Application Note 1108

# Comparison of Soxhlet and Accelerated Solvent Extraction for Leachable and Extractable Analysis of Packing Material

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**Key Words**
Pharmaceutical, Biopharma, U.S. FDA, Sample preparation, Packaging, Automated Extraction, HPLC, Pressurized fluid extraction

## Introduction

According to the regulations and guidelines set by regulatory agencies, including the U.S. Food and Drug Administration (US FDA),[1] the European Medicines Agency (EMA),[2] extractable and leachable information must be included in applications for medical devices and container closure systems packaging for human drugs and biologics. The specific applications include the New Drug Application (NDA), Abbreviated New Drug Application (ANDA) and Biologics License Application (BLA).

Leachables are compounds that can migrate from components of medical devices or container closure systems into the drug product.[3] They present safety concerns and may influence the effectiveness of a drug product. Extractables are compounds that can be extracted from components and are determined through Controlled Extraction Studies.[3] The main purpose of determining extractables is to obtain risk assessment data and to provide a basis for leachable studies.[3] In other words, determining to 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.

The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group have recommended incorporation of multiple extraction techniques that employ vigorous extractions using multiple solvents of varying polarity in Controlled Extraction Studies.[3] However, traditional Soxhlet or reflux techniques recommended by PQRI are labor intensive (>24 h/sample) and consume a large quantities of solvent (>150 mL/sample).

The accelerated solvent extraction technique is an automated technique with several advantages, including efficient extraction, reduced extraction time (<0.5 h/sample), reduced solvent use (<30 mL/sample), and flexibility in solvent selection. Using the method and sequence editor in the Dionex ASE 350 system, three solvents and their mixture can be used as extraction solvent in different methods in a single sequence run. The accelerated solvent extraction technique is an efficient technique to reliably extract compounds from polymeric materials.[4]

This application note compares Soxhlet and accelerated solvent extraction techniques in packing material extractable studies. A polypropylene pill bottle and a transdermal patch pouch were extracted with 2-propanol and n-hexane using the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system and Soxhlet, respectively. The extracts were analyzed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass spectrometer.

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## Equipment

### Sample Preparation Equipment

- Dionex ASE 350 system, 120 V (P/N 083114) or 240 V (P/N 083146)
- Dionex ASE Stainless Steel Extraction Cells, 10 mL (P/N 060070)
- Dionex ASE Sample Collection Vials, Clear, 60 mL (P/N 048784)
- Glass Fiber Filters, 27 mm, Type D28 (P/N 068092)
- Soxhlet apparatus with 250 mL flask
- Glass Fiber Thimble (P/N 088346)

### Analysis Equipment

- UltiMate 3000 LC system
- Thermo Scientific™ Accucore™ C18 column, 2.1 × 100 mm, 2.6 µm particle size (Cat. No. 17126-102130)
- Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer

## Solvents and Dispersants

2-propanol and n-hexane were selected as extraction solvents because they represent polar and non-polar solvents and are recommended by the Leachables and Extractables Working Group for Controlled Extraction Studies.[3] Ottawa sand was used as it is a naturally rounded grain of nearly pure quartz, produced by processing silica sand. It is important to use this as a dispersant since it is clean, with no extractable contaminants.

- N-hexane, HPLC grade (Fisher Scientific, P/N UN1208)
- 2-propanol, HPLC grade (Fisher Scientific, P/N UN1219)
- Ottawa Sand (Fisher Scientific, Cat. No. S23-3)

## Samples

Two packing material samples, an amber polypropylene (PP) pill bottle and a transdermal patch pouch, were selected for this study as they are the packages for the two most common dosage forms (oral drugs and transdermal patches).

Amber polypropylene (PP) pill bottles were purchased from a local pharmacy store.

Transdermal patch pouches were obtained from a pharmaceutical company.

## Sample Preparation and Extraction

The two packaging material samples were cut into ~2 mm$^2$ pieces to increase sample surface area and extraction efficiency. The samples were extracted with each solvent using the Dionex ASE 350 system (n = 3) and a Soxhlet apparatus (n = 1 due to the limited amount of samples and length of Soxhlet extraction).

### Accelerated Solvent Extraction

To prepare a Dionex ASE extraction cell, place a new glass fiber filter in an extraction cell cap. Weigh 0.5–1 g of ~2 mm$^2$ sample pieces into the thimble in a Dionex ASE cell, fill void volume with clean Ottawa sand, and tighten the cell cap to finger tight, as illustrated in Figure 1. Place the filled Dionex ASE extraction cells into the upper carousel and the Dionex ASE collection vials in the lower (Figure 2). Extract the Dionex ASE extraction cells with 2-propanol or n-hexane according to the conditions in Table 1. With help of sequence editor, multiple methods with different extraction conditions are accomplished in one run. Determine the extract solution volumes gravimetrically (Volume = Weight/Density). For the best precision, weigh each collection vial without the cap and septum before and after extraction.

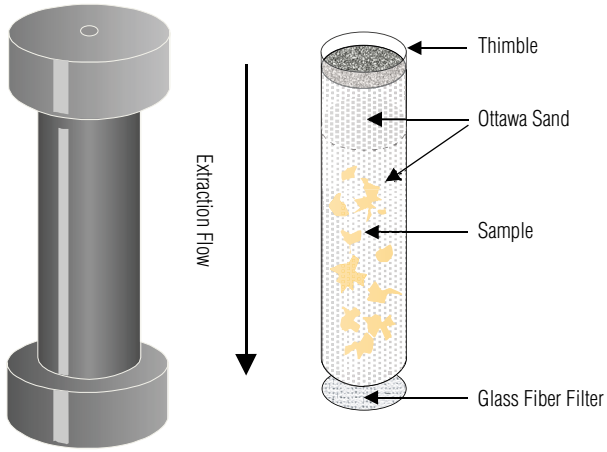


Figure 1. Illustration of an ASE extraction cell filled with sample.

Table 1. Accelerated solvent extraction conditions.

| | 2-Propanol Method | n-Hexane Method |
|---|---|---|
| Extraction solvent | 2-Propanol | n-hexane |
| Extraction cell size (mL) | 10 | 10 |
| Temperature (°C) | 125 | 100 |
| Pressure (psi**) | 1500 | 1500 |
| 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) | 28-29* | 26-27* |
| Total extraction time per sample (min) | 20 | 25 |

* This is the extract volume range of the method. The extract volume is different from cell to cell, and actual volume is determined gravimetrically.

** Pressure (psi) is 10 MPa/1500 psi. 1500 psi is equal to 10 MPa.

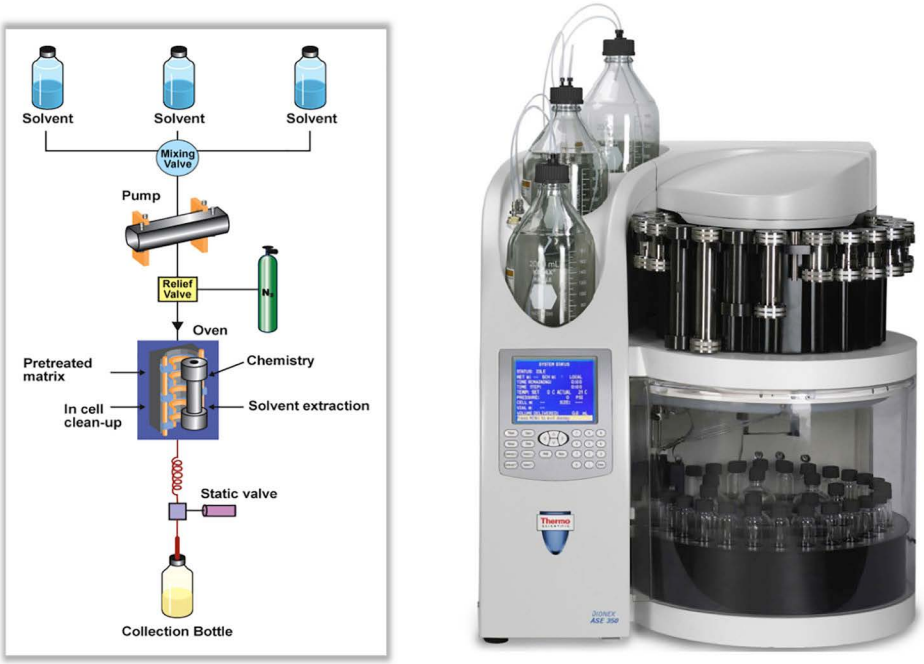


Figure 2. Schematic of the accelerated solvent extraction technique and a Dionex ASE 350 system.

### Soxhlet Extraction

Add 160 mL solvent into the flask; Set up the Soxhlet apparatus with a cycle time about 20 min. Weigh 0.5 to 2.0 g sample into the glass fiber thimble. Extract for 24 h. Determine the final volume of extract solution gravimetrically (volume=weight/density) after extraction.

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

**Conditions**

| Liquid Chromatography (HPLC) | |
|---|---|
| Column: | Accucore C18 column, 2.1 × 100 mm, 2.6 µm particle size |
| Mobile phase: | A: Water/0.1% Formic Acid<br>B: Acetonitrile ($CH_3CN$)/0.1% Formic Acid |
| Gradient: | 5% B (0–3 min),<br>5 to 95% B (3–21 min),<br>5% B (21.1–25 min) |
| Flow Rate: | 0.4 mL/min |
| Injection Volume: | 10 µL |

## Mass Spectrometry

The MS analyses were carried out using a Q Exactive mass spectrometer equipped with Thermo Scientific™ Heated Electrospray Ionization (HESI-II) Probe in the Electrospray Ionization (ESI) positive ion mode. High-resolution accurate mass (HRAM) full-scan MS and top 3 MS/MS spectra were collected in a data-dependent fashion at a resolving power of 70,000 and 17,500 at the Full Width of the peak at Half its Maximum height (FWHM) of *m/z* 200, respectively. The stepped NCE (Normalized Collision Energy) setting was 30, 40, 50.

## Data Analysis

The LC-MS data were collected and analyzed by Thermo Scientific™ Xcalibur™ software. The High Resolution Accurate Mass (HRAM) data were processed using differential analysis software Thermo Scientific™ SIEVE™ 2.1 for component extraction. ChemSpider database searching was carried out to obtain possible structures of extracted components. Thermo Scientific™ Mass Frontier™ 7.0 spectral interpretation software was used for structure elucidation.

## Results and Discussion

To verify no contamination from either extraction system, the Dionex ASE 350 system (with Ottawa sand filled cells) and Soxhlet system were extracted and analyzed by the same extraction and HPLC-MS methods prior to packing material samples extraction. Figure 3 shows the HPLC-MS chromatograms of HPLC mobile phase HPLC blank, extraction solvent (2-propanol) and the blank system extraction solutions. The results show identical chromatograms to the system blank, which demonstrates that both the Dionex ASE 350 and Soxhlet systems were clean prior to sample extractions.

### Extractables from the Pouch of a Transdermal Patch

To evaluate the extractables of the pouch used to contain the transdermal patch, the samples were extracted in triplicate in three separate cells by n-hexane and 2-propanol and compared against the solvent blank, the Dionex ASE system blank and the Soxlet extraction.

In Figure 4 the chromatograms of n-hexane extraction solutions from three independent Dionex ASE cells show identical extractable profiles with two analyte peaks, which indicate the consistency of the accelerated solvent extraction technique. The n-hexane Dionex ASE system blank chromatogram is again identical to the solvent (n-hexane), confirming acceptable ASE system blank with n-hexane.

As the extracts from three Dionex ASE extraction cells gave identical extractable profiles, representative results from one Dionex ASE cell were selected to compare with Soxhlet in the following figures and tables.

The same extractions were performed with 2-propanol and found to have similar results as using n-hexane. The predominant peaks are attributable to the solvent blank, confirming that the ASE extraction blanks are acceptable. Two unique peaks attributable to the transdermal patch pouch, retention times of 10.8 and 13.1 min, were found (Figure 5).

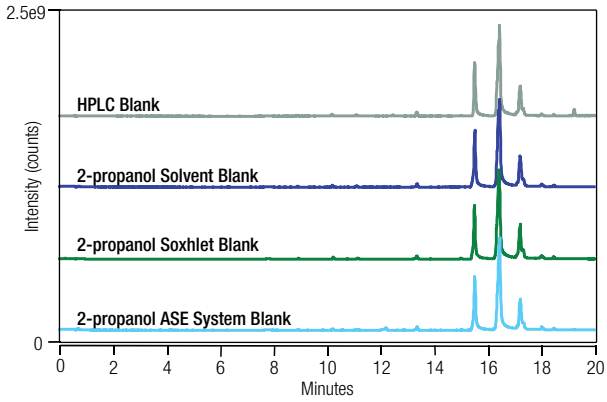


Figure 3. Chromatograms of 2-propanol and system blanks.

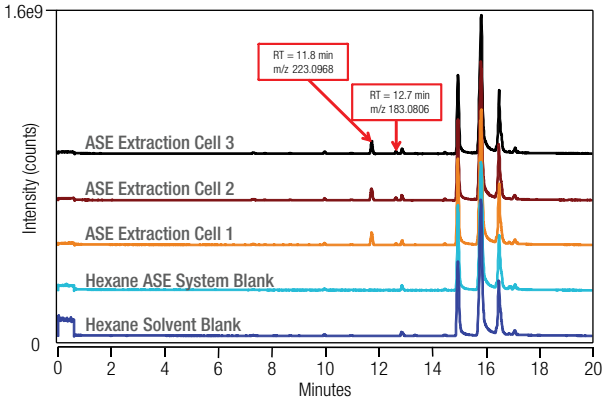


Figure 4. n-Hexane blanks and accelerated solvent extractions of transdermal patch pouch.

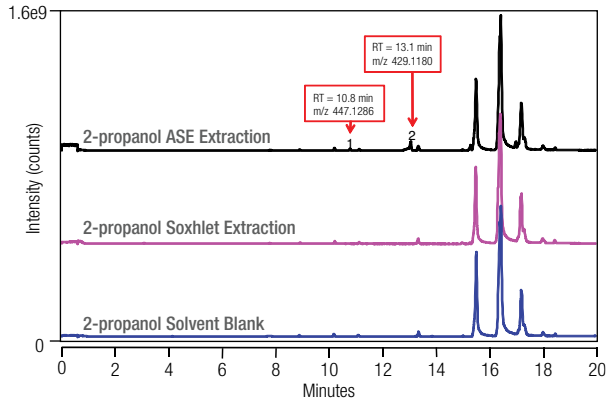


Figure 5. Extractable profile of a transdermal patch pouch using 2-propanol.

Table 2. Extraction conditions of a transdermal patch pouch using 2-propanol.

| Extraction Technique | Time (min) | Temp. (°C) | Solvent Used (mL) | Extract (mL) | Sample (g) | Intensity ratio Accelerated Solvent Extraction/Soxhlet ** |
|---|---|---|---|---|---|---|
| Soxhlet | 1440 | ~83* | 160 | 111 | 2.00 | 1.9 |
| Accelerated Solvent Extraction | 20 | 125.0 | 29.3 | 29.3 | 1.00 | 1.9 |

*Boiling point of 2-propanol

**Intensity ratio = $(\text{Sample Weight/Extract Volume})_{ASE}/(\text{Sample Weight/Extract Volume})_{Soxhlet}$

Table 3. Extractables in 2-propanol extracts of a transdermal patch pouch.

| Peak | Retention (min) | m/z | Soxhlet (*e7 counts) | Accelerated Solvent Extraction (*e7 counts) | Intensity ratio Accelerated Solvent Extraction/Soxhlet |
|---|---|---|---|---|---|
| 1 | 10.8 | 447.1286 | 0.26 | 1.38 | 5.2 |
| 2 | 13.1 | 429.1176 | 0.05 | 4.53 | 90.6 |

## Calculated Results

To compare the accelerated solvent extraction technique results directly with the Soxhlet results, the accelerated solvent extraction technique results must be corrected by factor of 1.9 for the differences in sample weight and volume used in Soxhlet extractions (Table 2).

The results revealed that the accelerated solvent extraction solutions were more concentrated than the Soxhlet solution (Table 3), e.g., 5.2× intensity of peak 1 and 90.6× of peak 2. Compared to Soxhlet extraction ~83 °C, at the boiling point of the extract solvent, accelerated solvent extractions at 125 °C delivered a more efficient extraction with less time (20 min vs. 24 h/sample) using less organic solvent (29.3 vs. 160 mL/sample). Accelerated solvent extraction technique employs pressure so organic solvents can be used at temperatures above their boiling points to deliver faster and more efficient extractions.

### Extractables from an Amber Polypropylene Pill Bottle

Extractions were also conducted on samples of an amber polypropylene pill bottle using 2-propanol and n-hexane. The results showed much higher extractables compare to the pouch of transdermal patch. Eighteen extractable compounds were found using 2-propanol and fifteen extractable compounds were found using n-hexane with both extract techniques. (Figures 6 and 7).

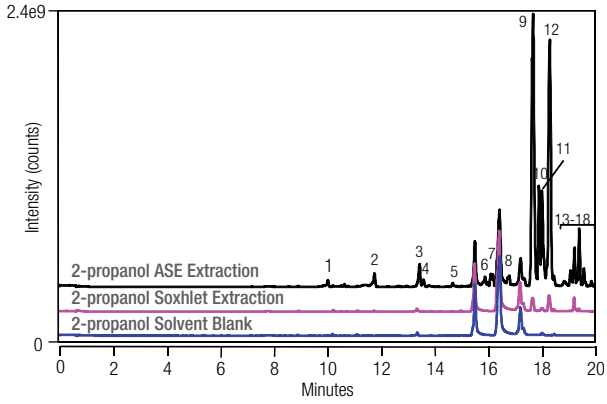


Figure 6. 2-propanol extractions of an amber polypropylene pill bottle.

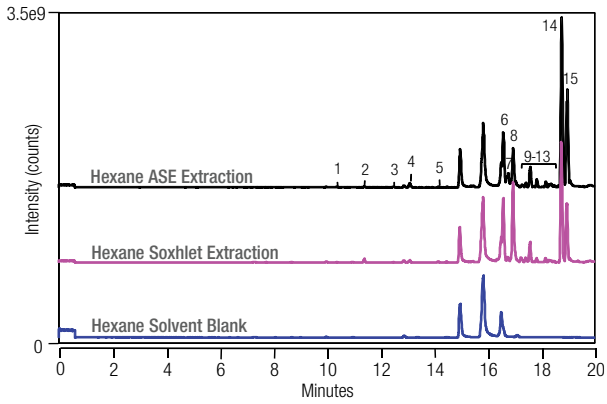


Figure 7. n-Hexane extractions of an amber polypropylene pill bottle.

Table 4. Conditions of 2-propanol extractions of an amber polypropylene pill bottle.

| Extraction Technique | Time (min) | Temp. (°C) | Solvent (mL) | Extract (mL) | Sample (g) | Intensity Ratio ASE/Soxhlet ** |
|---|---|---|---|---|---|---|
| Soxhlet | 1440 | ~83* | 160.0 | 122.0 | 0.70 | 4.1 |
| Accelerated Solvent Extraction | 20 | 125.0 | 27.9 | 27.9 | 0.65 | 4.1 |

*Boiling point of 2-propanol
**Intensity ratio = $(\text{Sample Weight/Extract Volume})_{ASE}/(\text{Sample Weight/Extract Volume})_{Soxhlet}$

Table 5. Extractables in 2-propanol extracts of an amber polypropylene pill bottle.

| Peak | Retention (min) | m/z | Soxhlet (*e7 counts) | Accelerated Solvent Extraction (*e7 counts) | Intensity Ratio Accelerated Solvent Extraction/Soxhlet |
|---|---|---|---|---|---|
| 1 | 10.0 | 257.2223 | 0.29 | 6.00 | 21.1 |
| 2 | 11.8 | 240.1959 | 1.06 | 11.50 | 10.8 |
| 3 | 13.5 | 415.2110 | 2.80 | 19.00 | 6.8 |
| 4 | 13.6 | 195.1016 | 0.54 | 6.05 | 11.3 |
| 5 | 14.7 | 200.2007 | 0.60 | 3.46 | 5.8 |
| 6 | 15.9 | 372.3459 | 1.04 | 8.46 | 8.1 |
| 7 | 16.1 | 308.2948 | 1.13 | 11.10 | 9.8 |
| 8 | 16.8 | 324.2892 | 2.80 | 9.49 | 3.4 |
| 9 | 17.7 | 336.3267 | 11.40 | 226.00 | 19.8 |
| 10 | 17.9 | 352.3201 | 1.17 | 83.60 | 71.5 |
| 11 | 18.0 | 352.3202 | 3.06 | 79.80 | 26.1 |
| 12 | 18.3 | 352.3206 | 13.30 | 204.00 | 15.3 |
| 13 | 18.9 | 352.3210 | 0.60 | 5.10 | 8.5 |
| 14 | 19.1 | 256.2634 | 1.07 | 14.00 | 13.1 |
| 15 | 19.2 | 282.2792 | 12.00 | 32.60 | 2.7 |
| 16 | 19.4 | 354.3369 | 2.16 | 48.40 | 22.4 |
| 17 | 19.6 | 308.2947 | 0.65 | 15.60 | 24.0 |
| 18 | 20.1 | 334.3101 | 0.61 | 15.30 | 25.1 |

The accelerated solvent extraction and Soxhlet conditions are compared inTable 4.

Based on the masses used and final extraction volumes, the accelerated solvent extraction solutions are expected to be 4.1× more concentrated than that of the Soxhlet extraction (Table 4). The results (Table 5) show the intensity ratio of different extractable compounds ranged from 2.7 to 71.5, which indicates comparable or higher concentration in the accelerated solvent extraction solution.

The results of hexane extractions of an amber polypropylene pill bottle are shown in Figure 7 and Tables 6 and 7.

The conditions of the n-hexane extractions are described in Table 6. However, significant solvent loss was observed in the Soxhlet extraction (160 mL to 69.4 mL) despite the presence of the condenser in the Soxhlet extraction. This solvent loss may impact the efficiency of the extraction.

Table 6. Conditions of n-hexane extractions of an amber polypropylene pill bottle.

| Extraction Technique | Time (min) | Temp. (°C) | Solvent (mL) | Extract (mL) | Sample (g) | Intensity ratio Accelerated Solvent Extraction /Soxhlet ** |
|---|---|---|---|---|---|---|
| Soxhlet | 1440 | ~68* | 160.0 | 69.4 | 1.20 | 1.1 |
| Accelerated Solvent Extraction | 25 | 100.0 | 26.3 | 26.3 | 0.50 | 1.1 |

*Boiling point of 2-propanol
**Intensity ratio = $(\text{Sample Weight/Extract Volume})_{ASE}/(\text{Sample Weight/Extract Volume})_{Soxhlet}$

Table 7. Extractables in n-hexane extracts of an amber polypropylene pill bottle.

| Peak | Retention (min) | m/z | Soxhlet (*e7 counts) | Accelerated Solvent Extraction (*e7 counts) | Intensity ratio Accelerated Solvent Extraction/Soxhlet |
|---|---|---|---|---|---|
| 1 | 10.4 | 163.1120 | 0.64 | 1.33 | 2.1 |
| 2 | 11.42 | 240.1958 | 4.40 | 1.91 | 0.4 |
| 3 | 12.51 | 191.1431 | 0.68 | 1.31 | 1.9 |
| 4 | 13.11 | 195.1016 | 2.76 | 4.98 | 1.8 |
| 5 | 14.18 | 200.2007 | 1.46 | 1.14 | 0.8 |
| 6 | 16.6 | 336.3264 | 68.60 | 58.50 | 0.9 |
| 7 | 16.79 | 352.3206 | 6.18 | 15.50 | 2.5 |
| 8 | 16.96 | 352.3208 | 85.20 | 41.90 | 0.5 |
| 9 | 17.26 | 352.3210 | 5.59 | 3.15 | 0.6 |
| 10 | 17.44 | 256.2634 | 5.27 | 5.26 | 1.0 |
| 11 | 17.61 | 282.2793 | 21.90 | 22.40 | 1.0 |
| 12 | 17.85 | 308.2947 | 5.12 | 8.90 | 1.7 |
| 13 | 18.18 | 334.3100 | 4.85 | 6.50 | 1.3 |
| 14 | 18.77 | 310.3106 | 128.00 | 180.00 | 1.4 |
| 15 | 18.99 | 327.1957 | 62.6 | 104.00 | 1.7 |

N-hexane extractions of an amber polypropylene pill bottle found 15 extractable compounds (Figure 7)

with similar (0.4-2.5×) concentrations in both extracts from the two extraction techniques (Table 7). Here the accelerated solvent extraction technique and Soxhlet extractions should be similar (1.1×) based on the sample weights and extract solution volumes (Table 6). Again, the accelerated solvent extraction technique, which extracted at higher temperature (100 vs. 68 °C), delivered comparable or slightly better extraction results with less time (25 min vs. 24 h/sample) and less organic solvent (25 mL vs. 160 mL/sample).

These results also demonstrate the importance of using different solvents for extractable studies. The pill bottle example shows that different solvents can result in different compounds extracted (Figures 6 and 7). These results also demonstrate the benefits of using the accelerated solvent extraction technique. The automated Dionex ASE 350 system provides a more convenient method accomplished in a single sequence while meeting Controlled Extraction Studies requirements to use different solvents at different temperatures.

## Identification of Extractable

In addition to extractable profiles of base peak chromatograms, the Q Exactive mass spectrometer also produces high quality high resolution accurate mass (HRAM) data, which can be used for molecular weight determination and structure elucidation. This HRAM data was obtained using full scan (m/z 150–2000, 70,000 resolution) followed by data dependent MS/MS at 15,000 resolution.

To demonstrate the application of HRAM, structure elucidation of the 2-propanol extractables from the transdermal patch pouch are shown here. One of the extractable compounds, eluted at 13.05 min had a base mass (m/z) of 429.1180. The data was processed with SIEVE™ 2.1 for component extraction and the extracted compound structure was identified via ChemSpider database search as $C_{22}H_{20}O_9$ (Figure 8). To further confirm the proposed structure, MS/MS data were used in conjunction with Mass Frontier™ 7.0 (Figure 9). Similarly, the peak at 10.08 min was identified to be $C_{22}H_{22}O_{10}$ (Figure 10). These two compounds are related with one water molecule difference.

There are many extractables from the amber polypropylene pill bottle, these compounds can be identified use same methodology but not shown in this application note.

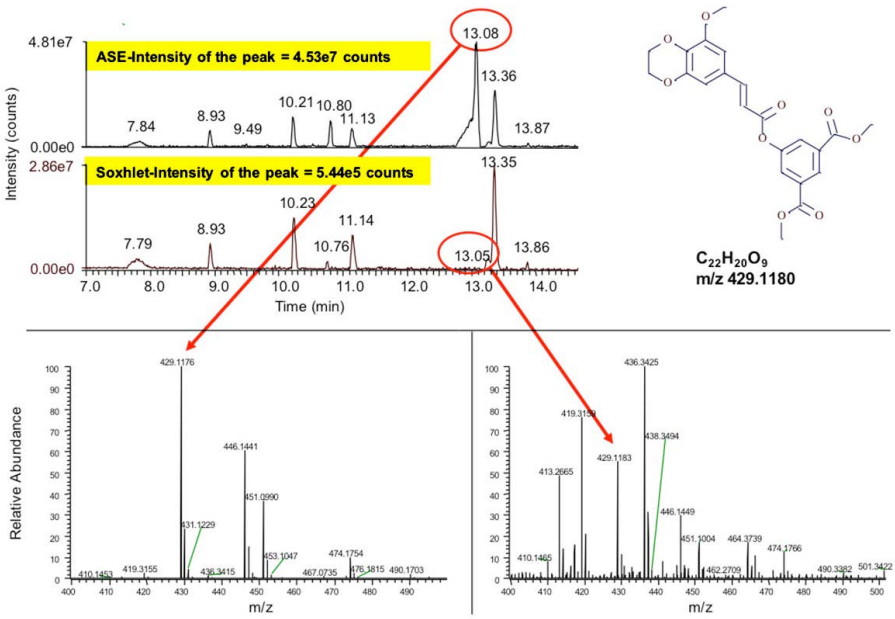


Figure 8. Identification of the peak at 13.05 min extracted from the transdermal patch pouch using 2-propanol.

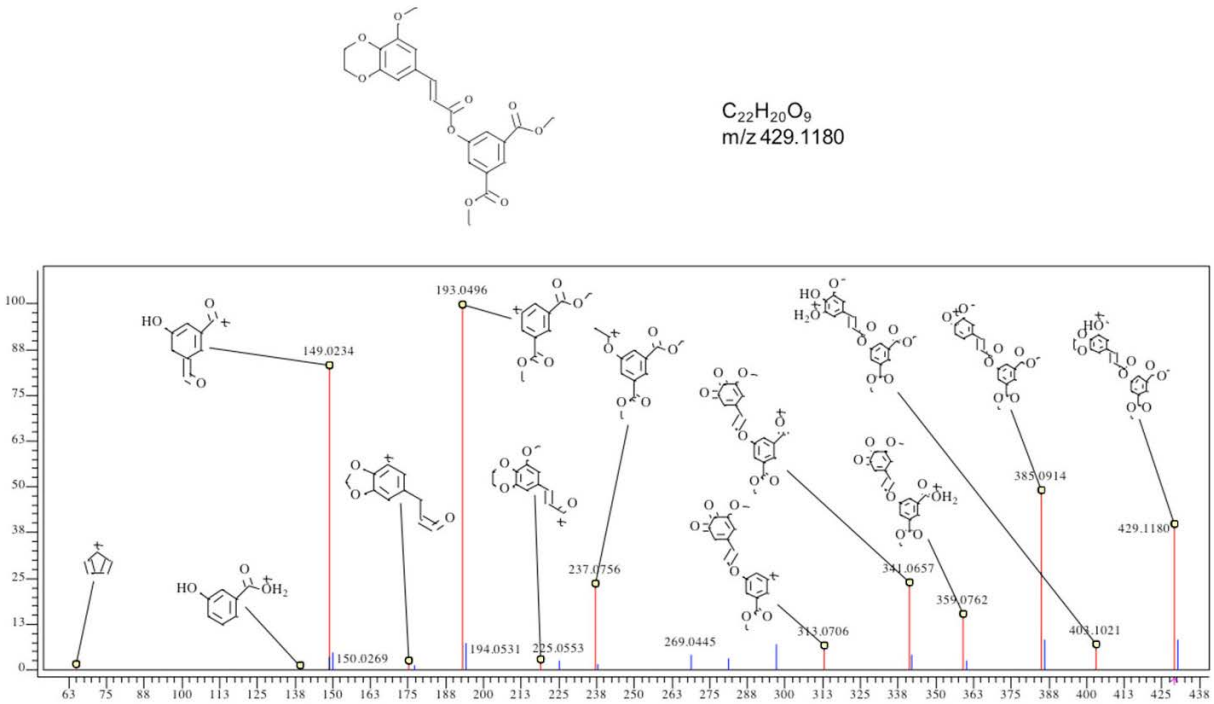


Figure 9. Auto-annotated MS/MS spectra of the proposed structure from 2-propanol extraction of the transdermal patch pouch.

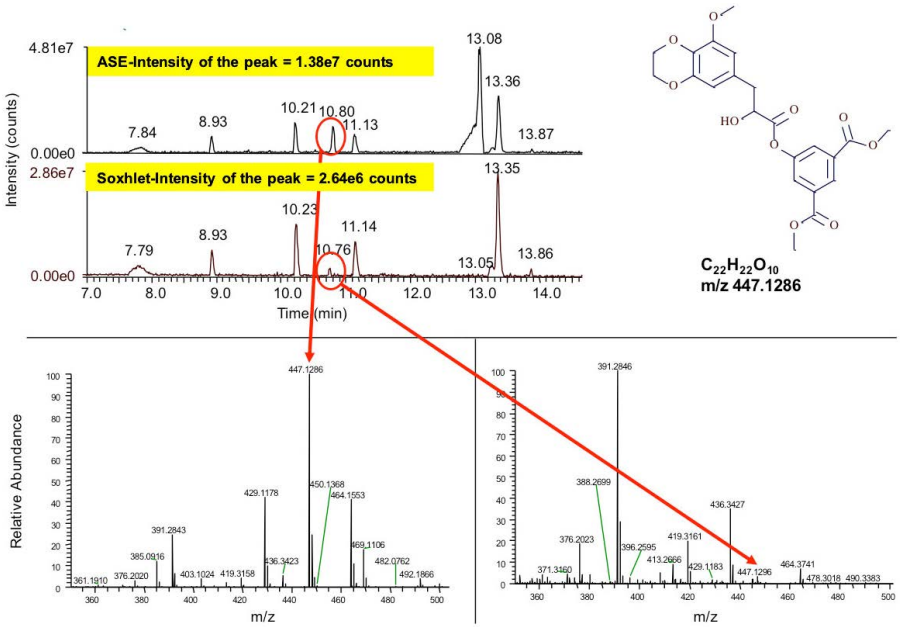


Figure 10. Identification of the 10.8 min peak extracted from the transdermal patch pouch using 2-propanol.

## Conclusion

The accelerated solvent extraction technique was demonstrated on a transdermal patch pouch and a polypropylene pill bottle using the automated Dionex ASE 350 system with multiple solvents in one sequence. The accelerated solvent extraction technique delivers comparable or more efficient extractions than the traditional Soxhlet extraction method using less time and less solvent. Extractions using two solvents of varying polarity is necessary in Controlled Extraction Studies, because different solvents can provide additional information on extractables.

The UltiMate 3000 LC system coupled to the Q Exactive mass spectrometer provides not only base peak chromatograms for extractable profiles, but also high quality HRAM data that enables molecular weight determination and structure elucidation of unknown extractables.

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