotated molecular weight, showing higher extraction efficiency for with the EXTREVA ASE system, in particular for high molecular weight extractables as evident by their positive Log2 Fold Change values

Under some conditions, the undesired degradation of extractables can occur during any extraction process. This has an impact on the extractables profiling particularly for antioxidant compounds, which are common additives in plastic materials and potential extractables, as reported in the literature.[5] To that end, the oxidation behavior of the new EXTREVA ASE system was compared to the extracts from overnight soaking, as well as a representative reflux extract that had been previously prepared from the twist-off port material with the same extraction solvents. As shown in Table 7, the ratio of detected MS peak areas for oxidized to unoxidized Irgafos 168 was lowest with the EXTREVA ASE system, possibly because of the extraction being carried out under nitrogen gas.

Table 7. Impact of extraction method on the relative amount of oxidized and unoxidized Irgafos 168, a common antioxidant additive found in extractables testing of plastics

Extraction method	Oxidized Irgafos 168 MS peak area	Irgafos 168 MS peak area	Peak area ratio (O-I168 / I168)
EXTREVA ASE	3.00e8	7.31e8	0.41
Overnight soaking	3.24e7	2.87e7	1.13
Reflux	4.04e7	3.85e7	1.05

Profiling of extractables using the multi-detector platform

To demonstrate the successful detection and identification of extractable compounds from the twist-off port extracts prepared with the EXTREVA ASE system, one of the extract samples was analyzed after spiking with 2,5-bis(5-tert-butyl-benzoxazol-2-yl) thiophene as an internal standard at a concentration of 1.0 µg/mL, corresponding to an analytical evaluation threshold (AET) of 0.5 µg per component.

The data from this sample were processed using Chromeleon CDS to detect compounds from the CAD and UV traces, which are known to show lower variance in the relative signal response than the ESI-MS signal.[7,9] The detected compounds were filtered using the peak area of the internal standard, after adjustment for commonly employed uncertainty factors of 2 and 5 for the CAD and UV detectors, respectively, with a total of ten components exceeding the adjusted AET. Figure 7 shows the chromatograms from the analysis, overlaid with the extraction blank, labeling the peaks exceeding the adjusted AET, which are also summarized in Table 9.

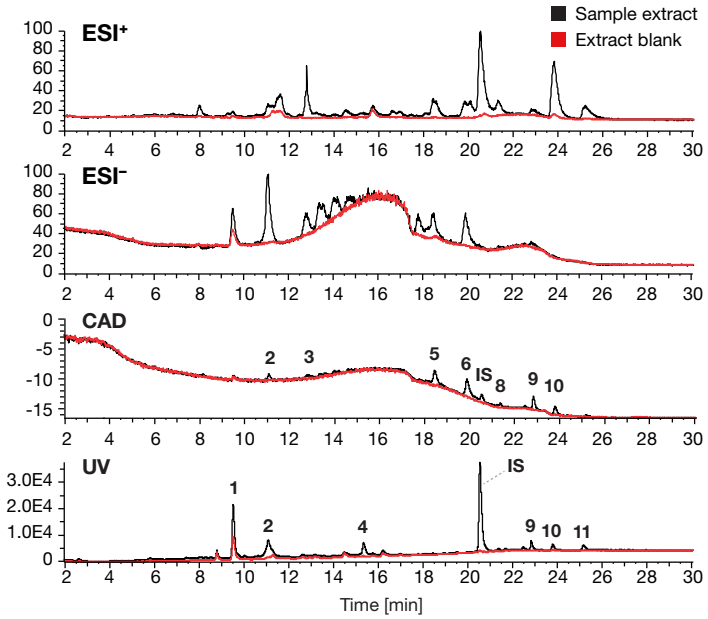

Figure 7. Overlay of the twist-off sample extract and extraction blank chromatogram traces for the different detectors, with the detected extractables labeled

The data were then processed in the Compound Discoverer software to detect compounds in the mass spectral data that correlated with the UV or CAD components and determine their identity. Notably, this drastically lowered the time spent investigating the identity of compounds that showed disproportionally high MS response but were present at concentrations below the relevant AET.

The detected compounds were automatically assigned an elemental composition based on the accurate mass and isotopic pattern and then annotated using multiple data sources, including the mzCloud™ and NIST™ HRAM MS/MS spectral libraries, an in-house E&L mzVault™ library and E&L-specific mass lists included in Compound Discoverer software.

For example, **compound 9** (MW 1176.7843 @ 22.84 min) could be confidently identified as Irganox 1010, a common extractable compound, based on matches of the fragmentation spectra in both positive and negative polarities to reference spectra in the mzCloud and mzVault libraries (Figure 8).

In addition to the direct annotation of compounds for which reference spectra were contained in the spectral libraries, other compounds could be annotated based on similarity searches. This allowed the identification of several other compounds related to Irganox 1010, such as **compound 8** (MW 916.6065 @ 21.36) shown in Figure 9.

In total, nine of the ten major extractable compounds could be annotated in this fashion, and their structures are shown in Figure 10. Additionally, the use of the spiked internal standard allowed their semi-quantitative concentration determination based on the CAD trace (or UV, in the absence of a CAD peak). The results for all ten extractables exceeding the AET were summarized in Table 8.

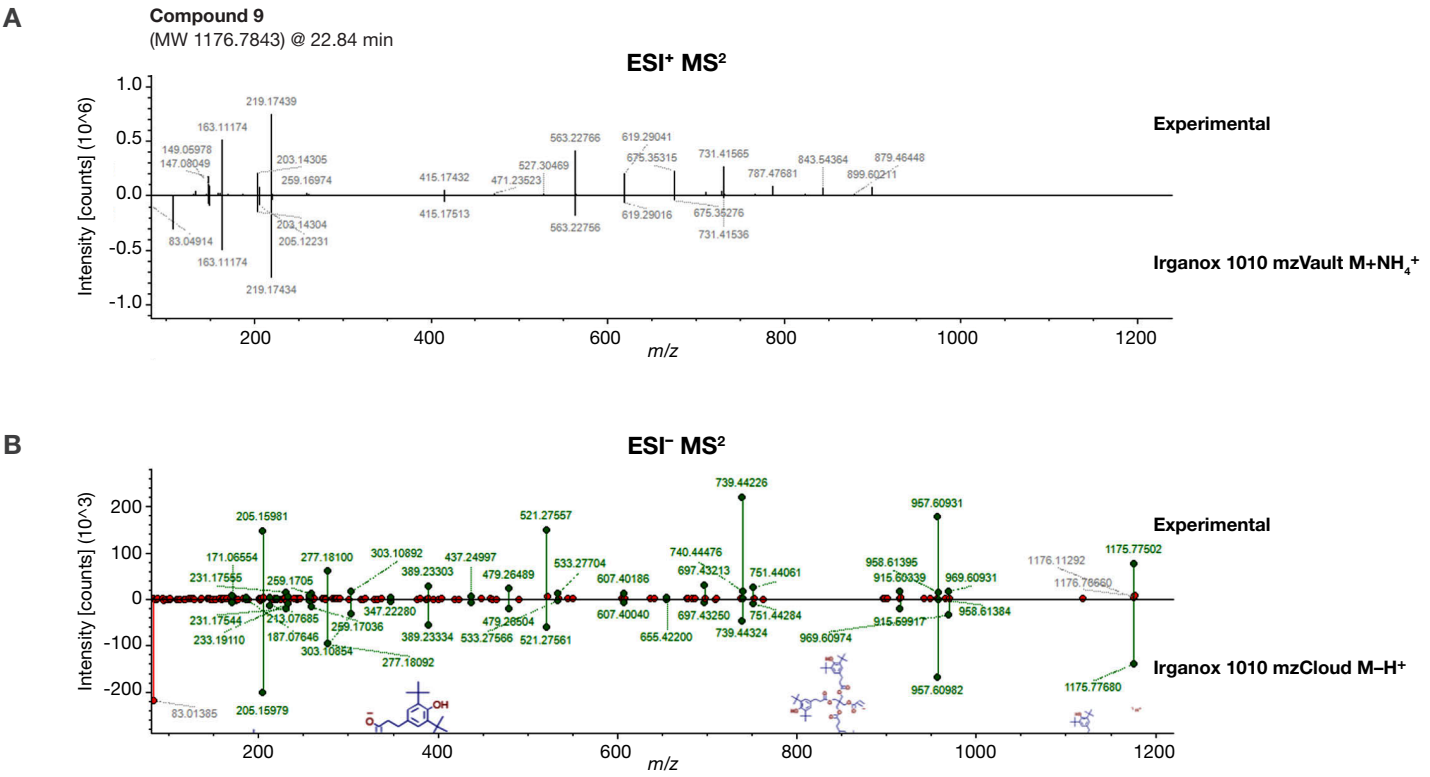

Figure 8. Confident identification of extractable compound 9 (MW 1176.7843) as Irganox 1010 visualized with the mirror plots (A) and (B) of the positive mode and negative mode MS² fragmentation spectra to reference data in the in-house mzVault library and the mzCloud spectral library, respectively

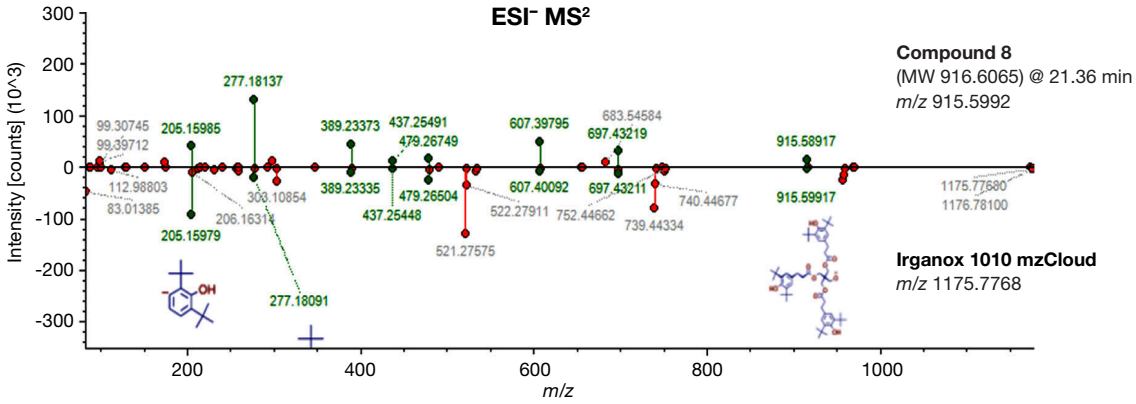

Figure 9. Mirror plot of the negative mode MS² fragmentation spectra of **compound 8** (MW 916.6065) and the library entry for Irganox 1010, showing matching fragment ions in green, allowing the identification of the **compound 8** as a degradation product of the latter

Table 8. Summary of compounds in the twist-off plug sample detected in the CAD or UV data and their annotation based on the corresponding mass spectral data

Peak #	RT (min)	UV peak area (µAU/min)	CAD peak area (µAU/min)	Estimated conc. (µg/component)	MS compound MW (Da)	Main ion
1	9.52	1394.72	n.d.	0.14[a]	294.1831	M–H
2	11.09	1282.33	0.150	0.53[b]	278.1881	M–H
3	12.77	92.87	0.086	0.30[b]	396.2510	M+EtNH$_2$+H
4	15.35	1096.94	n.d.	0.11[a]	418.2178	M–H
5	18.47	26.05	0.359	1.27[b]	330.2770	M+HOAc-H
6	19.90	n.d.	0.506	1.79[b]	358.3084	M+HOAc-H
IS	20.55	4952.13	0.141	–	430.1713	M+H
8	21.36	97.80	0.100	0.35[b]	916.6065	M+NH$_4$
9	22.84	398.54	0.285	1.01[b]	1176.7843	M+NH$_4$
10	23.80	295.89	0.238	0.84[b]	662.4463	M+NH$_4$
11	25.18	322.14	0.063	0.22[b]	646.4514	M+H$^+$

Peak #	Formula	Formula ΔMass (ppm)	Compound annotation	Annotation based on
1	$C_{17}H_{26}O_4$	-0.07	3-(3',5''-di-t-butyl-1'-hydroxy-4'-oxacyclohexa-2',5'-dienyl) propanoic acid (Irganox 1010 degradation product)	E&L Masslist
2	$C_{17}H_{26}O_3$	-0.31	3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoic acid (Irganox 1010 degradation product)	E&L Masslist
3	$C_{22}H_{36}O_6$	-0.40	Pentaerythritol 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1010 degradation product)	E&L Masslist
4	$C_{24}H_{34}O_4S$	-0.01	–	–
5	$C_{19}H_{38}O_4$	-0.60	glycerol palmitate	NIST HRAM MS/MS Library
6	$C_{21}H_{42}O_4$	-0.50	glycerol stearate	mzCloud and NIST HRAM MS/MS Library
IS	$C_{26}H_{26}N_2O_2S$	-0.40	2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene (Internal Standard)	mzCloud
8	$C_{56}H_{84}O_{10}$	0.06	Pentaerythritol tris(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate) (Irganox 1010 degradation product)	E&L MassList and MS² similarity to **9**
9	$C_{73}H_{108}O_{12}$	0.18	Pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate) (Irganox 1010)	mzCloud and E&L mzVault
10	$C_{42}H_{63}O_4P$	-0.04	Tris(2,4-ditert-butylphenyl)phosphate	E&L Masslist
11	$C_{42}H_{63}O_3P$	-0.14	Tris(2,4-di-tert-butylphenyl) phosphite (Irgafos 168)	mzCloud

[a] based on UV data
[b] based on CAD data

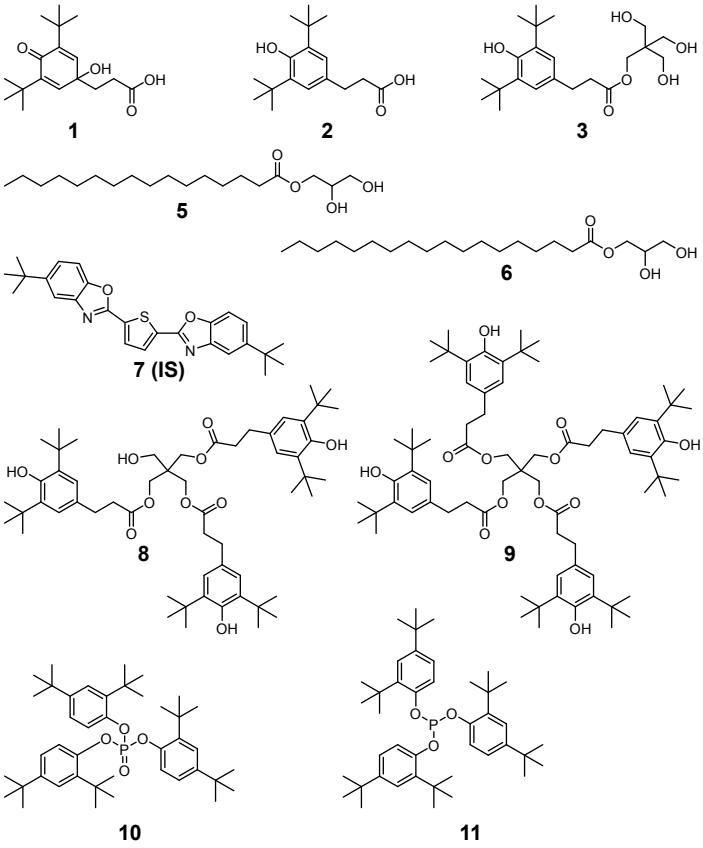

Figure 10. Proposed structures of the annotated extractable compounds listed in Table 8

Conclusion

In this work, extraction using the EXTREVA ASE system followed by LC-based multi-detector analysis was found to be an efficient method for the profiling of extractables, as shown for a medical device component. After optimization of the extraction and evaporation parameters, the high reproducibility of the EXTREVA ASE system as well as the benefit of the automated extract concentration was demonstrated. Additionally, the multidetector platform allowed for the confident detection and estimated quantitation of extractables from the twist-off port on the basis of the CAD and UV data, which were readily annotated using the high-quality mass spectral data obtained with the Orbitrap Exploris 120 MS and spectral libraries in the Compound Discoverer software.

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