INTRODUCTION

The optimal conditions for electrospray ionisation 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 mass spectrometry but ultimately have a negative impact on the electrospray process. Modifying the electrospray droplet ionization and desolvation process. Altering solvent properties post-column addition can be an effective technique to improve electrospray ionisation 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 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 solution (Figure 1). The two channels meet after the chromatographic separation and prior to the emitter leaving insignificant dead volume. The PCA iKey was used for the post-column addition of a liquid or gaseous modifier can be an effective technique to improve electrospray ionisation 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.

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 samples showed a significant increase in sensitivity when 0% (green) vs. 5% (red) IPA was used as a post-column modifier solution (Figure 2). Examples of modifiers that include solutions of ammonium fluoride, triethylamine, and ammonium formate are introduced into the electrospray environment by post-column addition of modifiers to the LC solvent. The post-column addition of iliopropyl alcohol enabled enhanced detection of the more hydrophilic compounds, including ibuprofen metabolites.

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 hydrophobic 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.

2. Post-Column Addition of Vapor Modifiers

2.1. Effect of Ammonia on Estrogens

There are numerous ways that post-column addition can be used to displace an ion pair with the analyte with an additive forming a weaker ion pair. This methodology can be used to improve electrospray sensitivity. The following modifiers were experimented with: ammonium fluoride, ammonium formate, ammonium hydroxide, triethylamine and ammonia gas. One example of the vapor effect is illustrated in Figure 2. The analysis of estrogens in negative ion mode using vapor assisted electrospray showed a significant increase in sensitivity when exposed to gaseous ammonia.

CONCLUSION

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

- Post-column addition of solvents, such as isopropanol, facilitates the electrospray process by reducing the surface tension.
- Post-column addition of gases, such as ammonia gas, can be used to facilitate deprotonation and enhance sensitivity in shotgun proteomics.

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, HFA) forming stronger ion pairs with the analytes with an additive forming weaker ion pairs (propanol acid). This methodology is known as the “TFA Fix.”