Dosing Syringe Extractables Analysis using Bench-top Orbitrap Mass Spectrometer

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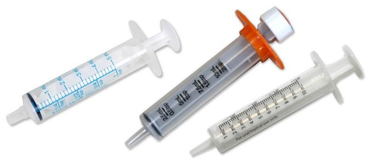
ABSTRACT

Identify the extractables from dosing syringes using HR-LCMS and node-based data processing software.

INTRODUCTION

There are many commercially available containers used for medicine dispensing. The quality of these containers can have an impact on the patient, especially infants and young children due to their early stage of development. In this study, extractable analyses were carried out on three different types of commercially available dosing syringes. LC-HRMS analysis was carried out to identify the non-volatile extractables. Data was processed using small molecule analysis software Compound Discoverer 2.0.

The study found noticeable variation in the syringes extraction profiles, and the extractables identified included substances of concern.



MATERIALS AND METHODS

Sample preparation

Three types of commercially available dosing syringes (syringe #1, syringe #2, and syringe #3) were filled with pH3 water, pH9 water, and EtOH/H_2O (1:1). The filled syringes were capped using aluminum foil and placed in the oven at 40 ^{0}C for 48 hours.

Liquid Chromatography

Liquid Chromatography separations were carried out on an Ultimate™ 3000 LC system (Thermo Fisher Scientific) using mobile phases composed of: A: H_2O/0.1% formic acid, and B: ACN/0.1% formic acid with gradient on an Accucore C18 column (2.1X100 mm 2.6µm). The flow rate was 0.4 mL/min.
Gradient:

Time	0	0.5	5.0	31.0	37.0	37.1	40.0
B%	5	5	20	95	95	5	5

Mass Spectrometry

The MS analysis was performed on a Thermo Scientific Q Exactive Plus bench-top high resolution mass spectrometer using electrospray ionization (ESI). High resolution full scan MS and data-dependent top 3 MS/MS data were collected in a data-dependent fashion at a resolving power of 70,000 and 17,500 (FWHM m/z 200) with polarity switching.

Ionization mode: positive ESI
Scan Range (Full MS): 120-1200 amu
Ion source: HESI-II
Spray voltage (KV):+3.5
Spray volltage(KV):-3.0
Heated capillary temp (^{o}C): 300
S-lens RF level: 55.0
Heater temp (^{o}C): 430
Sheath Gas::50

RESULTS AND DISCUSSION

The extract solutions were directly used for LCMS analysis. The samples were chromatographically separated, and high resolution mass spectrometry analysis was conducted on Q Exactive plus MS using full scan and data-dependent HCD MS/MS with polarity switching. The resolutions used were 70,000 (full scan) and 17,500 (MS/MS). The MS base peak chromatograms are shown in Figures 1, 2, and 3.

The High Resolution Accurate Mass (HRAM) data increased confidence for component identification and elemental composition assignment. HRAM HCD MS/MS fragments provide ample information for structure elucidation, see Figure 4. The polarity switching feature made it possible to detect structurally diverse compounds in a single run, see Figure 5.

Figure 1. Syringe #1 Extracts MS Base Peak Chromatogram (+)

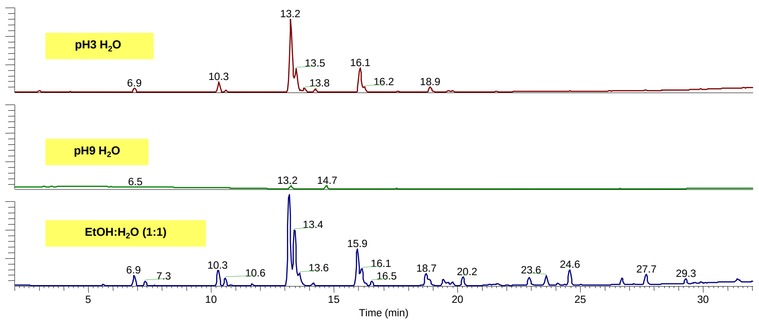


Figure 2. Syringe #2 Extracts MS Base Peak Chromatogram (+)

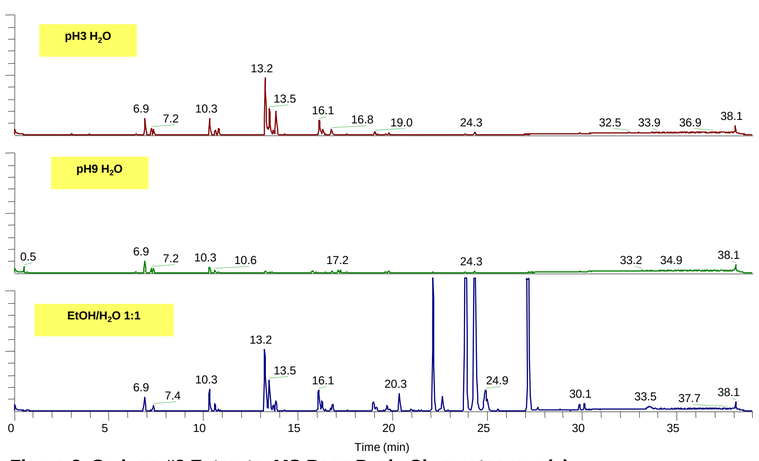


Figure 3. Syringe #3 Extracts MS Base Peak Chromatogram (+)

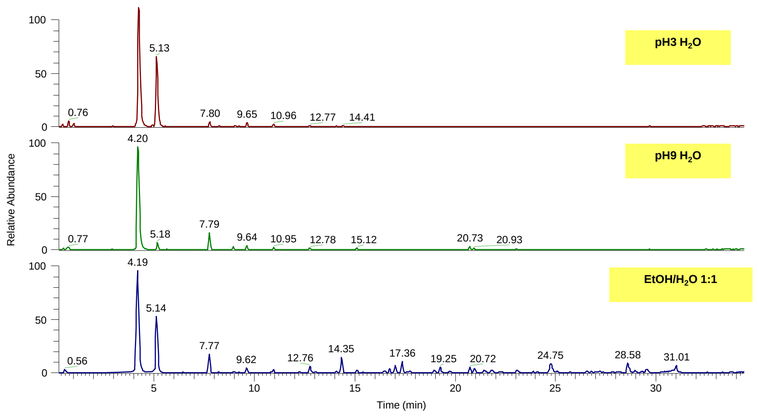
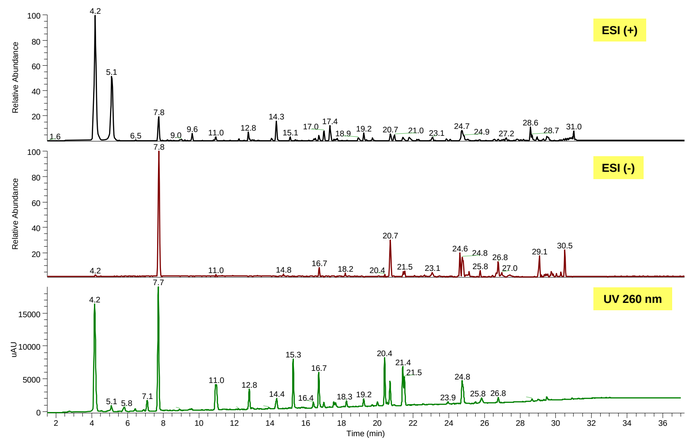


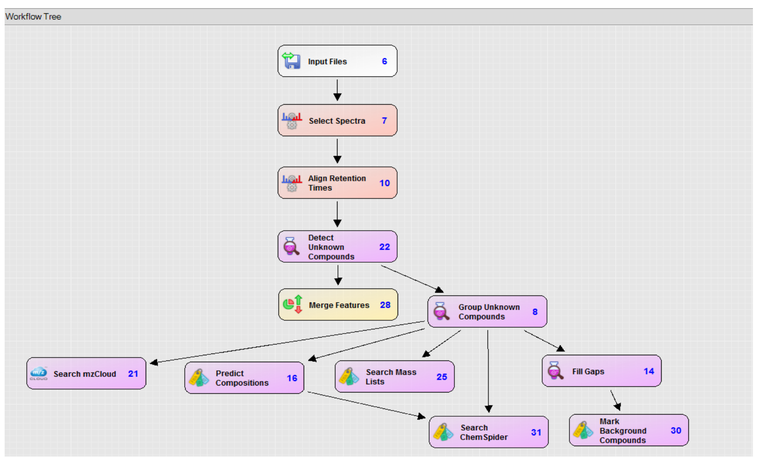
Figure 4. Positive and Negative MS Full Scan & UV Chromatograms of Syringe #3 EtOH/H_2O (1:1) Extract



DATA ANALYSIS

The data was processed with “Compound Discoverer 2.0” (CD 2.0), a node-base small molecule structure analysis software by Thermo Fisher Scientific. The process workflow was build by following the “New Study and Analysis Wizard” and using the workflow template called “Extractables and Leachables”. This is an unknown workflow, which detects unknown compounds with composition predictions, automatic database searching on high resolution spectral database mzCloud, ChemSpider, and the default E&L compound list, see Figure 5.

Figure 5. Compound Discoverer 2.0 Node-Based Workflow



The comprehensive “Result View” is shown in Figure 6. The “compounds” table listed the identified compounds, the predicted formula, and their molecular weight.
The database search results from mzCloud, ChemSpider, and E&L compound list were summarized in each corresponding table with compound name, molecular weight, structure, and link to the database. mzCloud search results have “Best Match” score, and the hitting compound can be further checked by viewing the mirror plot of MS/MS spectra with library reference, see Figure 7.

Figure 6. Result View

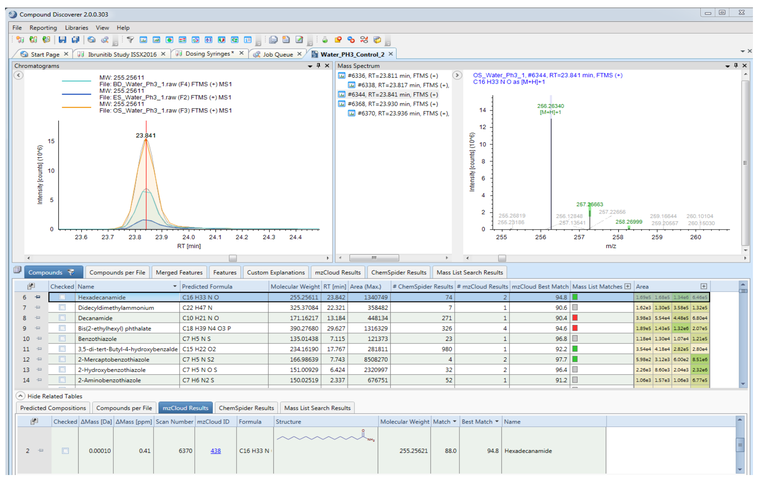
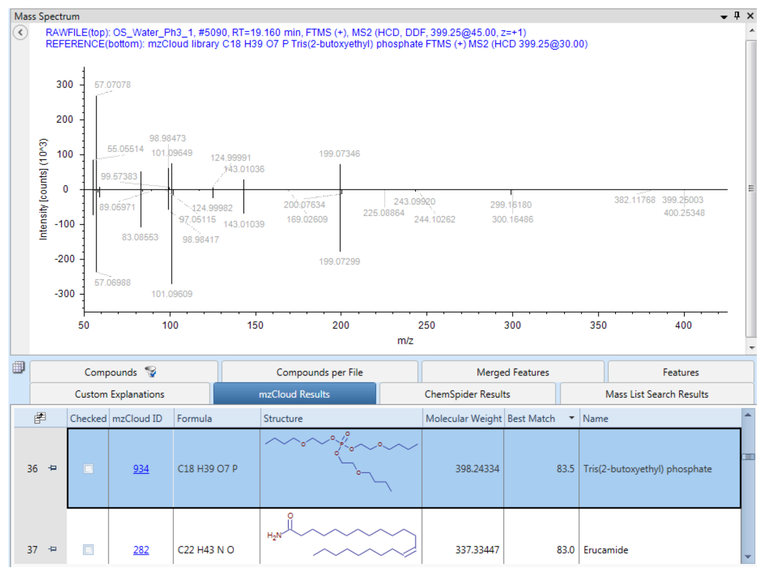


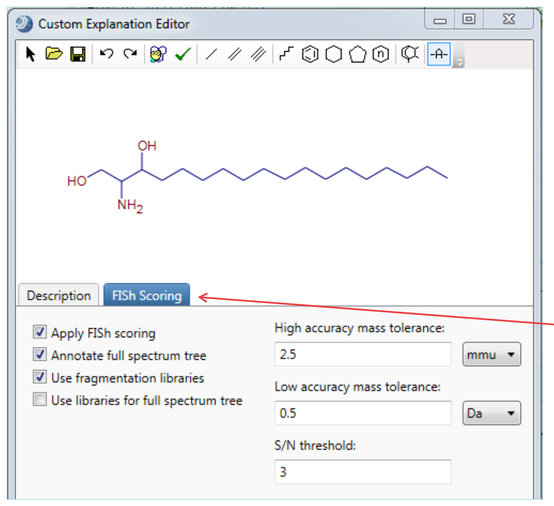
Figure 7. mzCloud Spectral Database Search Result - Mirror Plot



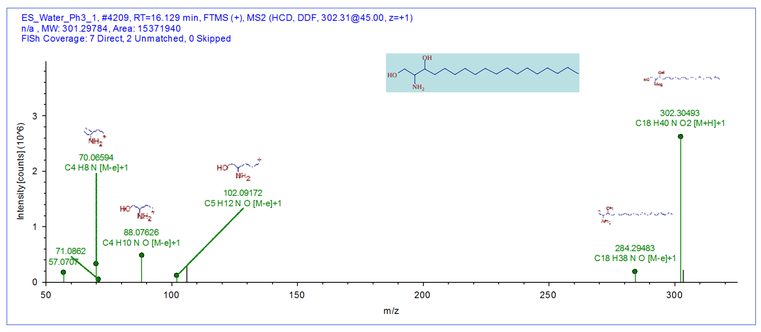
Unknown Structure Elucidation Using “Custom Explanations” Feature

The predicted formula provided useful information for unknown component identification and structure elucidation. The unknown compounds in “Compounds per File” sub-table were added to the “Custom Explanations” table in CD 2.0. The putative structure of unknown compounds were propose based on the “Predicted Formula” and MS/MS spectra, then using the “FISh Scoring” function (FISh stands for: Fragment Ion Search) searching the internal “Fragments and Mechanism” library, the matching fragments were auto-annotated with structure, molecular weight, elemental composition, and charge state, see Figure 7.

Figure 8. Unknown Structure Elucidation – Custom Explanation Editor and FISh Scoring



FISh Scoring



Data Reporting

The data report was generated using the report template. For each identified extractable, the database search and custom explanation information are included in the report, see Figure 9.

Figure 9. CD 2.0 Reporting Pages 1 and 4 Shown as Examples

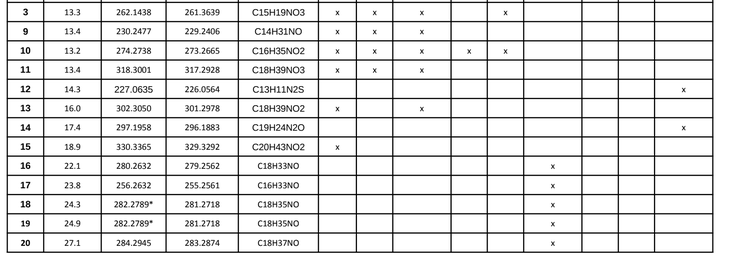


Figure 10. Structure of Compounds Identified Using CD 2.0 (partial list)

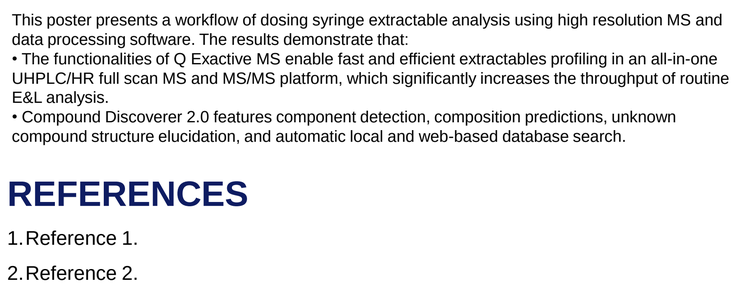


Table 1. Compounds Identified Using CD 2.0 (partial list)

Peak	RT [min]	M/Z (+)	Molecular Weight	Formula	Syringe #1			Syringe #2			Syringe #3		
					pH3	pH9	EtOH/H2O	pH3	pH9	EtOH/H2O	pH3	pH9	EtOH/H2O
1	4.2	212.1181	211.1110	C13H13N3							x	x	x
2	5.1	198.1279	197.1205	C14H15N1							x	x	x
4	7.7	167.99362	166.9864	C7H5NS2							x	x	x
5	6.9	218.2115	217.2042	C12H27NO2	x			x	x				
6	7.2	216.1957	215.1885	C12H25NO2	x			x	x				
7	10.3	246.2427	245.2354	C14H31NO2	x			x	x				
3	13.3	262.1438	261.3639	C15H19NO3	x	x	x		x				
9	13.4	230.2477	229.2406	C14H31NO	x	x	x						
10	13.2	274.2738	273.2665	C16H35NO2	x	x	x	x	x				
11	13.4	318.3001	317.2928	C18H39NO3	x	x	x						
12	14.3	227.0635	226.0564	C13H11N2S									x
13	16.0	302.3050	301.2978	C18H39NO2	x		x						
14	17.4	297.1958	296.1883	C19H24N2O									x
15	18.9	330.3365	329.3292	C20H43NO2	x								
16	22.1	280.2632	279.2562	C18H33NO						x			
17	23.8	256.2632	255.2561	C16H33NO						x			
18	24.3	282.2789*	281.2718	C18H35NO						x			
19	24.9	282.2789*	281.2718	C18H35NO						x			
20	27.1	284.2945	283.2874	C18H37NO						x			

CONCLUSIONS

This poster presents a workflow of dosing syringe extractable analysis using high resolution MS and data processing software. The results demonstrate that:
• The functionalities of Q Exactive MS enable fast and efficient extractables profiling in an all-in-one UHPLC/HR full scan MS and MS/MS platform, which significantly increases the throughput of routine E&L analysis.
• Compound Discoverer 2.0 features component detection, composition predictions, unknown compound structure elucidation, and automatic local and web-based database search.

REFERENCES

1. Reference 1.
2. Reference 2.

ACKNOWLEDGEMENTS

List optional acknowledgements here, such as “We would like to thank Professor Smith from the University of Texas for supplying the purified samples.”