Organic Flush Solutions to Remove Sample Matrix Interferences in LC-MS Systems

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OVERVIEW

Purpose:

- To demonstrate the workflow of using MB124 isopropanol: acetonitrile: acetone (45:45:10) for online sample preparation and matrix removal from TurboFlow™ column
- Effective removal of background contamination offline in LC-MS using isopropanol: acetonitrile: dichloromethane: cyclohexane (50:25:10:15)

Methods:

- Prelude SPLC™ system equipped with TurboFlow column and Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer with two-channel multiplexing were used to increase the instrument uptime and sample throughput in combination with automatic sample preparation and matrix removal
- Sample preparation efficiency and matrix removal was evaluated using Atenolol, Warfarin, Lidocaine and Imipramine spiked into synthetic urine (surine) on TurboFlow column
- Thermo Scientific Quantiva™ triple quadrupole system and Q-Exactive™ Focus Orbitrap™ system coupled with UltiMate™ 3000 LC system were used to monitor baseline noise and identify common contaminants

Results:

- MB124 was effective in sustaining a continuous workflow for online sample preparation and TurboFlow column cleanup as observed from consistent retention time and peak area of the targeted compounds spiked into synthetic urine.
- Blend of isopropanol: acetonitrile: dichloromethane: cyclohexane removed effectively the matrix background contamination commonly encountered in mass spectrometers.

INTRODUCTION

- Chemical contamination is a common issue in LCMS analysis.
  - The source of contamination can be from low grade analytical solvents, sample matrix, solvent reservoir, solvent filters, LC pump, PEEK tubing, ESI spray needle and ubiquitous airborne contaminants.
  - Contaminants such as polyethylene glycol (PEG) and surfactants (both separated by 44 Da in ESI$^+$), polypropylene glycol (PPG, separated by 58 Da in ESI$^+$), phthalates, metal ions, detergents, and polysiloxanes (separated by 74 Da in ESI$^+$) are frequently observed. Phthalates from plasticizers usually appeared in ESI$^+$ as m/z 391, 419 and 447, which are related to diisooctylphthalate, dinonylphthalate and diisodecyl phthalate, respectively. They can form dimers and adduct ions with sodium, potassium, and ammonium.
  - Fatty acid and trifluoroacetic acid are observed as contaminants in negative mode (ESI$^-$).
- Polar mobile phases such as methanol and acetonitrile fail to remove resistant polar and nonpolar contaminants.
- Isolation of analytes from biological matrices (such as urine and plasma) using SPE cartridge and TurboFlow column need extensive washing to remove biological matrices.
- In this study, we report efficient removal of matrix interferences that remained on column after sample preparation using a flush solution containing isopropanol:acetonitrile:acetone (MB124). Moreover, use of isopropanol:acetonitrile: dichloromethane:cyclohexane blend effectively removed offline the background contamination which is commonly encountered in mass spectrometers.
- The automated approach using MB124 for matrix removal during sample preparation enabled high sample throughput and consistent results by multiplexing across multiple LC channels using Thermo Scientific Prelude SPLC and Transcend™ TLX systems.

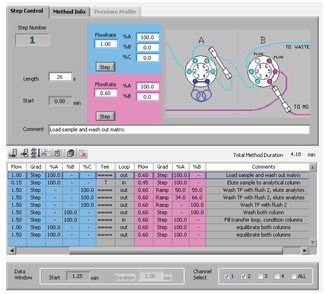
METHODS

- Sample preparation
  - Atenolol, Warfarin, Lidocaine and Imipramine were spiked into Surine™ (Dyna-Tech Industries, Lenexa, KS) at the concentration of 10 ng/µL
- LC Mobile Phase
  - LPA: 10 mM ammonium formate in water with 0.05% formic acid (MB123)
  - LPB: 10 mM ammonium formate in methanol with 0.05% formic acid (MB122)
  - LPC: acetonitrile: isopropanol: acetone (45:45:10) (MB124)
  - EPA: 10 mM ammonium formate in water with 0.05% formic acid (MB123)
  - EPB: 10 mM ammonium formate in methanol with 0.05% formic acid (MB122)
  - Wash 1: water: methanol (40:60) with 0.05% formic acid
  - Wash 2: acetonitrile: isopropanol: acetone (45:45:10) (MB124)
- Column
  - TurboFlow column: CYCLONE-P COLUMN, 0.5 x 50 mm (CH-953289)
  - Temperature for TurboFlow column: 30º C
  - Analytical column: ACCUCORE-PFP, 2.6 µm, 50 X 2.1 mm (17426-052130)
  - Temperature for analytical column: 40º C

Online Sample Preparation and Matrix Removal

- **Fig. 1** depicts the Prelude SPLC system used in TX mode. The system was equipped with TurboFlow column and an analytical column. LPA, LPB and LPC are used as mobile phases for loading pumps (blue), and EPA and EPB for eluting pumps (pink).
- Sample was injected into TX injector, loaded to TurboFlow column and washed the column with LPA for 26 sec to remove sample matrix such as proteins, salts and sugars to waste and eluted with LPB stored in 100 µL transfer loop of divert valve A by pumping LPA at 0.15 mL/min and EPA at 0.45 mL/min to analytical column through special T valve.
- After targeted compounds were eluted to analytical column, the TurboFlow column was further washed with MB124 (LPC) for 60 sec to remove all remaining polar and nonpolar contaminants contained in biological fluids. MB124 is compatible with most sample preparation techniques, such as Sep-Pak cartridge or TurboFlow column.
- Column was reconditioned (after sample matrix was removed from column) with LPB for 60 sec followed by LPA for 60 sec. Transfer loop in divert valve A was refilled with LPB and ready for the next injection.
- In parallel, the target compounds were eluted from analytical column using gradient of EPA and EPB, and sent to MS for analysis. As a comparison, sample was injected directly to analytical column in LX mode.

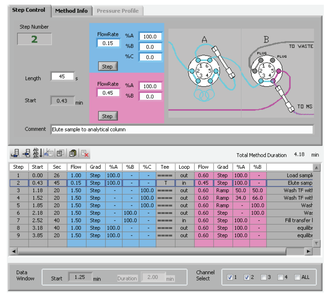
Figure 1. TX Injector



Sample was injected in TX injector, and loaded on sample preparation column

- **Fig. 2** indicates that the analyte was eluted from TurboFlow column to analytical column through a special T valve and sent to MS for analysis.
- The solvent strength in the transfer loop was diluted with both LPA and EPA so that the analytes can be condensed on the front of the analytical column as a sharp band.

Figure 2. TurboFlow Column to Analytical Column



Analytes were eluted from TurboFlow column to analytical column through a special T valve and sent to MS for analysis

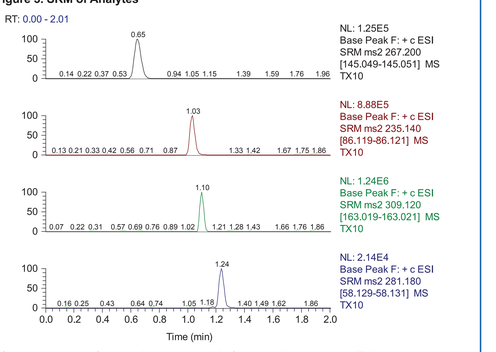
- Mass spectrometry: a TSQ Endura MD triple quadrupole mass spectrometer with a HESI probe in positive mode was used as the detector.
- Data analysis: all data acquisition for this method was performed using Thermo Scientific Aria™ MX software version 2.2 and Thermo Scientific Xcalibur™ software version 3.0.
- MS condition:
  - Spray voltage: 3200
  - ITT temp (ºC): 350
  - Sheath gas: 45
  - Aux gas: 15
  - Sweep gas: 0
  - Vaporizer temp (ºC): 400
- Scan type: SRM
  - Q1 resolution (FWHM): 0.7
  - Q3 resolution (FWHM): 0.7
  - Dwell time: 400 ms
  - CID gas (m Torr): 2
- MRM Transitions:

Compound	Precursor Ion	Product Ion	Collision (V)	RF Lenses (V)
Atenolol	267.20	145.05	26	125
Lidocaine	235.14	86.12	18	89
Warfarin	309.12	163.02	15	93
Imipramine	281.18	58.13	31	100

Removal of Background Contamination Offline

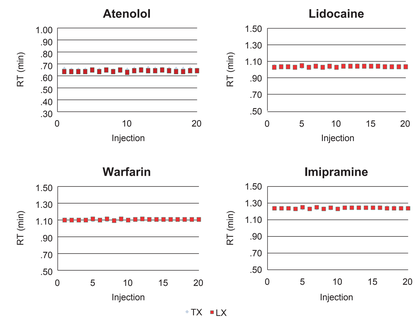
- For offline system cleanup, Thermo Scientific Q Exactive Focus Orbitrap mass spectrometer and Thermo Scientific Quantiva triple quadrupole mass spectrometers were used.
- LCMS grade methanol (Fisher Scientific, A456) was infused to Q Exactive Focus through UltiMate 3000 RS pump at 350 µL/min to collect the baseline noise in full scan in the mass range of 50-750 Dalton.
- The system was washed with flush solution isopropanol: acetonitrile: dichloromethane: cyclohexane (50:25: 10:15) for 2 hrs at the flow rate of 0.4 mL/min.
- After residual solvent was removed from system, baseline noise was monitored again by pumping through the same LCMS methanol under the same condition for comparison.

Figure 3. SRM of Analytes



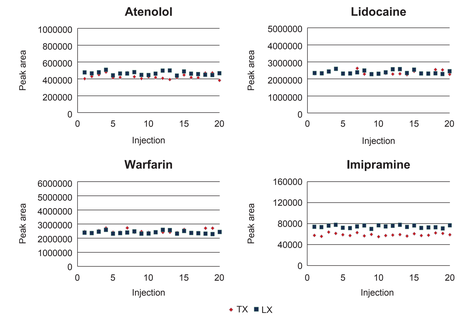
Chromatograms of Atenolol, Lidocaine, Warfarin and Imipramine in TX mode

Figure 4. Consistent Retention Time



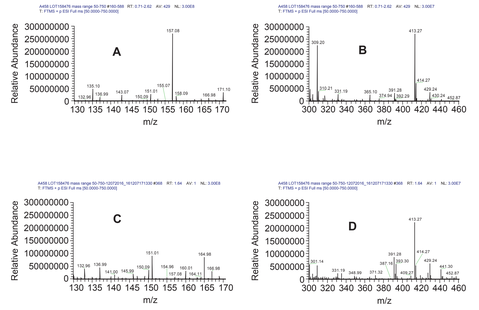
Retention times of Atenolol, Lidocaine, Warfarin, and Imipramine from 20 injections in both TX and LX modes

Figure 5. Consistent Peak Area



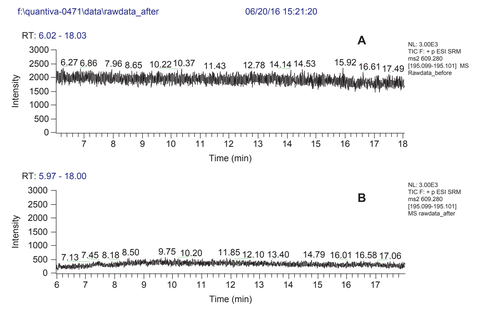
Peak areas of Atenolol, Lidocaine, Warfarin, and Imipramine from 20 injections in both TX and LX modes

Figure 6. Baseline Noise Reduction by Flush Solution



Baseline noise of methanol in Q Exactive Focus with UltiMate 3000 LC system before (A and B) and after (C and D) flush solution cleanup

Figure 7. Background Noise Reduction



Background noise of methanol:water (90:10) with 0.05% formic acid at a flow rate of 350 µL/min before (A) and after (B) flush solution cleanup

RESULTS AND DISCUSSION

- To mimic a typical bioanalytical application, Atenolol, Lidocaine, Warfarin and Imipramine mixture were spiked in Surine at 10 ng/µL. Samples were injected in TX injector with both TurboFlow and analytical columns and LX injector with analytical column only for comparison (**Table 1**).
- **Fig. 3** depicted the SRM chromatograms of Atenolol, Lidocaine, Warfarin and Imipramine.
- **Fig. 4** showed that all four compounds have consistent retention time (RT) with %RSD less than 1% from 20 injections in both TX and LX injectors.
- **Fig. 5** depicted the %RSD of peak area of Atenolol, Lidocaine, Warfarin and Imipramine from 20 injections in both TX and LX injectors.
- **Table 1** shows the %RSD of peak area and recovery percentage in TX mode.

Table 1

	Atenolol	Lidocaine	Warfarin	Imipramine
% RSD - TX	6.7	4.4	4.9	4.4
% RSD - LX	5.0	4.0	4.1	3.2
% Recovery	91.7	98.8	103.6	79.5

- **Fig. 6** showed that cleanup with flush solution significantly reduced the ions at m/z 157.08 and m/z 309.20. Based on accurate mass in Q Exactive, these two ions are identified as polypropylene glycol adduct ions. Ions at m/z 391.28 and 413.27 were diisooctyl phthalate adducts. Phthalates are more difficult to remove and require the system to be washed with flush solution for a longer time.
- A similar process was used to collect data in MS/MS mode using Quantiva mass spectrometers to monitor the effectiveness of flush solution to remove background noise. The data was collected in the system verification during new Quantiva mass spectrometer installation.
- The baseline noise level of the system remained at 2000 counts infused with A456 methanol : W6 water (90:10) with 0.05% LCMS grade formic acid at a flow rate of 350 µL/min after the system was cleaned with methanol and baked out at high temperature for a few days.
- **Fig. 7** showed that the baseline noise of LCMS in MRM positive mode had significantly decreased from 2000 counts to about 400 counts, and was infused with the same mobile phase at the same flow rate after system was cleaned with 50 mL of flush solution.

CONCLUSIONS

- Prelude SPLC or Transcend TLX equipped with automated approach for matrix removal using MB124 during sample preparation:
  - doubles the sample throughput,
  - improves instrument up time, and
  - minimizes matrix effects while maintaining consistency of results from injection to injection.
  - It also saves consumable cost and avoids laborious manual sample preparation process.
- The extraction efficiency and recovery for Atenolol, Lidocaine, Warfarin and Imipramine are good in TX mode in comparison to LX mode.
- Isopropanol/acetonitrile/dichloromethane/cyclohexane flush solution effectively removed PPG as well as other unknown impurities, and it reduced background contamination significantly to improve the performance of instrument.