Reactive Flowing Atmospheric Pressure Afterglow for Derivatization Analytes in *Real-time*

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Abstract

Plasma-based ambient mass spectrometry (AMS) allows desorption/ionization of analytes in real-time to minimize the typically long time required for sample preparation in mass spectrometry. In addition, the unique reactive plasma environment of plasma-based AMS sources has been proposed for performing real-time, on-line derivatization chemical reactions to improve the desorption and ionization efficiency of analytes that have low vapor pressure or proton affinity. The flowing atmospheric pressure afterglow (FAPA) AMS source has been shown to be very effective in the analysis of many different compounds.[1] In this study, reactive FAPA is used for derivatization of benzaldehyde, cyclohexanone and acetic anhydride in *real-time* using methylamine (MA) as derivatization reagent. Imine formation takes place in derivatization of benzaldehyde while acylation reaction takes place in derivatization of acetic anhydride. The reactive FAPA source relative position, discharge current and helium gas flow rate were optimized. The reactive FAPA using MA as derivatization reagent was also implemented in applications to improve limits of detection (LOD) of steroids and UV filter analytes.

Introduction

Ambient mass spectrometry allows direct sample desorption and ionization with minimal-to-no sample preparation. Plasma-based AMS sources employ various types of plasmas to generate reagent species that ionize analytes with a wider polarity range than spray-based AMS. However, plasma-based AMS techniques may present inadequate LOD for some classes of analytes. A solution to overcome these limitations is derivatization (chemical modification of analytes' properties) but this is typically a time-consuming sample preparation step. In contrast, plasma-based AMS provides a unique reactive environment with potential for online/real-time derivatization.

Steroids are important components of cell membranes. However, they are only present in low abundance so that improving the detection sensitivity of them is important. Pregnenolone acetate (P5A) is a synthetic pregnane steroid that can be used for a skin-conditioning and skin anti-aging reagent. Ethylhexyl methoxycinnamate (EMC) and octocrylene (OCR) are used in UV filters to protect skin from UV light. However, their concentrations are strictly regulated. The detection sensitivity of these compounds is low due to inadequate desorption/ionization efficiency. To solve this problem, recently there were reports implementing derivatization in real-time combined with low temperature plasma (LTP) AMS to enhance the detection sensitivity.[2][3]

In the present study, reactive flowing atmospheric pressure afterglow (FAPA) is developed to overcome low desorption or ionization efficiency limitations for some groups of analytes.

Method

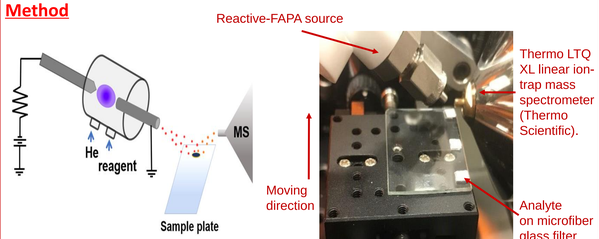


Figure 1. Reactive FAPA source geometry

Figure 2. High-throughput sampling

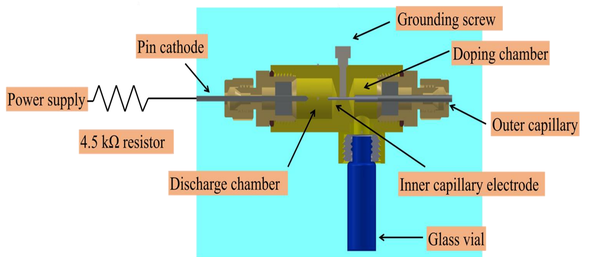


Figure 3. Reactive FAPA source design

Method

The reactive FAPA source design has a pin-to-capillary configuration. A negative DC voltage is applied to the pin cathode through a ballast resistor of 4.5 kΩ while the inner capillary electrode is grounded, leading to a glow discharge generated in the discharge chamber between the two electrodes with a voltage of ~450 V and current ~30 mA. The inter-electrode distance is set to 6 mm and helium is used as the discharge gas. A 2 mL glass vial served as the derivatization reagent container is connected to the ceramic doping chamber and the derivatization reagent vapor is incorporated into the effluent by the venturi effect. Analytes on the microfiber glass filter passing by the MS sampler one by one via the motorized stage. The mass spectra are collected by Thermo LTQ XL linear ion-trap mass spectrometer (Thermo Scientific).

Mechanism of Imine Formation Reaction

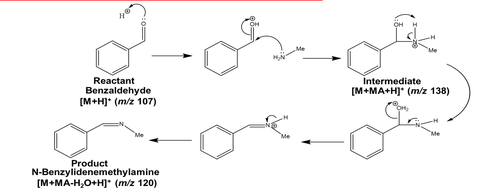


Figure 4. Reaction mechanism of benzaldehyde derivatized by methylamine

Full MS of benzaldehyde in reactive FAPA

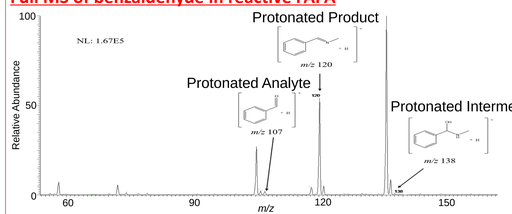


Figure 5. Full MS of benzaldehyde in reactive-FAPA

Full MS of cyclohexanone in reactive FAPA

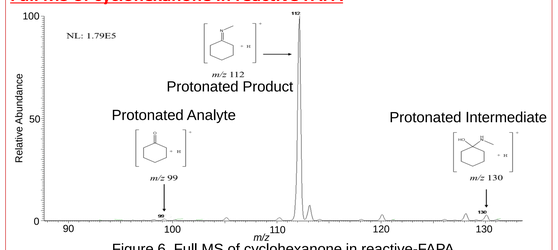


Figure 6. Full MS of cyclohexanone in reactive-FAPA

Full MS of acetic anhydride in reactive FAPA

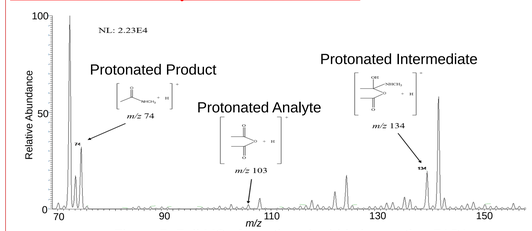


Figure 7. Full MS of acetic anhydride in reactive-FAPA

Optimization of FAPA operating conditions for Benzaldehyde

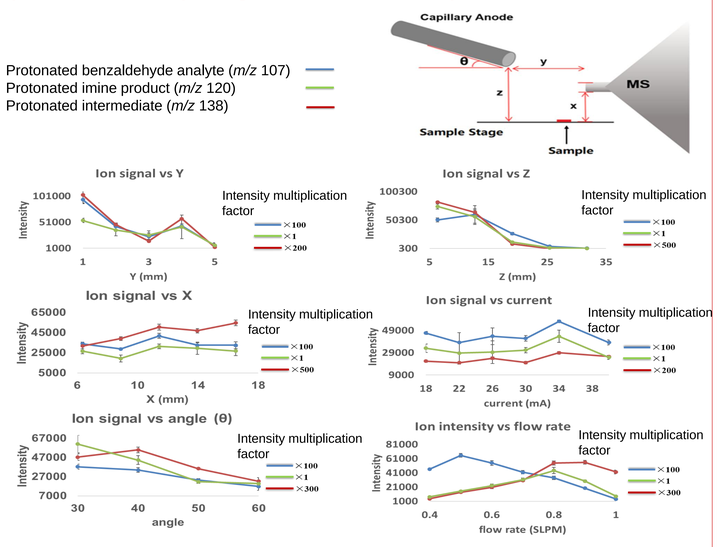


Figure 8. Optimization of reactive FAPA parameters for benzaldehyde.
Note: All signal intensities of protonated benzaldehyde analyte (*m/z* 107, blue trace) and protonated intermediate (*m/z* 138, red trace) were multiplied by a factor shown in the plot legends for clarity.

Optimized parameters: X: 11 mm, Y 1 mm, Z: 11 mm, angle: 40° Current: 34 mA, helium flow rate: 0.8 SLPM.

Optimization of FAPA operating conditions for Octocrylene (OCR)

Protonated OCR analyte (*m/z* 362)
Protonated intermediate (*m/z* 393)

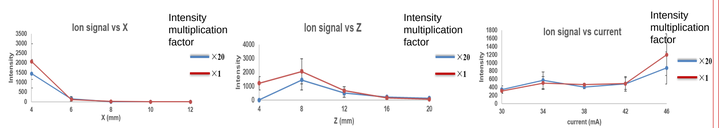
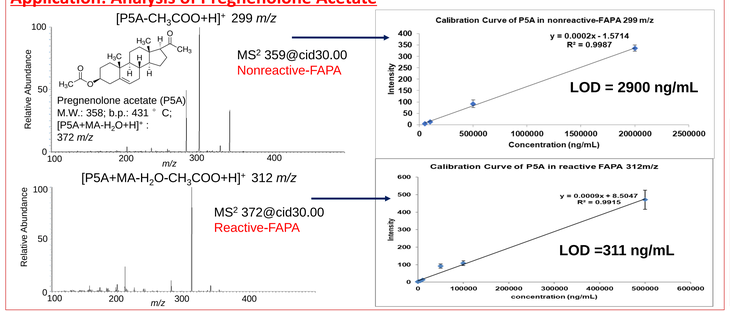
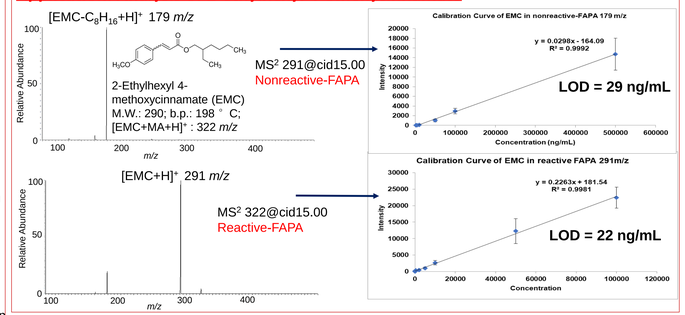


Figure 9. Optimization of reactive FAPA parameters for octocrylene.
Note: Protonated product is not detectable.
Optimized parameters: X: 4 mm, Z: 8 mm, current: 46 mA, other parameters were similar to small molecules as benzaldehyde.

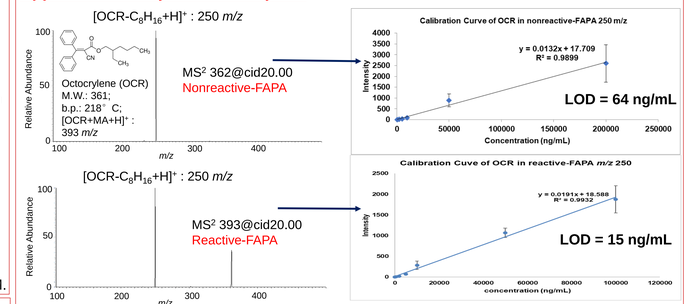
Application: Analysis of Pregnenolone Acetate



Application: Analysis of 2-ethylhexyl 4-ethoxycinnamate



Application: Analysis of Octocrylene



Conclusion

1. Reactive FAPA MS was successfully implemented for the on-line derivatization of benzaldehyde, cyclohexanone, and acetic anhydride using methylamine as the derivatization reagent.
2. The trends in the optimization plots show that desorption is the limiting factor at different FAPA source relative positions since both intermediate and product follow the same trends as analyte. However, residence/reaction time will become the limiting factor when the flow rate is increased.
3. Quantitation of steroid-type analyte as well as UV filter model compounds by reactive-FAPA using methylamine as derivatization reagent shows LODs down to tens of ng/mL scale. The enhancement factor compared with nonreactive-FAPA is up to 10X higher.
4. Reactive-FAPA AMS shows the potential for analyzing steroids in drugs and UV filters in personal-care products in real-time.

Future Work

1. Variety of ketone-based steroid analytes will be tested.
2. Other derivatization reagents will be further studied for imine formation and acylation reactions.
3. Different derivatization reactions such as esterification will be studied.

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Reference

[1] Shelley, J.T., Wiley, J.S., & Heiftje, G. M. (2011). Ultrasensitive ambient mass spectrometric analysis with a pin-to-capillary flowing atmospheric-pressure afterglow source. Analytical chemistry, 83(14), 5741-5748.

[2] Ding, X. L.; Garikapati, V.; Spengler, B.; Heiles, S.(2018). Analysis of ketone-based neurosteroids by reactive low temperature plasma mass spectrometry. Rapid Communications in Mass Spectrometry, *32*(16), 1439-1450.

[3] Ding, X. L.; Gerbig, S.; Spengler, B.; Schulz, S. (2018). Reactive Low Temperature Plasma Ionization Mass Spectrometry for the Determination of Organic UV Filters in Personal Care Products, Talanta, 178, 780-787.