

Poster Reprint

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Getting the most out of your Extractables and Leachables LC/MS Analysis with the Proposed US FDA CDRH CLAP Standard Set

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Introduction

Non-targeted Analysis of Extractables and Leachables using the Chemicals List for Analytical Performance (CLAP).

Robust and sensitive analysis of extractable and leachable (E&L) compounds by LC/MS is a crucial part of the safety risk assessments for medical devices and drug delivery products. To help improve the quality of data submissions for chemical characterization of medical devices, a group at the US Food and Drug Administration (US FDA CDRH) proposed a Chemicals List for Analytical Performance (CLAP) that covers a wide range of chemical classes, molecular weights, solubility, LogP, pKa, boiling points, and response factors.¹

In LC/MS, variation in ionization efficiency and presence of structural isomers makes compound identification and quantitation a challenge, particularly for E&L analyses. Robust, high-resolution hardware and sensitive, reproducible methods are crucial for successful E&L analysis.

Experimental

Broad Range of E&L Standards for Representative Monitoring of Method Performance

Standards for 82 LC/MS-amenable compounds from the CLAP analyte list were acquired from AChemTek and prepared as 0.2, 0.5, and 1 ppm solutions. Prepared standard mixtures were analyzed under a series of chromatographic and MS conditions.



Figure 1. The Agilent 1290 Infinity III LC system paired with the Agilent Revident LC/Q-TOF provide a robust platform with simultaneous high-resolution and extended in-scan dynamic range for E&L analysis.

Experimental

Optimized Method for LC/Q-TOF Yields Improved Performance for Extractables and Leachables Analysis

The original FDA CDRH CLAP LC/MS method was adapted and optimized for the Agilent Revident LC/Q-TOF. Common reverse phase columns, mobile phase compositions, and ionization strategies were compared to achieve a sensitive, robust, and reproducible method for LC/MS E&L analysis.

Table 1. Optimized LC Conditions

1290 LC Conditions															
Analytical column	Agilent Poroshell 120 Aq-C18, 2.1 x 150 mm, 2.7 μ m PN: 693775-742														
Delay column	Agilent Poroshell 120 EC-C18 4.6 x 50 mm, 2.7 μ m PN: 699975-902														
Column temperature	20 °C														
Autosampler temperature	5 °C														
Needle wash	10 s; 50% isopropanol, 25% methanol, 25% acetonitrile														
Mobile phase	A: 5 mM ammonium formate, 0.1% formic acid, 5 μ M ammonium fluoride in water B: 5 mM ammonium formate, 0.1% formic acid, 5 μ M ammonium fluoride in methanol														
Flow rate	0.4 mL/min														
Gradient program	<table border="1"><thead><tr><th>Time</th><th>%B</th></tr></thead><tbody><tr><td>0.0</td><td>0</td></tr><tr><td>5.0</td><td>0</td></tr><tr><td>20.0</td><td>35</td></tr><tr><td>40.0</td><td>100</td></tr><tr><td>50.0</td><td>100</td></tr><tr><td>55.0</td><td>0</td></tr></tbody></table>	Time	%B	0.0	0	5.0	0	20.0	35	40.0	100	50.0	100	55.0	0
Time	%B														
0.0	0														
5.0	0														
20.0	35														
40.0	100														
50.0	100														
55.0	0														
Total run time	60 min														

Table 2. Revident LC/Q-TOF AJS Source Conditions

Dual AJS ESI Source Conditions	
Gas Temperature, Flow	200 °C, 11 L/min
Nebulizer Pressure	35 psi
Sheath Gas Temperature, Flow	250 °C, 11 L/min
Nozzle Voltage	2000 V
Capillary Voltage	3500 V

Results and Discussion

Mobile Phases Impact Sensitivity, Retention Times, and Background Ion Abundances.

Among mobile phase solvents, signal response for many compounds in the CLAP dataset showed marked improvement with the use of methanol rather than acetonitrile as mobile phase B. In addition, inclusion of ammonium formate as well as formic acid in the mobile phases also yielded higher signal response than formic acid alone (Figure 2), particularly for compounds that preferentially ionize as ammonium adducts, like Cyanox 425 and Irganox 1076.

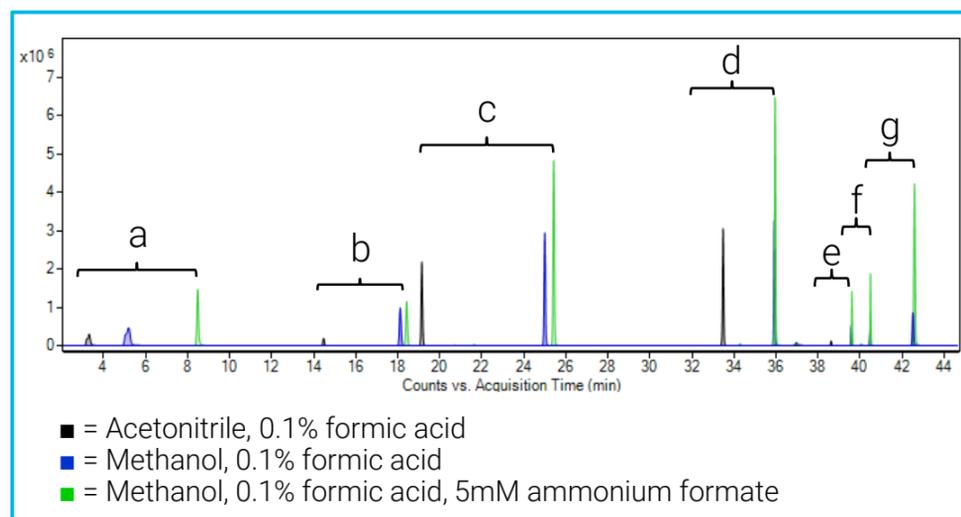


Figure 2. EICs of (a) 4,4'-diaminobiphenyl methane, (b) triacetin, (c) triethyl citrate, (d) acetyltributyl citrate, (e) octrizole, (f) tinuvin 320, (g) triisooctyl trimellitate at 1ppm show examples of differing retention time and response with different mobile phase compositions.

The inclusion of ammonium fluoride as an additional mobile phase additive further improves sensitivity and selectivity by promoting the formation of protonated and ammoniated ions over sodium adducts. Reducing the presence of sodium adducts is particularly important for MS/MS analyses as sodiated ions generally do not produce usable fragment ions.

Solvent Delay Column Separates Target Sample Analytes from Mobile Phase Contaminants

Obtaining sufficiently high purity LC/MS solvents is a particular challenge for E&L analysis, where many of the target analytes may also be present as contaminants. Adding a delay column plumbed at the outlet of the LC pump can aid in distinguishing between analytes in the sample and identical contaminant compounds in the mobile phase.

With a delay column, analytes originating from the sample maintain expected narrow, gaussian peak shape and retention time, while their contaminant counterparts elute later with broader peak shapes (Figure 3).

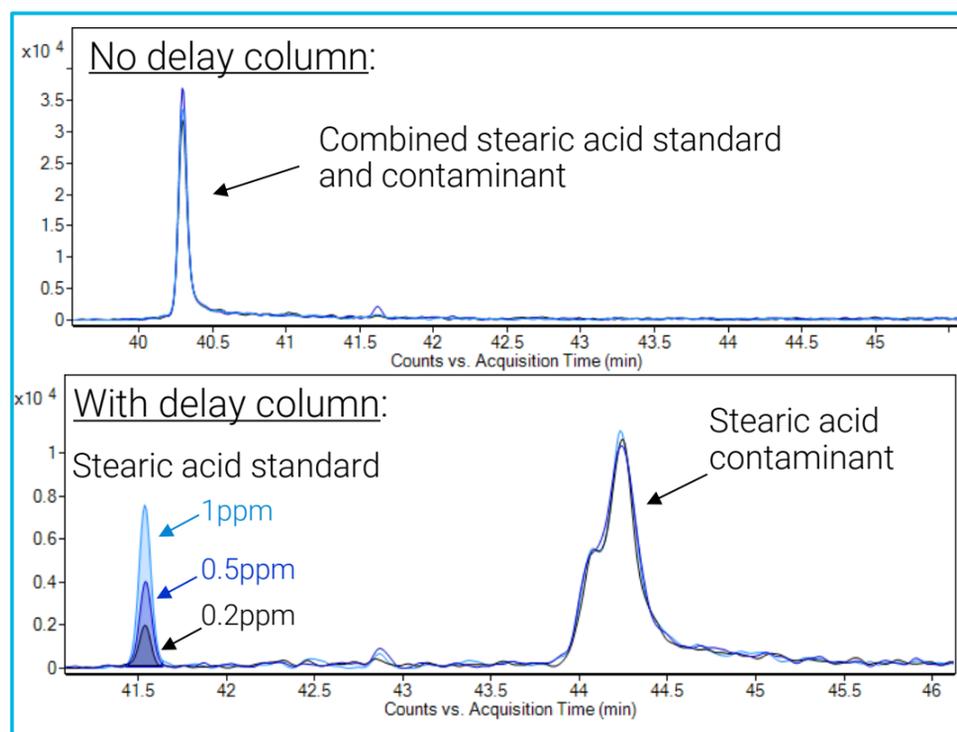


Figure 3. EICs of stearic acid injected at 1 ppm, 500 ppb, and 200 ppb without a solvent delay column (top) and with a solvent delay column (bottom) to separate the injected standard from the background contaminant.

Poroshell 120 Aq-C18 Column Improves Retention of Early-eluting Analytes and Facilitates Separation of Near-eluting Isomers.

Compared to a more traditional fully porous Zorbax Eclipse Plus C18 column, the superficially porous Poroshell 120 Aq-C18 is designed for compatibility with 100% aqueous mobile phases to aid in the retention of challenging polar analytes with reverse phase. Early-eluting analytes were retained longer (Figure 4) without compromising isomer separation (Figure 5) using the Poroshell 120 Aq-C18 column.

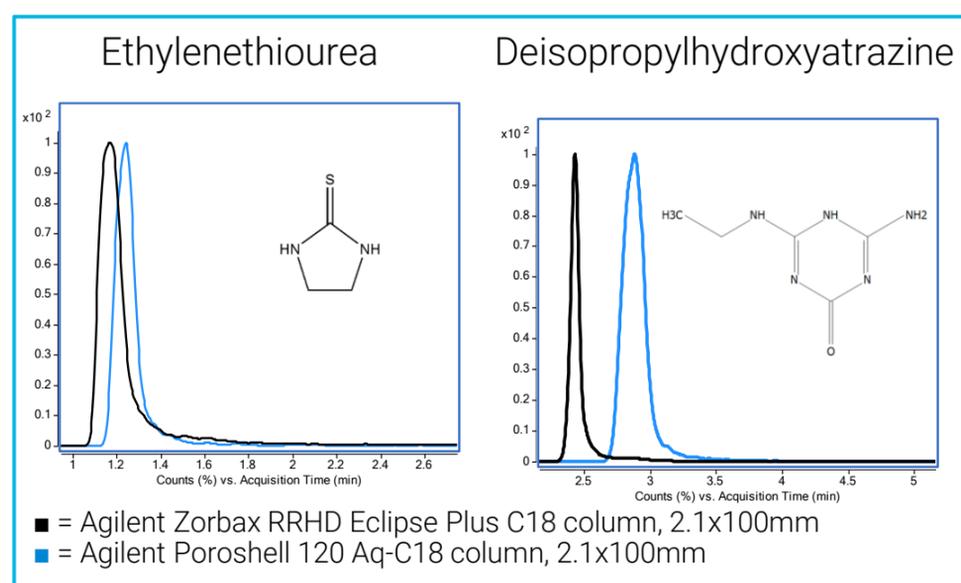


Figure 4. EICs of ethylenethiourea (left) and deisopropylhydroxyatrazine (right) demonstrate improved retention of early-eluting bases using a Poroshell 120 Aq-C18 analytical column.

Results and Discussion

Application of a 150 mm Poroshell 120 Aq-C18 column provided separation of all LC/MS-amenable isomer sets in the CLAP dataset (Figure 5), with the exception of diphenyl phthalate and diphenyl terephthalate, which can be distinguished using GC/MS as an alternative analytical platform.

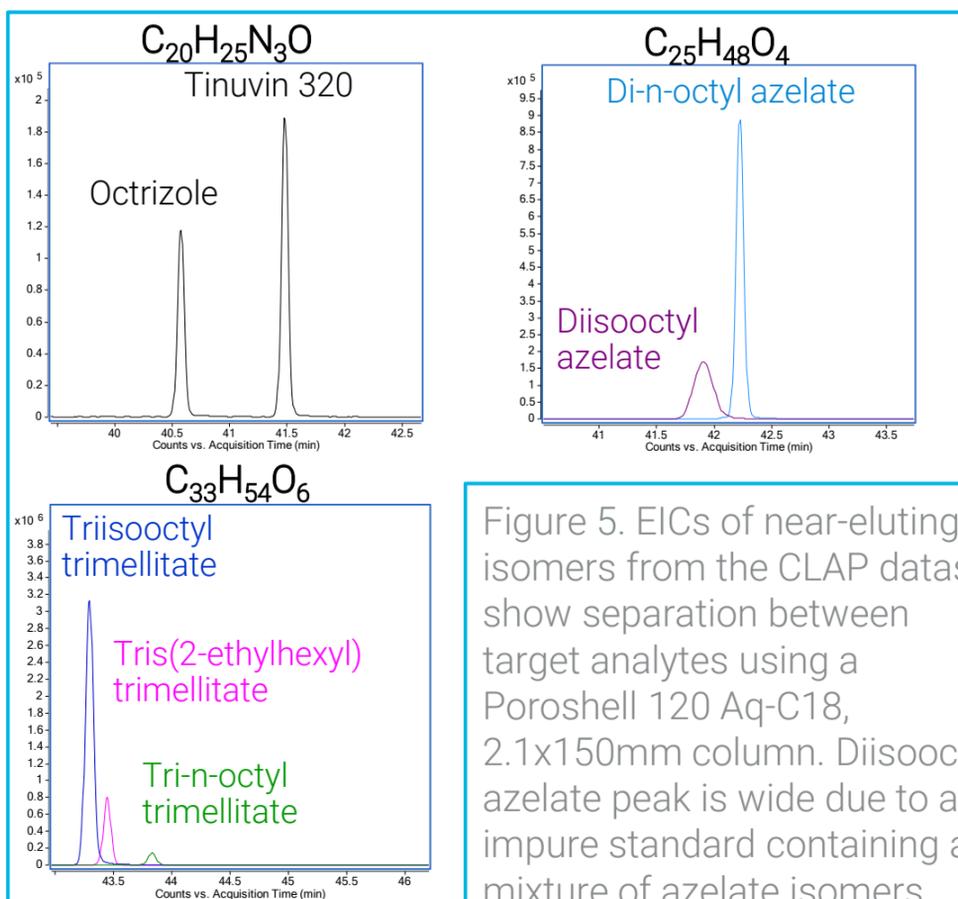


Figure 5. EICs of near-eluting isomers from the CLAP dataset show separation between target analytes using a Poroshell 120 Aq-C18, 2.1x150mm column. Diisooctyl azelate peak is wide due to an impure standard containing a mixture of azelate isomers.

The compatibility of the Poroshell 120 Aq-C18 column with 100% water additionally aided in the reduction of background by allowing post-run column re-equilibration to be conducted with just the aqueous mobile phases, reducing exposure of the LC/MS system to the typically higher levels of contaminants present in organic solvents.

Relative Response Factors Demonstrate Method Reliability and Accuracy across Analyses

The detectability of a variety of extractable and leachable analytes using the CLAP dataset was assessed based on their relative response factors (RRF), determined as the ratio of the analyte signal intensity to the internal standard signal intensity at the same concentration. RRFs were calculated from standard mixtures at 0.2, 0.5, and 1 ppm with butyryl trihexyl citrate as the positive ion internal standard and 2,4-dihydroxybenzophenone as the negative ion internal standard.

Calculated RRF values were in line with those previously reported by US FDA CDRH,¹ with an additional 30 CLAP analytes identified by Revident LC/Q-TOF with optimized chromatography, which were not detected by LC/MS using the alternative HRMS platform employed to collect the original US FDA CDRH CLAP dataset.¹

<https://www.agilent.com/en/promotions/asms>

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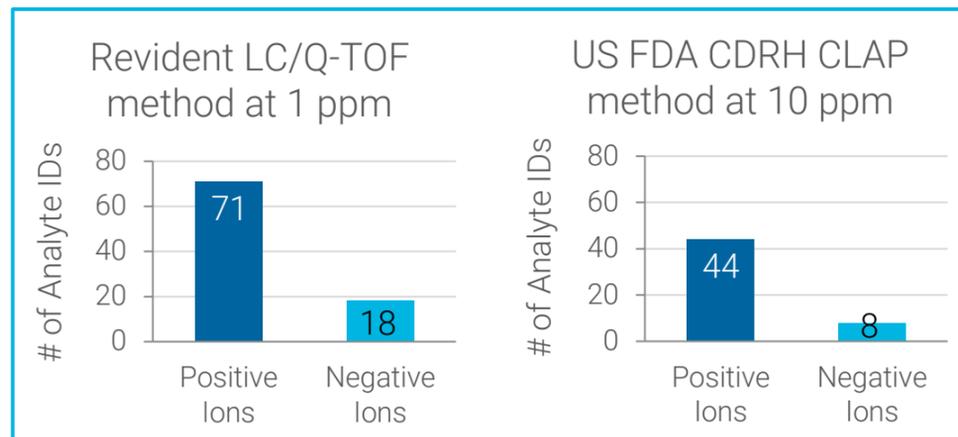


Figure 6. 79 total CLAP analyte IDs were achieved using the optimized Revident LC/Q-TOF method at more than 10x lower concentrations compared to only 49 total LC/MS IDs originally reported by US FDA CDRH CLAP using alternative instrumentation.

Conclusions

Mobile Phase, Column, and Ion Source Selection Drive Non-targeted Extractable and Leachable Method Performance.

- Agilent Poroshell 120 Aq-C18 column compatibility with 100% aqueous mobile phases retains early-eluting polar compounds better, allowing for analysis of all LC/MS-amenable CLAP analytes on one gradient.
- Use of methanol-based mobile phase containing ammonium formate substantially increased signal response for the majority of CLAP analytes.
- Use of solvent delay column and measurement of RRFs distinguish sample analytes from background contaminants, increasing confidence in extractable and leachable analyte identifications.
- Revident LC/Q-TOF with optimized method facilitates detection of 30 additional CLAP analytes at >10x lower concentration compared to the original US FDA CDRH method.

References

¹ United States Food and Drug Administration Center for Devices and Radiological Health Chemicals List for Analytical Performance (CLAP), <https://cdrh-rst.fda.gov/chemicals-list-analytical-performance-clap>

COI: Sierra D. Durham and David A. Weil are employees of Agilent Technologies.