Comparison of Soxhlet and Accelerated Solvent Extraction for Packing Material Leachable and Extractable Studies Using LC-MS

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Overview

Purpose: To compare Soxhlet and accelerated solvent extraction (ASE) techniques for leachable and extractable analysis.

Methods: A transdermal patch pouch and a polypropylene pill bottle were extracted with 2-propanol and hexane using both the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system and Soxhlet. The samples were analyzed by an LC-MS system consisting of a Thermo Scientific™ UltiMate 3000 LC system and a Thermo Scientific™ Q Exactive™ Quadrupole-Orbitrap Mass Spectrometer.

Results: Compared to Soxhlet, the Dionex ASE 350 system produces equivalent or more efficient extraction results, but with less time (<0.5 vs. 24 h per sample) and reduced organic solvent use (<30 vs. 160 mL per sample), thereby saving money and time.

Introduction

According to the regulations and guidelines of the U.S. Food and Drug Administration (US FDA) [1], the European Medicines Agency (EMA), and other regulatory agencies, extractable and leachable information must be included in each application: New Drug Application (NDA), Abbreviated New Drug Application (ANDA) and Biologics License Application (BLA) for medical devices and container closure systems packaging human drugs and biologics.

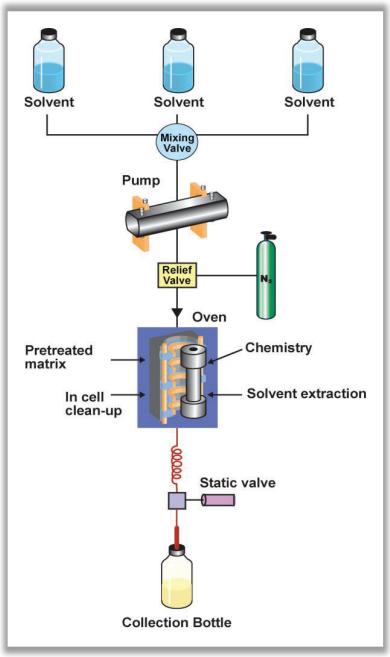
The main purposes of determining extractable are to obtain data for risk assessment and to provide a basis for leachable studies. Determining what extent compounds can be extracted establishes the maximum amount of material that could be leached. Therefore, sample extraction is an important first step for a successful extractable and leachable study.

Extractions traditionally have been carried out using the Soxhlet or reflux techniques[2] which require a lengthy extraction (>24 h/sample) and consume a large quantity of solvent (>150 mL/sample). Accelerated solvent extraction technique is an automated extraction technique which provides many advantages over traditional methods. One advantage of using a Dionex ASE 350 system is that up to 24 samples and three solvents can be processed unattended following a single setup. Figure 1 shows a schematic of the accelerated solvent extraction technique.

The accelerated solvent extraction technique employs pressure so that organic solvents can be used at temperatures above their boiling points to deliver extractions equivalent to or even better than traditional techniques, but at greatly reduced extraction time (<0.5 hr/sample) and solvent use (<30 mL/sample). ASE has been shown to be a powerful technique to reliably extract compounds from polymer materials.[3]

In this study, accelerated solvent extraction technique is compared to Soxhlet extraction for the analysis of extractable from packaging material.

FIGURE 1. Schematic of the Accelerated Solvent Extraction Technique



Methods

Sample Preparation

Two packaging material samples, an amber polypropylene pill bottle purchased from a local pharmacy and a transdermal patch pouch obtained from a pharmaceutical company, were extracted using the Dionex ASE 350 system (n = 3) and Soxhlet apparatus (n = 1) with 2-propanol and hexane. Prior to extraction, both samples were cut into ~ 2 mm^2 to increase surface area of the sample and therefore the extraction efficiency.

Accelerated solvent extraction: Pack ~ 0.5 g of sample into each Thermo Scientific Dionex ASE extraction cell to carry out extraction. Figure 2 illustrates an Dionex ASE extraction cell filled with sample. To prepare an Dionex ASE extraction cell, place a new glass filter in each extraction cell cap, weigh sample into Dionex ASE cell, fill void volume with clean Ottawa sand (Fisher Scientific Cat # S23-3) and hand tighten the cell cap. Figure 3 shows an Dionex ASE 350 system, in which filled Dionex ASE extraction cells were placed into the upper carousel and the collection vials in the lower. Accelerated solvent extraction conditions are listed in Table 1. For the best precision, weigh each collection vial without the cap and septum before and after extraction, divide the sample weight by solvent density to determine the volume.

FIGURE 2. Filled Dionex ASE Extraction Cell

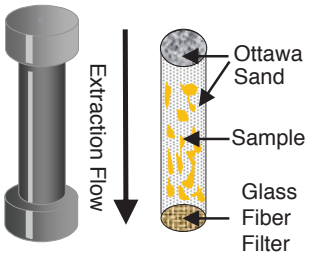


FIGURE 3 Dionex ASE 350 System

TABLE 1. Accelerated Solvent Extraction Conditions

	2-propanol Method	Hexane Method
Extraction solvent	2-propanol	Hexane
Extraction cell size (mL)	10	10
Temperature (°C)	125	100
Static extraction time (min)	5	10
Number of static cycles	3	2
Rinsing volume (%)	100	100
Purge time (Sec)	120	120
Total solvent volume per sample (mL):	~28	~25
Total extraction time per sample (min):	20	25

Soxhlet: Add 160 mL solvent to flask. Set up the Soxhlet apparatus with cycle time about 20 min. Weigh and record sample weight (0.5 to 1.0 g) into glass fiber thimble; start extraction and extract for 24 hours. Determine the final volume of the extract solution by weight after extraction is completed.

After extraction, transfer 1 mL of the extract into an HPLC vial for HPLC/MS analysis.

Liquid Chromatography

Column: Thermo Scientific™ Dionex™ Accucore C18 column, 2.1 × 100mm, 2.6 μm
Mobile phase: A: Water /0.1% Formic Acid; B: Acetonitrile/ 0.1% Formic Acid
Gradient : 5% B(0 – 3 min), 5% to 95% B (3 – 21 min), 5% B (21.1 – 25 min)
Flow Rate: 0.4 mL/min
Inj. Volume: 10 μL
Column Temp.:30°C

Detection: Q Exactive mass spectrometer was operated in positive ionization mode with a HESI-II probe (Sheath Gas: 45, Aux Gas: 10, Porbe heater temp.400 °C). Data was acquired at full scan (m/z 150-2000, 70,000 resolution) followed by data dependent MS/MS at 15,000 resolution).

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS). software and Thermo Scientific™ Xcalibur™ software

Results and Discussion

Prior to running sample extractions, Dionex ASE 350 (with sand filled cells) and Soxhlet system blanks were collected to verify that there was no contamination from the systems. Figure 4 shows the chromatograms of 2-propanol blanks. The chromatograms of HPLC , 2-propanol, and blank ASE and Soxhlet extracts are identical, with three major peaks between 15 to 18 min. The blanks demonstrate that both Dionex ASE 350 and Soxhlet systems were clean prior to sample extractions.

FIGURE 4. Chromatograms of 2-propanol blanks

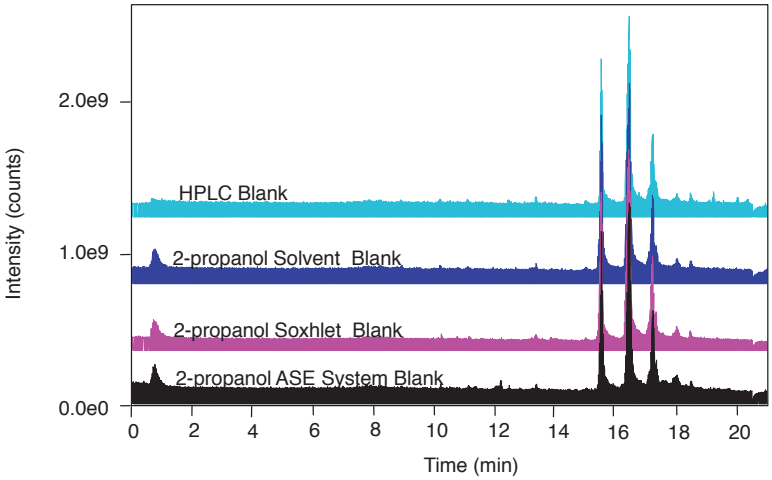
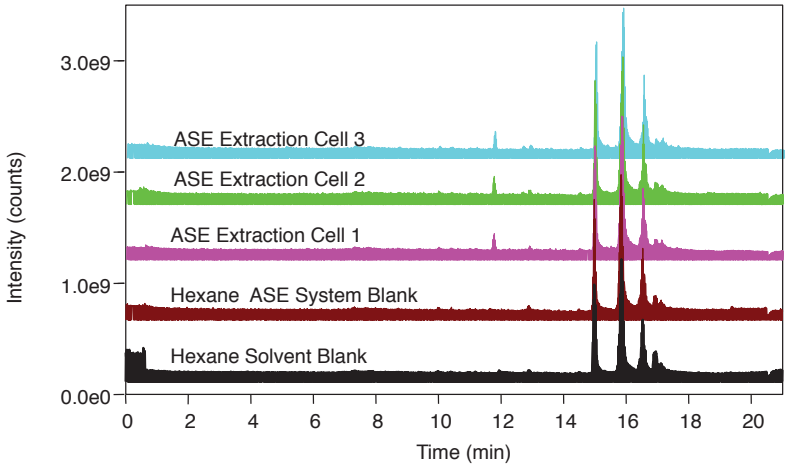


Figure 5 shows hexane blanks and ASE extractions of a transdermal patch pouch. The chromatograms of hexane solvent blank and ASE system blank again confirm the cleanness of the Dionex ASE 350 system. The chromatograms of extract solution from three independent Dionex ASE cells are almost identical, which shows the consistency of the accelerated solvent extraction technique.

FIGURE 5. Hexane Blanks and ASE Extractions of Transdermal Patch Pouch



As the extracts from three Dionex ASE extraction cells have similar results, only one is included in the Figure 6 to Figure 8.

Figure 6 is results of 2-propanol extraction of transdermal patch pouch. Two compounds were extracted with retention time of 10.8 and 13.1 minutes.

FIGURE 6. 2-propanol Extractions of a Transdermal Patch Pouch

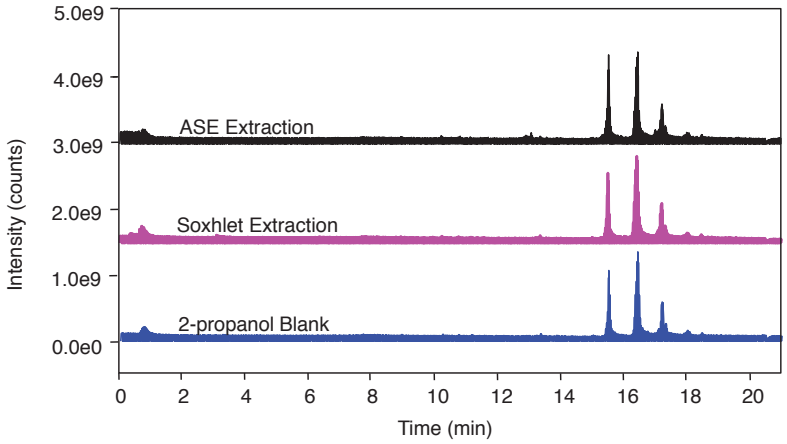


Figure 7 compares the chromatograms of 2-propanol extractions of an amber polypropylene pill bottle. Compared to blank, the extracts from both accelerated solvent extraction and Soxhlet show additional peaks indicating there are extractable compounds from the pill bottle. Despite the presence of the condenser in the Soxhlet extraction, significant solvent was evaporated after the 24 h Soxhlet extraction. Based on the sample weight and volume, the extraction solution from the accelerated solvent extraction technique should be 4x concentrated of the Soxhlet solution. However, it was shown the extraction solution from the accelerated solvent extraction technique had a significantly higher number of peaks at higher responses (>4x) than the Soxhlet solution. In conclusion, compared to Soxhlet extraction at the 2-propanol boiling point of 82.5°C, accelerated solvent extraction at 125°C had delivered more efficient extraction results in a shorter time (~0.5 vs. 24 h/sample) using less organic solvent (20 vs. 160 mL/sample).

FIGURE 7. 2-propanol Extractions of an Amber Polypropylene Pill Bottle

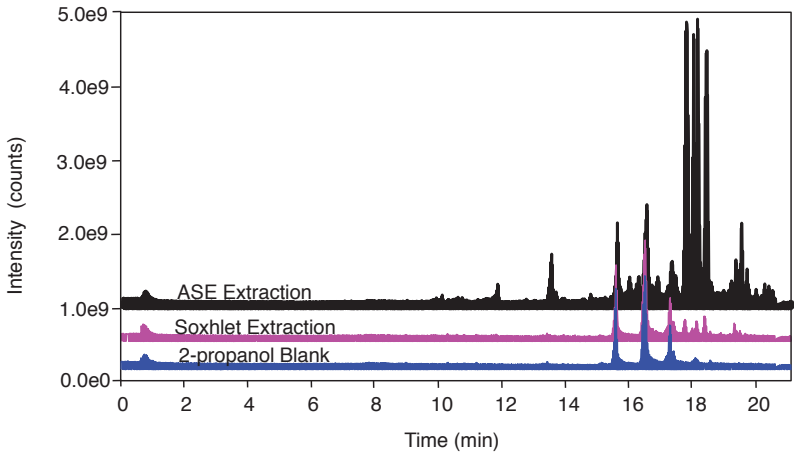
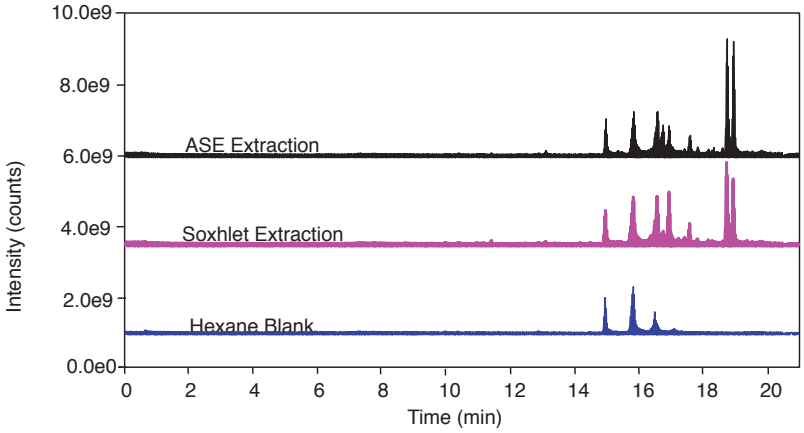


Figure 8 shows the chromatograms of hexane extractions of an amber polypropylene pill bottle. Here the accelerated solvent extraction technique and Soxhlet extractions should be similar (1.1x) based on the similar sample weights and extract solution volumes. Again the accelerated solvent extraction technique, which extracted at higher temperature, had more efficient extraction results with less time (25 min vs.24 h/sample) and less organic solvent (25 vs.160 mL/sample).

These results also demonstrate the importance of using different solvents for extractable studies. As seen in this pill bottle example, different solvents can result in different compounds extracted (Figures 7 and 8).

FIGURE 8. Hexane Extractions of an Amber Polypropylene Pill Bottle



Note: The data was acquired at full scan followed by data dependent MS/MS at 15,000 resolution using Q Exactive mass spectrometer. Better than 1ppm mass accuracy help identify the unknown extractable compounds. See related poster "High Resolution Mass Spectrometer and mzCloud Database Search for Extractable and Leachable Analysis" for more detail.

Conclusion

- Accelerated solvent extraction technique was demonstrated on a transdermal patch pouch and a polypropylene pill bottle using the automated Dionex ASE 350.
- Accelerated solvent extraction technique provides automated and more efficient extractions than the traditional Soxhlet extraction method and require less time and less solvent.
- Using different solvents can provide useful information on extractable.

References

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2. PQRI, Leachables and Extractables Working Group, *Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products*, 2006. [Online}] http://www.pqri.org/pdfs/LE_Recommendations_to_FDA_09-29-06.pdf (accessed Apr 17, 2014).
3. Dionex , now part of Thermo Scientific, Application Note 331: *Accelerated Solvent Extraction (ASE) of Additives from Polymer Materials*. Sunnyvale, CA. [Online] http://www.dionex.com/en-us/webdocs/4467-AN331-ASE-Additives-Polymers-14Apr2011-LPN0987-04.pdf (accessed Apr 17, 2014).

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