Sample Preparation

Comprehensive extractables analysis using novel automated parallel extraction and concentration coupled with a multi-detector LC/UV/CAD/HRAM Orbitrap MS system

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Abstract

Purpose: 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.

Methods: LC/MS analysis of extractables of unknown compounds above AET has been developed using the combination of the UV, CAD and MS detection. Extreva ASE automated extraction and evaporation system enabled fast and reproducible extractions and sample pre-concentration while minimizing hands-on time..

Results: This method allows confident extractable testing of medical device components using a new automated parallel extraction and evaporation sample preparation system and the multidetector approach

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 AN1401 to allow semi-quantitation of the unknown extractables.[7]

Materials and methods

Sample Preparation and extraction

The polypropylene twist-off ports were extracted using the EXTREVA ASE Accelerated Solvent Extractor system (P/N 22184-60101).
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 listed in AN001950.[8] An empty cell was also extracted in parallel to serve as a matrix blank.
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 AN1401.[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 parameters listed in AN001950.[8]

Data Analysis

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 MS2 (ddMS2) experiments. The MS source conditions for both methods and important MS experiment parameters are detailed in in AN001950.[8]

Thermo Scientific™ EXTREVA™ ASE™ system

Accelerated solvent extraction (pressurized fluid extraction)

Figure 1. EXTREVA ASE system

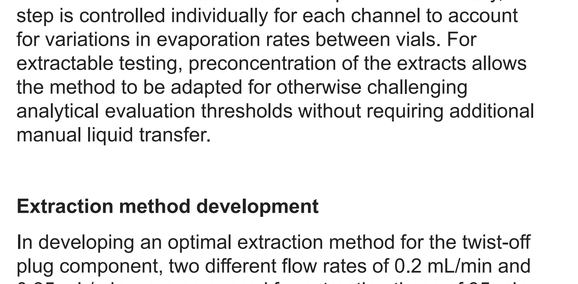
Results

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.

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.

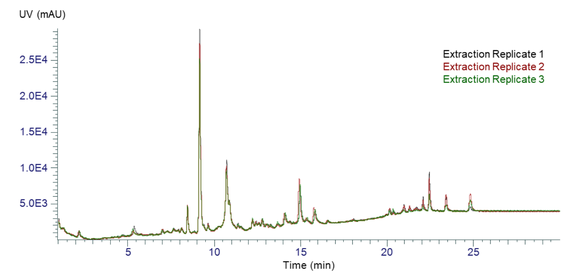


To allow better detection of the extractables from the twist-off plug material using the multidetector platform, the evaporation function of the EXTREVA ASE system was used to concentrate the extract from a volume of approximately 28 mL down to 1 mL in an autosampler vial. During the evaporation step, 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%).

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 3 shows the overlay of the UV chromatogram traces from three replicate extractions, indicating the excellent reproducibility.

Figure 3. Overlay of the UV chromatogram traces for the three extraction replicates from the extractions carried out with the EXTREVA ASE system.



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 4 shows the chromatograms from the analysis, overlaid with the extraction blank, labelling the peaks exceeding the adjusted AET, which are also summarized in Figure 5. All those molecular entities identified through exact mass determination are shown

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

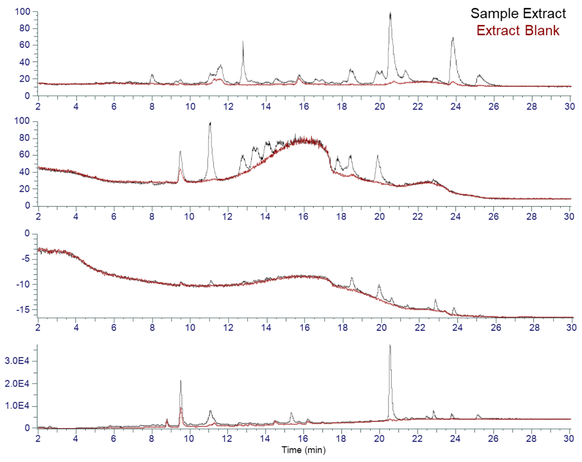


Figure 5. Confident identification of extractable **compound 9** (MW 1176.7843) as Irganox 1010 visualized with the mirror plots (A) and (B) of the positive mode and negative mode MS2 fragmentation spectra to reference data in the in-house mzVault library and the mzCloud spectral library, respectively.

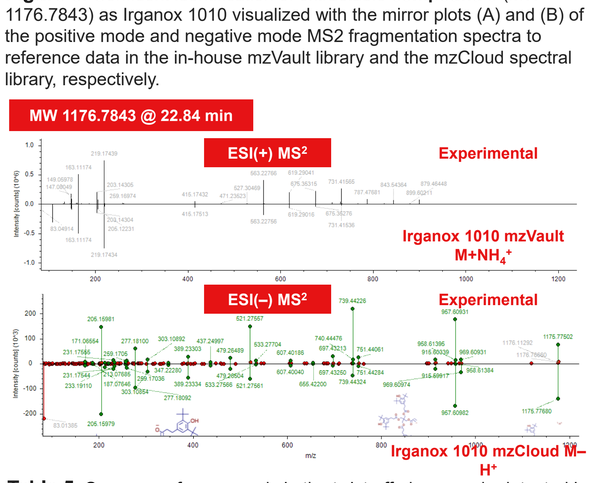


Table 5. Summary of compounds in the twist-off plug sample detected in the CAD or UV data and their annotation based on the corresponding mass spectral data.

Peak #	Formula	Formula ΔMass (ppm)	Compound annotation	Annotation based on
1	$C_{17}H_{36}O_4$	-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	$C_{17}H_{36}O_3$	-0.31	3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoic acid (Irganox 1010 degradation product)	E&L Masslist
3	$C_{32}H_{66}O_6$	-0.40	Pentaerythritol 3-(3,5-di-tert-butyl- 4-hydroxyphenyl)propionate (Irganox 1010 degradation product)	E&L Masslist
4	$C_{34}H_{70}O_2S$	-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	$C_{58}H_{94}O_{10}$	0.06	Pentaerythritol tris(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate) (Irganox 1010 degradation product)	E&L MassList and MSP 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

Conclusions

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.
- 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
- Confident annotation of extractables against comprehensive HRAM spectral libraries was achieved with the Compound Discoverer software.

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