POST-COLUMN ADDITION AS A TOOL TO ENHANCE PERFORMANCE IN MICROFLOW LC/MS

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OVERVIEW

- **Post-column addition of modifiers to facilitate deprotonation and enhance the ionization process.**
- **Post-column addition does not effect chromatographic separation, therefore optimization of the ESI MS conditions can be achieved without compromising LC conditions**

INTRODUCTION

The optimal conditions for electrospray ionization mass spectrometry may not always be compatible with optimal LC conditions. Modifications of the mobile phase (e.g. addition of buffers, changing the pH and solvent strength) may be beneficial to the chromatography, but ultimately have a negative impact on the electrospray droplet ionization and desolvation process. Altering solvent properties by post-column addition of a **liquid or gaseous** modifier can be an effective technique to improve electrospray ionization without affecting the chromatographic separation. We experimented with several solvent properties, such as surface tension, pH, polarity or boiling point, in an attempt to better understand and consequently improve the electrospray process.

METHODS

The experiments were performed with the ionKey/MS system composed of the ACQUITY UPLC M-Class, the ionKey source, and Xevo® G2-XS Qtof or Xevo TQ-S Mass Spectrometer.

1. Post-Column Addition of Liquid Modifiers

The post-column addition (PCA) iKey contains two channels, a 150 µm I.D. channel packed with sub-2-µm particles, and an open channel used for post column addition of solvent (Figure 1). The two channels meet after the chromatographic separation completes and prior to the emitter leaving insignificant dead volume. The PCA iKey was used for the post-column addition of the liquid modifier. The flow rates used in this study varied from 1- 4µl/min and the separation gradients were adjusted for different applications. Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The post column addition modifier flow rate was optimized for each application. The flow rates ranged from 100 to 1000 nl/min.

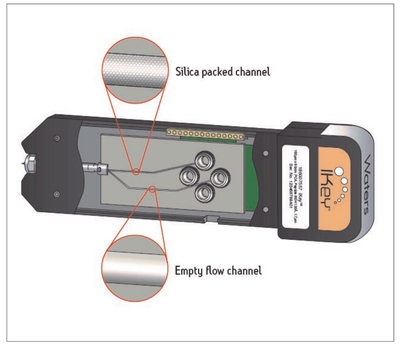

Figure 1. ***Post-column addition (PCA) iKey. The analytical channel is connected to the upper port and the post-column addition channel is connected to the right port.***

2. Post-Column Addition of Vapor Modifiers

In another experiment, we carried out in-situ modification of electrospray droplets (instead of the mobile phases or analyte solution) with vapors. Vapors are introduced into the electrospray environment by flowing the nebulizer gas over the headspace of a modifier solution (Figure 2.). Examples of modifiers include solutions of ammonium fluoride, triethylamine, and ammonium hydroxide, or ammonia gas. The presence of the vapor modifiers facilitates ionization and increases sensitivity.

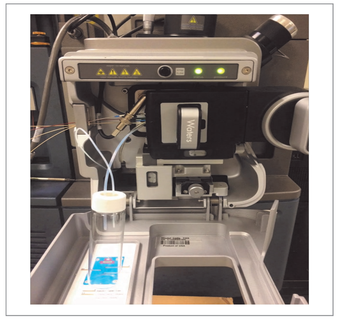

Figure 2. ***Setup for vapor assisted electrospray***

RESULTS AND DISCUSSION

1.Post-Column Addition of Liquid Modifiers

1.1. Effect of IPA on urine analysis

The analysis of ibuprofen and its metabolites in human urine in negative ionization mode using post-column addition of isopropanol showed a significant increase in sensitivity (Figure 3 and 4). Pre-dose urine and ibuprofen-metabolite containing urine samples were collected from a healthy male volunteer. Under standard gradient conditions, in the absence of isopropanol, not all compounds were detected. Post-column addition of isopropanol enabled enhanced detection of the more hydrophilic compounds, including ibuprofen metabolites.

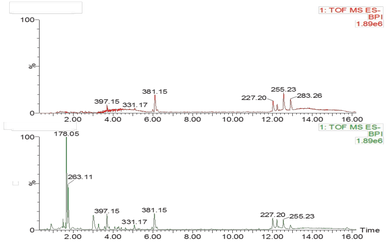

Figure 3. ***Chromatographic separation of human urine when 0 (red), and 1 (green) µl/min IPA was used as a post -column modifier. The chromatograms are scaled to the highest relative intensity among the two chromatograms.***

The extracted ion chromatograms of *m/z* 397.1499, corresponding to the hydroxylated glucuronide metabolites of ibuprofen, are shown in Figure 4. For the most intense peak the signal increased by over 50% when IPA was used (bottom chromatogram).

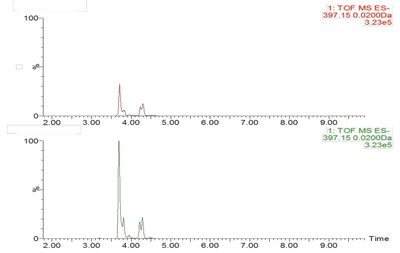

Figure 4. ***Extracted ion chromatograms of the hydroxylated glucuronide metabolites of ibuprofen; top (red) chromatogram in the absence of IPA; bottom (green) chromatogram with IPA.***

1.2. Effect of DMSO on protein digest

Increasing the electrospray responses of peptides by adding a low percentage of dimethylsulfoxide (DMSO) to the LC solvent has been investigated in previous studies. DMSO is a polar aprotic solvent with an elution strength similar to acetonitrile. Therefore, addition of DMSO to the LC solvents requires adaptation of the elution gradient to avoid the loss of hydrophilic peptides. The post-column addition iKey enables the introduction of DMSO through a side channel without influencing chromatographic performance as illustrated in Figure 6. The peak shape of the hydrophilic peptides is significantly deteriorated when DMSO is added to the mobile phase. The change in retention times depends on the amount of DMSO added in the mobile phases, whereas with the post-column addition the retention times are the same regardless of the DMSO concentration.

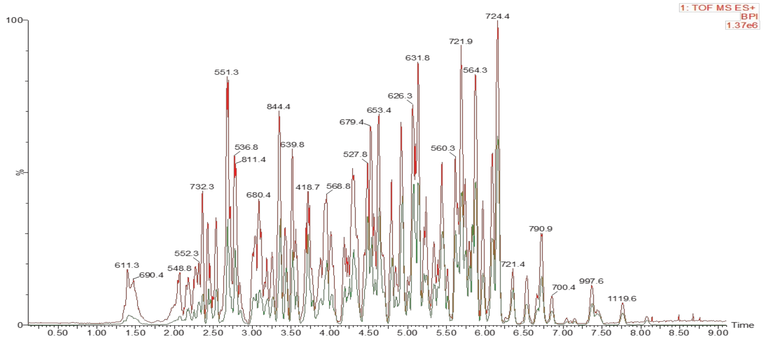

Figure 5. ***Improved LC-MS/MS performance using DMSO. Base peak intensity chromatograms of a four protein digest (MassPREP protein digestion standard mixture 1) in the presence (red) and absence (green) of 5% DMSO.***

The addition of DMSO may result in lowering the charge states of certain peptides, and therefore, proper identification and updating of the MRM transitions while performing targeted analysis is critical for the post column DMSO addition. In the example presented in Figure 7, the dominant species of the peptide TIAQYAR is the doubly charged ion *m/z* 411.7. However, with the post-column addition of DMSO, the singly charge ion *m/z* 822.4 became the most abundant species.

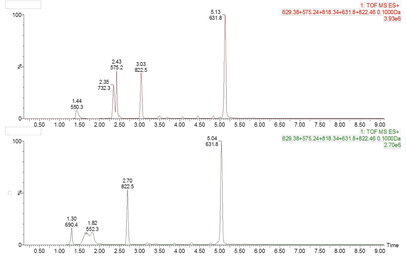

Figure 6. ***DMSO effect on chromatography. Post-column addition of DMSO (red) vs. in-solution addition of DMSO (green).***

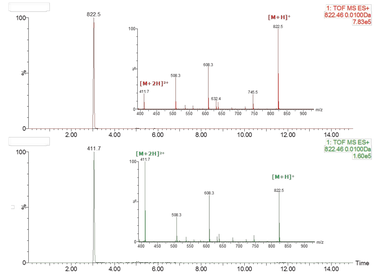

Figure 7. ***Extracted Ion Chromatograms and Mass Spectra of TIAQYAR peptide in the presence (red) and absence (green) of 5% DMSO***

2. Post-Column Addition of Vapor Modifiers

2.1. Effect of Ammonia on Estrogens

In this study we evaluated the post column addition of modifiers in vapor phase to enhance the ionization process and consequently the sensitivity. The following modifiers were experimented with: ammonium hydroxide, ammonium fluoride, ammonium bicarbonate, triethylamine and ammonia gas. One example of the vapor effect is illustrated in Figure 8. The analysis of estrogens in negative ion mode using vapor assisted electrospray showed a significant increase in sensitivity when exposed to gaseous ammonia.

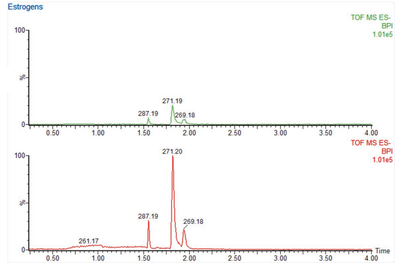

Figure 8. ***Chromatographic separation of estrogens in the presence (red), and absence (green) of ammonia gas. The chromatograms are scaled to the highest relative intensity among the two chromatograms.***

CONCLUSION

There are numerous ways that post-column additions can be employed to improve electrospray sensitivity, as listed below:

- Post-column additon of solvents, such as isopropanol, facilitates the electrospray process by reducing the surface tension.
- Post-column additon of DMSO can be used to enhance sensitivity in shotgun proteomics.
- Addition of gases post column can facilitate the ionization process and significantly increase sensitivity.

Other possible applications include:

- Derivatization of a sample to improve electrospray sensitivity can be performed post column.
- Post-column addition can be used to displace an additive (e.g. TFA, HFBA) forming stronger ion pairs with the analytes with an additive forming weaker ion pairs (propionic acid). This methodology is known as the "TFA Fix".

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