Application note | 001434

Sample preparation

Identification of plastic additives in pharmaceutical packaging using a fully automated parallel extraction evaporator system and UHPLC-HRMS

Authors

Jingli Hu, Jeffrey Rohrer, Thermo Fisher Scientific, Sunnyvale, CA, USA

Keywords

E&L, inhaler, pressurized fluid extraction, Thermo Scientific EXTREVA ASE Accelerated Solvent Extractor, ASE

Goal

To demonstrate a method for the extraction of plastic additives in packaging using the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor, a fully automated parallel extraction and evaporation system

Introduction

Polymeric materials are widely used in the pharmaceutical industry to manufacture medical devices such as containers, syringes, and inhalers. During storage or use, chemicals might leach from these materials into the pharmaceutical product and affect the drug efficacy and/or safety. The United States Food and Drug Agency (FDA) has previously stated that “Drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality or purity of the drug beyond the official or established requirements.”[1] The FDA provided further definition of the requirements for extractables and leachables (E&L) testing for final container/closures, with an emphasis on the data required for a New Drug Application (NDA) or a Biologics License Application (BLA) submission, with the Guidance for Industry: Container Closure Systems for Packing Human Drugs and Biologics (1999).[2] Consequently, E&L studies are now a crucial component of product release.

In 2006, the Product Quality Research Institute (PQRI) joined with the FDA on recommendations for the evaluation of E&L in Orally Inhaled or Nasal Drug Products (OINDP).[3] The recommendations have been recognized by FDA and global regulatory authorities. In 2015 the U.S. Pharmacopeial (USP) Convention provided more detailed guidelines for E&L testing of pharmaceutical manufacturing and packaging materials in two USP General Chapters on Extractables and Leachables: <1663>, <1664>.[4] These chapters provide a framework for the design, justification, and execution of E&L assessments for pharmaceutical packaging systems.

An E&L study is composed of two individual yet interlinking projects. The extractables study identifies species from manufacturing components (where applicable) and the packaging system that could migrate into the pharmaceutical product upon storage under normal conditions. This establishes a baseline for the following leachables study, a series of tests carried out at predefined time-points on the pharmaceutical product for the duration of its shelf-life. Extractables screening is crucial to provide a profile of potential substances that may also leach into the bioprocess. The components under investigation are extracted in isolation from the pharmaceutical product. Key points to consider are the number of components and material types that are to be tested, and the solvents with which to perform the extractions. The PQRI protocol recommends performing vigorous extraction with multiple solvents of varying polarity, incorporating multiple extraction and analytical techniques, to provide comprehensive information for both material characterization and forecasting potential leachables. Common solvents include isopropanol and *n*-hexane.

The extraction from polymers has been traditionally performed by Soxhlet extraction or boiling under reflux. These methods typically are time-consuming and require large volumes of solvent. Accelerated solvent extraction (ASE) is a pressurized fluid extraction (PFE) technique that uses organic solvents at high pressures therefore temperatures above the solvent's boiling point can be used, resulting in increased efficiency and reduction of extraction times. ASE is recommended by the PQRI[5] and proposed by USP<1663>[6] as one of the analytical techniques for extractables testing. ASE is widely used for plasticizers and additives from polymers within the pharmaceutical and food industries.[7]

It was previously reported that replacing a Soxhlet system with a PFE system (Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor) significantly saves solvent and time, especially when the accelerated solvent extraction is combined with the Thermo Scientific™ Rocket™ Evaporator.[8] The EXTREVA ASE Accelerated Solvent Extractor (Figure 1) is a system based on many proprietary technologies including gas-assisted solvent delivery technology[9] and parallel accelerated solvent extraction.[10] This fully automated system combines the extraction and evaporation capabilities in one instrument, and it can be conveniently used for extracting and concentrating/drying compounds from up to 16 solid and semi-solid samples.

The work reported here explores the performance of the EXTREVA ASE system as an extraction technique for characterization of an inhaler. Accelerated solvent extraction conditions in Thermo Scientific Application Note AN1108 using Dionex ASE 350 system were converted to EXTREVA ASE system extraction conditions. Plastic additives were identified using ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry. Extraction efficiency was compared with the traditional solid liquid extraction method.

Figure 1. EXTREVA ASE Accelerated Solvent Extractor

Experimental

Equipment and consumables

- Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor (P/N 22184-60101)
- Thermo Scientific™ Dionex™ ASE™ Collection Vials, 60 mL (P/N 048784)
- Thermo Scientific™ Dionex™ Extraction Cell Filters, Cellulose, 27 mm (P/N 068093)
- Stainless Steel Extraction Cells 10 mL (P/N 060070)
- 10 mL Thermo Scientific™ Dionex™ ASE™ 150/350 Cellulose Thimbles (P/N 088345)

Liquid chromatography

- Thermo Scientific™ Vanquish™ Flex UHPLC System including:
  - Thermo Scientific™ Vanquish™ Quaternary Pump F (P/N VF-P20-A) with 200 µL static mixer (P/N 6044.5110)
  - Thermo Scientific™ Vanquish™ Split Sampler FT (P/N VF-A10-A)
  - Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A) with 2-position/6-port valve (P/N 6036.1560)
  - Thermo Scientific™ Vanquish™ Diode Array Detector FG (P/N VF-D11-A) with 2.5 µL titanium flow cell (P/N 6083.0550)
  - Thermo Scientific™ Vanquish™ System Base F (P/N VF-S01-A)
- Thermo Scientific™ Accucore™ C18 column, 2.1 × 100 mm, 2.6 µm particle size (P/N 17126-102130)

Mass spectrometer

- Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap Mass Spectrometer
- Peak Scientific™ Genius™ 1022 nitrogen generator (P/N 10-6022 (230v))

Software

- Thermo Scientific™ TraceFinder™ Software, version 5.0
- Thermo Scientific™ Compound Discoverer™ Software, version 3.2

Reagents and Chemicals

- Hexanes, HPLC grade (Fisher Scientific™, P/N H302-4)
- Isopropanol (IPA), Optima™ for HPLC and GC, (Fisher Chemical™, P/N A464-4)
- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Ottawa Sand (Fisher Chemical™, P/N S23-3)
- Acetonitrile, Optima™ LC/MS Grade (Fisher Scientific™, P/N A955-1)
- Thermo Scientific™ Pierce™ Formic Acid, LC-MS Grade, 10 × 1 mL (P/N 28905)
- Thermo Scientific™ Pierce™ 0.1% Formic Acid (v/v) in Acetonitrile, LC-MS Grade (P/N 85174)
- Thermo Scientific™ Pierce™ 0.1% Formic Acid (v/v) in Water, LC-MS Grade (P/N 85170)
- Thermo Scientific™ Pierce™ LTQ Velos ESI Positive Ion Calibration Solution (P/N 88323)
- Thermo Scientific™ Pierce™ Negative Ion Calibration Solution (P/N 88324)

Samples

Blank nasal inhalers were purchased from the Amazon™ store.

Sample preparation and ASE procedure

Blank nasal inhalers were cut into ~2 mm^2 pieces using scissors to increase sample surface area and extraction efficiency. Weigh 0.5–1 g of sample pieces into a cellulose thimble. Place a cellulose filter at the bottom of the cell and tighten the cell cap. Place the thimble with the sample into the cell and fill about 50% of the void volume with clean Ottawa sand and tap the cell to mix the sample with sand. Fill the rest of the void volume with clean Ottawa sand.

Place another cellulose filter on the top of the cell body and hand tighten the second end cap. The cell was placed on the EXTREVA ASE Accelerated Solvent Extractor, which was programmed as indicated in the accelerated solvent extraction and evaporation conditions table. After extraction and evaporation, 1 mL of acetonitrile was added to reconstitute the dried sample, and then it was centrifuged for 10 min at 10,000 × g. The supernatant was placed in a vial for analysis.

Notes:

Dispersion in the sand will keep the polymer particles from agglomerating when heated. This ensures an efficient extraction. Sand may contain trace contamination. If necessary, wash sand with 50% IPA/hexane using a large volume ASE cell.

Extraction thimbles for the Thermo Scientific™ Dionex™ ASE™ 350 Extraction Cells are used to hold solid and semi-solid samples when extracting analytes from complex matrices during accelerated solvent extraction. These thimbles can be used to make cell cleaning easier when extracting samples that melt at high temperatures or stick to the walls of the cell such as polymer samples. Thimbles can also act as an additional filter when working with fine particulate samples that can clog the cell.

Table 1. Extraction and evaporation conditions

Extraction		
Extraction solvent	IPA	Hexane
Extraction cell size	10 mL	
Temperature (°C)	125	100
Pressure	200 psi	
Extraction time (min)	20	25
Rinsing volume post rinse	10 mL	
Purge time	120 s	
Gas-assisted mode	N_2 at 10 mL/min each channel	
Cell fill volume	50%	
Solvent flow rate	0.35 mL/min	
Dead volume (mL)	5	5
Initial fill volume (mL)	10 × 50% = 5	10 × 50% = 5
Dynamic extraction volume (mL)	0.35 mL/min × 20 min = 7	0.35 mL/min × 25 min = 8.75
Total solvent volume (mL)	~17	~19
Evaporation		
Vacuum	2 psi (100 torr)	
Mode	Dryness	
Collection bottle	60 mL vial	
Evaporation temperature	60 °C	
N_2 flow rate	50 mL/min per channel	
Evaporation time	45 min	30 min

Table 2. HPLC and MS conditions

HPLC conditions	
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Gradient	5% B (0–1 min) 5–99% B (1–18 min) 99% B (18–25 min) 5% B (25.1–30 min)
Flow rate	0.4 mL/min
Injection volume	2 µL
Column temperature	45 °C
Run time	30 min
MS conditions	
Ion source	Electrospray ionization (ESI), positive/ negative polarity switch mode
HESI source	Sheath gas flow rate: 50 Aux gas flow rate: 10 Sweep gas flow rate: 0 Spray voltage (kV): 3.5 Capillary temp. (ºC): 300 S-lens RF level: 60 Aux gas heater temp (ºC): 430
Experiments	Full MS/ddMS^2
Chrom peak width	4 s
Method duration	30 min
Full MS	
Resolution	120,000
AGC target	3e6
Maximum IT	100 ms
Scan range	150 to 1500 *m/z*
dd-MS^2	
Resolution	60,000
AGC target	2e5
Maximum IT	50 ms
Loop count	3
Top N	3
Isolation window	2 Da
NCE stepped	30, 50, 80
Dynamic exclusion	2.5 s

Results and discussion

A schematic diagram of the EXTREVA ASE system is shown in Figure 2. The EXTREVA ASE system is a fully automated sample preparation platform designed for extracting and concentrating organic compounds from a variety of solid and semi-solid matrices. This system can use up to six different extraction solvents (or mixtures of them) and extract up to four cells in parallel. The sample is added to a stainless-steel sample cell. A liquid solvent and a gas are added to the sample cell during the extraction. This process is referred to as gas-assisted solvent extraction. The addition of the gas is controlled to establish an elevated pressure (~200 psi) in conjunction with a backpressure valve within the sample cell. The liquid solvent is heated to an elevated temperature that is below the boiling temperature of the liquid solvent at elevated pressure. The analyte can dissolve from the solid sample into the liquid solvent. Next, some portion of the liquid solvent containing the dissolved analyte continuously flows into the collection bottle. The evaporation process starts immediately after the completion of the extraction step without user interaction. The extract can be evaporated to dryness as was done in this experiment. The extracts can also be concentrated in 2 mL vials, with the final volume controlled by artificial intelligence machine vision.

Inhaler samples were extracted with hexane in four replicates using the four channels of the EXTREVA ASE system. The extracts were analyzed by UHPLC coupled with high resolution accurate mass (HRAM). The superior quality of the MS/MS data provides confidence for both nontargeted and targeted approaches. Full scan HRAM spectrometry is well-suited for multi-residue screening of low-level substances and detecting unexpected entities that would otherwise be missed. Figure 3 shows representative base peak chromatograms, as well as extracted ion chromatograms (XIC) for three identified additives, demonstrating the performance and confidence of the applied analytical method for the detection and identifications of the extracted compounds. A broad spectrum of E&Ls was identified from the inhaler by library identity search using Compound Discoverer software, version 3.3 by matching of the MS/MS fragmentation spectra to the *mz*Cloud™ spectral library. The major three extractable compounds identified are shown in Table 3. Those are common antioxidant additives and their degradation products in polymer materials. Tris(2,4-di-tert-butylphenyl) phosphite, often referred to under the trade name Irgafos 168, is a secondary antioxidant stabilizer that is commonly added to resin formulations of the kinds of polymers from which flexible bioprocess equipment is made. Lately, this antioxidant has gained importance because one of its degradation products, bis(2,4-di-tert-butylphenyl)phosphate (bDtBPP), showed detrimental effects on cells.[11, 12] In this study, other degradations products of Irgafos 168 were also found in the extracts, such as tris(2,4-di-tertbutylphenyl) phosphate, commonly called Irgafos oxidized form. A common polymer additive Irganox 1010 by BASF was also identified.

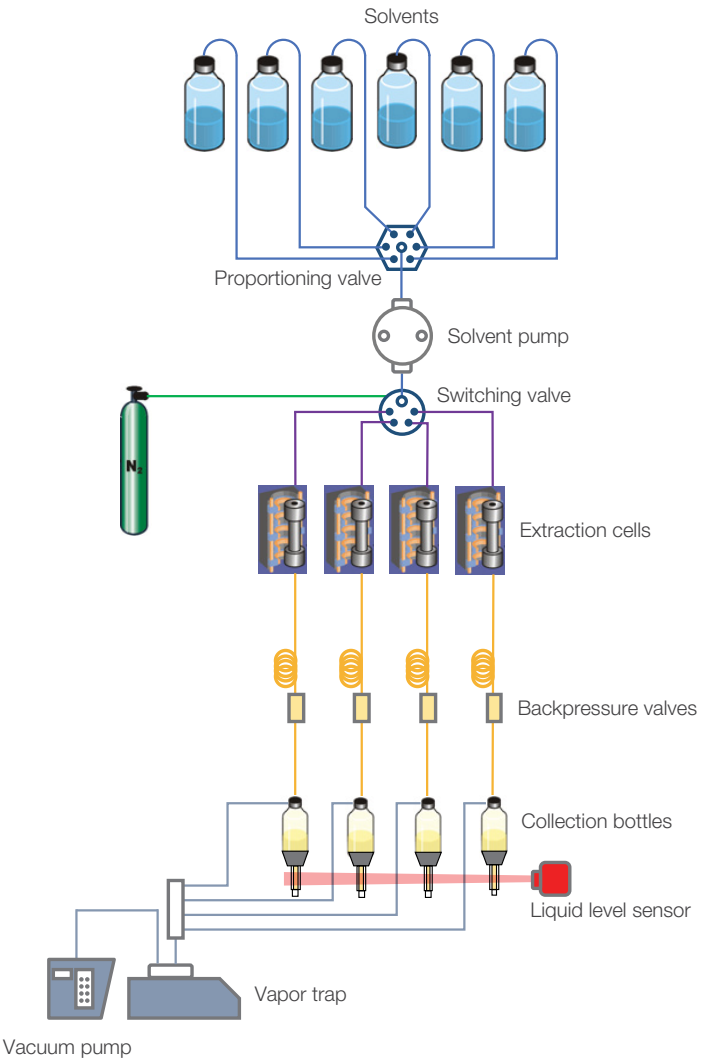


Figure 2. Schematic diagram of the EXTREVA ASE Accelerated Solvent Extractor

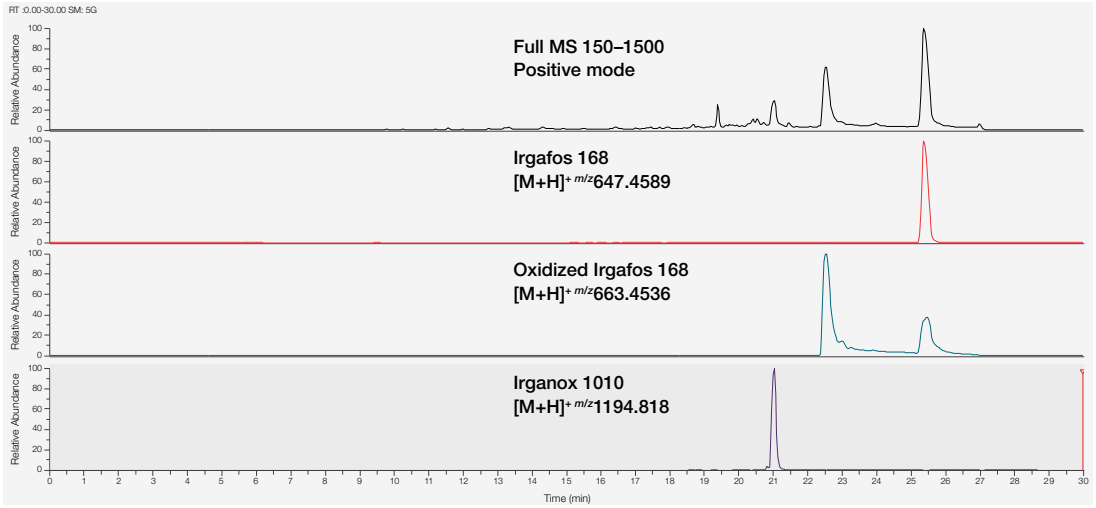


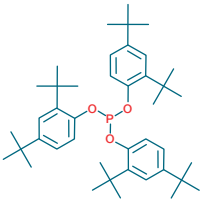
Figure 3. Total ion chromatogram (positive mode) of the inhaler hexane extract and extracted ion chromatograms of the three identified major compounds in the extract.

Table 3. Identifications

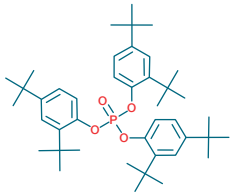
Name	Formula	Calc. MW	*m/z*	RT [min]	Reference ion
Irgafos 168	$C_{42}H_{63}O_3P$	646.4516	647.4589	25.36	$[M+H]^{+1}$
Tris[2,4-bis(2-methyl-2-propanyl)phenyl] phosphate (Oxidized Irgafos 168)	$C_{42}H_{63}O_4P$	662.4463	663.4536	22.48	$[M+H]^{+1}$
Irganox 1010	$C_{73}H_{108}O_{12}$	1176.7840	1194.8180	21.02	$[M+NH_4]^{+1}$

Structures

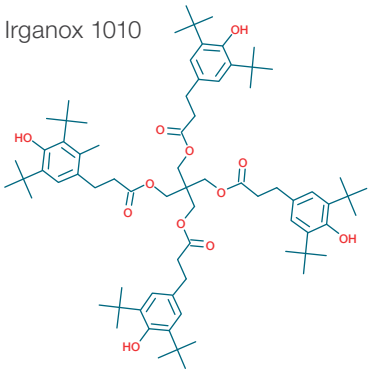
Irgafos 168



Oxidized Irgafos 168



Irganox 1010



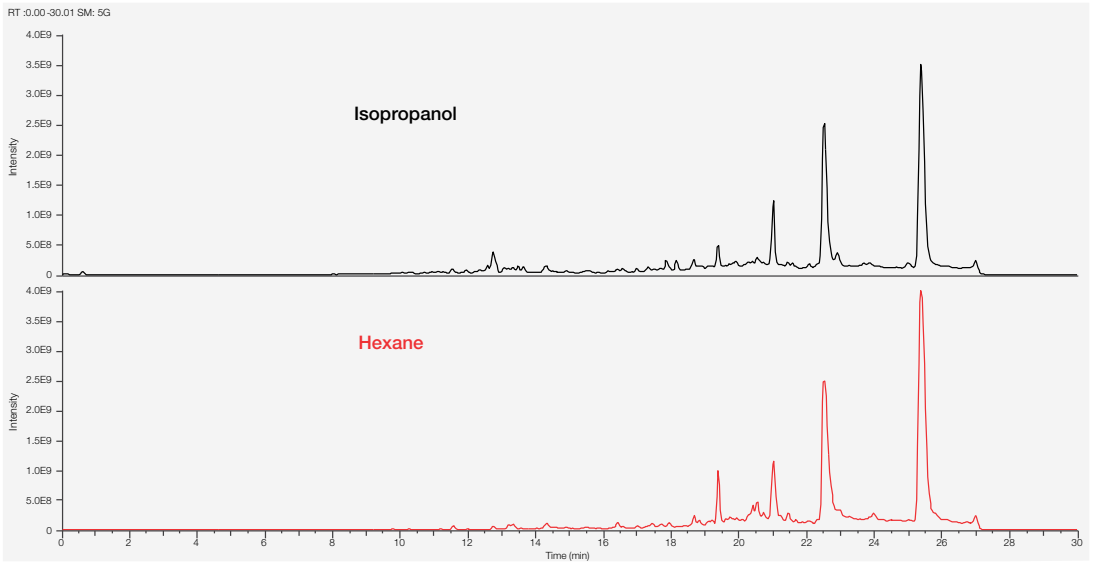


Figure 4. Total ion chromatogram of inhaler isopropanol and hexane extracts

The PQRI guideline recommends using a solvent of different polarity for inhaler E&L studies. Inhaler samples were extracted with IPA in four replicates using the four channels of the EXTREVA ASE system. Figure 4 shows the chromatogram of IPA and hexane extracts. The most abundant three identified peaks for the IPA extract are the same as the hexane extract, but present at a lower concentration as shown in Figure 5.

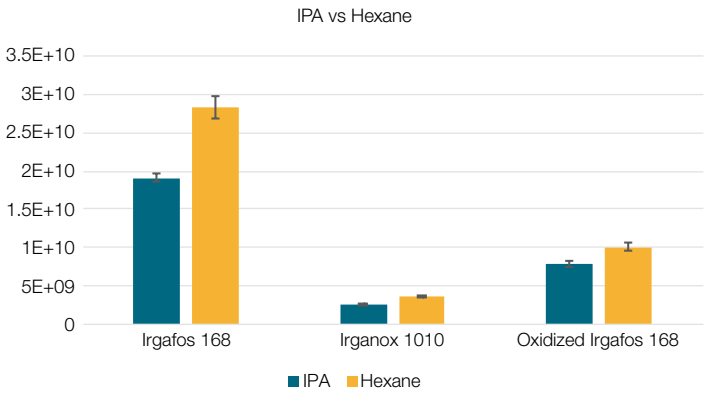


Figure 5. Extraction of three additives using IPA vs hexane

As higher temperatures and pressure are used in ASE, care must be taken to avoid the dissolution of the polymer. The ideal situation is one where the extraction is performed at a temperature that causes the maximum swelling without dissolving the polymer. IPA is among the solvents least likely to dissolve a polymer such as polypropylene. Hexane tends to dissolve the polymer at high temperatures used in ASE. Temperature is an important parameter to optimize because it increases extraction efficiency but too high a temperature can lead to polymer melting. Two extraction temperatures, 90 °C and 100 °C, were compared in this study for hexane extractions. Three additives were found in the sample. Hence, the optimization study was limited to these additives. 100 °C shows better extraction efficiency than 90 °C at 14%, 13%, 16%, respectively for Irgafos 168, Irganox 1010, and oxidized Irgafos 168 (Figure 6). Extraction time generally improves extraction. Two extraction times, 20 and 25 min, were compared. Twenty-five min shows higher extraction efficiency than 20 min at 6%, 12%, and 13%, respectively for Irgafos 168, Irganox 1010, and oxidized Irgafos 168 (Figure 7).

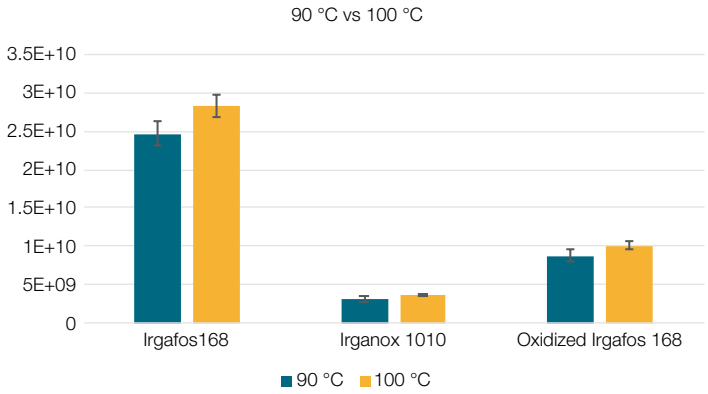


Figure 6. Extraction of three additives using two different extraction temperatures

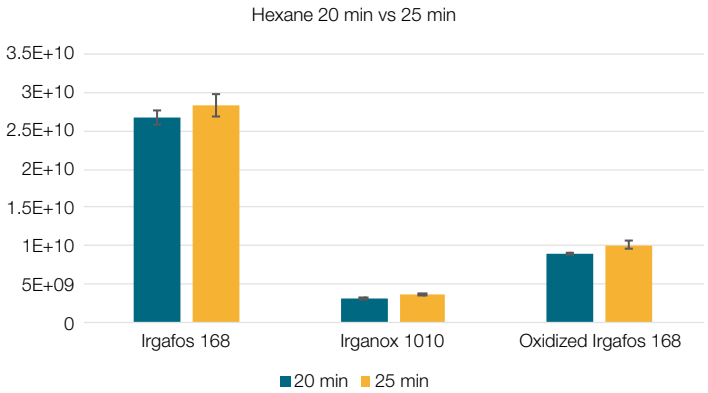


Figure 7. Extraction of three additives using hexane with two different extraction times

It was reported that high temperature used during the ASE extraction oxidized partially or completely the phosphite-group of these antioxidant additives. Traditional solid liquid extraction (SLE) for 10 h with hexane solvent was selected as the preferred method to preserve phosphite stability during extraction.[6] In this work, the Dionex ASE 350 system and the EXTREVA ASE system were compared with the 10 h SLE method. Irgofas 168 oxidation was observed using the Dionex ASE 350 system following the method described in AN1108, which agrees with the publication. However, the EXTREVA ASE system achieves equivalent extraction efficiency to the 10 h SLE method for the three additives as shown in Figures 8 and 9. This is possibly due to the special gas-assisted dynamic extraction mechanism of the EXTREVA ASE system. The nitrogen used for gas-assisted extraction may protect the Irgafos 168 from oxidation.

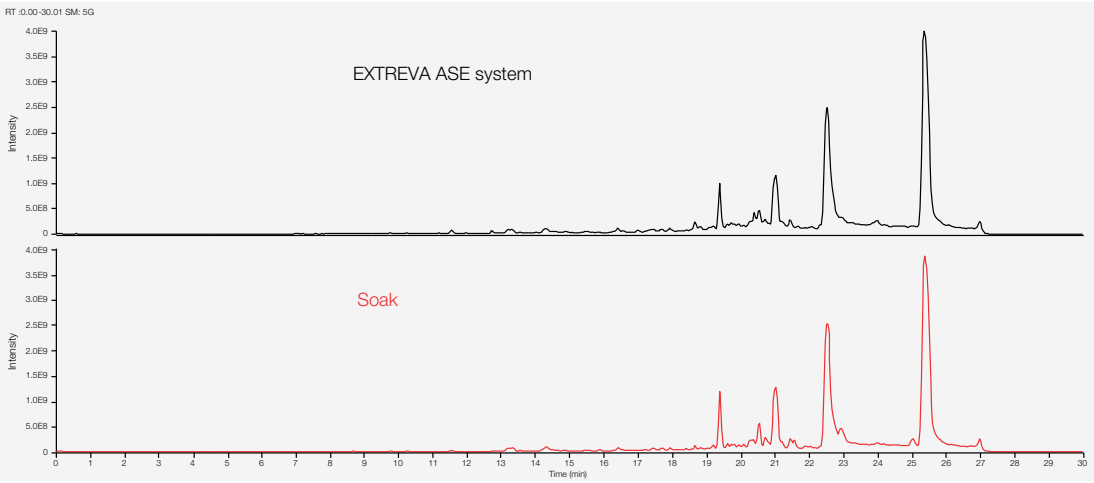


Figure 8. Chromatogram comparison of hexane extraction by the EXTREVA ASE system and 10 h SLE

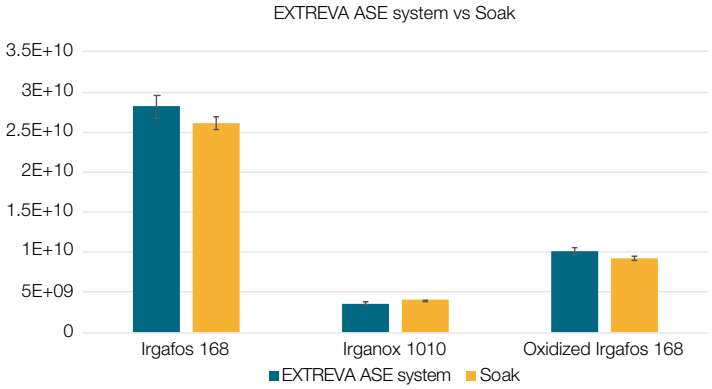


Figure 9. Comparison of hexane extraction by the EXTREVA ASE system and 10 h SLE

Conclusions

This application note demonstrates the successful use of the fully automated the EXTREVA ASE system for the extraction of plastic additives in packaging. Additives were confidently identified by liquid chromatography and Orbitrap-based high-resolution accurate mass (HRAM) mass spectral analysis. The EXTREVA ASE technique delivers comparable or more efficient extractions than the traditional 10 h static liquid extraction. The EXTREVA ASE system combines extraction and evaporation in one unit, which significantly reduces manual labor cost and increases sample throughput.

References

1. U.S. FDA, Code of Federal Regulations, 21CFR211.94 - http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.94
2. https://www.fda.gov/media/70788/download
3. https://pqri.org/wp-content/uploads/2015/08/pdf/LE_Recommendations_to_FDA_09-29-06.pdf
4. https://www.gmp-compliance.org/gmp-news/new-usp-general-chapters-on-extractables-and-leachables-1663-1664
5. D.L. Norwood, L.M. Nagao, C.L. Stults, Perspectives on the PQRI extractables and leachables "safety thresholds and best practices" recommendations for inhalation drug products, PDA J. Pharm. Sci. Technol. 67 (5) (2013) 413–429.
6. U.S.P. Convention, <1663>Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems, USP 40 General Chapter, Rockville, MD, United States, 2017, pp. 2020–2035.
7. C. Moreta, M.T. Tena, Determination of plastic additives in packaging by liquid chromatography coupled to high resolution mass spectrometry, J. Chromatogr. A 1414 (2015) 77–87.
8. Thermo Scientific Application Note 1108: Comparison of Soxhlet and Accelerated Solvent Extraction for Leachable and Extractable Analysis of Packing Material.
9. Kannan Srinivasan and Rahmat Ullah, Method and Device to Extract Analyte from a sample with Gas Assistance (U.S. Patent No. US 9,440,166 B2) Sep 13, 2016.
10. Kannan Srinivasan and Rahmat Ullah, Apparatus for parallel accelerated solvent extraction (U.S. Patent No. US 11,123,655 B2) Sep 21, 2021.
11. Hammond, M.; Nunn, H.; Rogers, G.; Lee, H.; Marghitoiu, A. L.; Perez, L.; Nashed-Samuel, Y.; Anderson, C.; Vandiver, M.; Kline, S. PDA J. Pharm. Sci. Technol. 2013, 67, 123-134.
12. Kelly, P. S.; McSweeney, S.; Coleman, O.; Carillo, S.; Henry, M.; Chandran, D.; Kellet, A.; Bones, J.; Clynes, M.; Meleady, P.; Barron, N. Biotechnology progress 2016, 32, 1547-1558.

Learn more at **thermofisher.com/extreva**