Fast Reaction Monitoring of Reductive Amination using Chip-Based FAIMS-MS

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Overview: A chip-based FAIMS device has been evaluated for fast reaction monitoring of reductive amination, in combination with time-of-flight (ToF) mass spectrometry. The technique provides good separation of the structurally similar imine and amine ions, and allows monitoring of both ions over time and results show that chip-based FAIMS-MS can be used to monitor reaction kinetics

1. Introduction

UPLC-MS is commonly used for reaction monitoring within the pharmaceutical industry. While the methodology is well-established and reliable, there are some disadvantages. Water-sensitive intermediates can be hydrolyzed during LC runs and short-lived intermediate may not survive the typical 2-5 minutes needed for the state-of-the-art LCMS. In addition, during the reaction optimization stage, where hundreds of reaction conditions are explored simultaneously, a much faster analytical methodology with high specificity is desired. High-field asymmetric waveform ion mobility spectrometry (FAIMS) separates ions on the basis of differential mobility under alternating low field and high field conditions and is orthogonal to LC and *m/z* separation[1-3] and chip-based microscale FAIMS in particular offers the fast scanning speeds desirable for reaction monitoring.

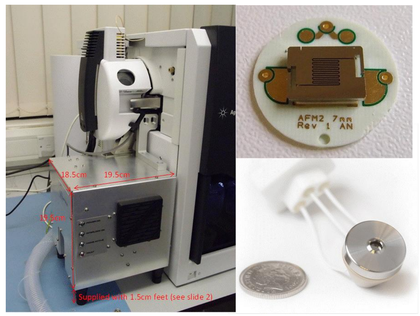
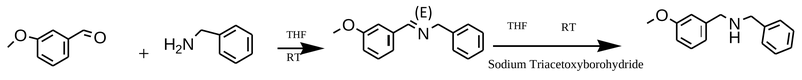


Figure 1 Prototype chip-based FAIMS device installed on Agilent TOF, with close-ups of the FAIMS chip

2. Methods

In this study, ultrafast chip-based FAIMS-MS for reaction monitoring is evaluated for a reductive amination reaction.



m- anisaldehyde Benzylamine imine Benzyl-(3-methoxy-benzyl)-amine

10 µL of all samples were diluted in 20 mL acetonitrile (ACN) with 0.1% formic acid and infused into infused into an Agilent 6230TOFMS equipped with chip-based FAIMS system (Owlstone Ltd). Electrospray conditions were optimised as described in Table 1.

Table 1: Experimental conditions

MS/ESI parameters	Initial Experiments	Reaction monitoring experiment
Drying gas temperature	150C	150C
Drying gas	10 L min^{-1}	7 L min^{-1}
Nebulizer	15 psig	20 psig
Sheath gas temperature	250C	250C
Sheath gas flow	12 L min^{-1}	12 L min^{-1}
Liquid flow rate	50 µL min^{-1}	50 µL min^{-1}
Capillary voltage	3500V	3500V
Nozzle voltage	2000V	2000V

3. Results

Initial experiments were carried out to optimise the separation of the imine intermediate ion (*m/z* 226.12), which would be invisible by HPLC-MS, and the amine product ion (*m/z* 228.14).

FAIMS sweeps were performed at dispersion fields (DFs) of 200 to 300 Td with compensation field (CF) sweep from 0Td to 6Td. Ion chromatograms were extracted using custom Agilent software to plot ion abundance against CF value. Runs were carried out both without modifier vapors and with ACN at various concentrations. Figure 2 shows the original (non-modifier) best separation (DF 240Td) and the separation obtained with ~2% ACN (also at DF 240Td) – note that peaks are shifted to more negative CFs with this modifier, therefore the CF sweep covered a -4 Td to +2 Td range.

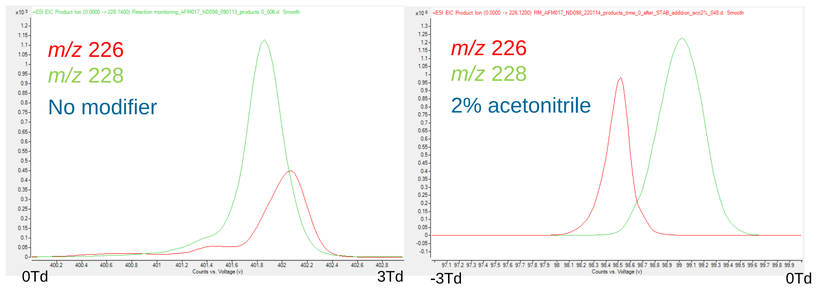


Figure 2 Optimised separations of imine intermediate ion (*m/z* 226.12) and the amine product ion (*m/z* 228.14) at a DF of 240 Td (left) without drying gas modifier and (right) with 2% acetonitrile in the drying gas.

A set of time series experiments to monitor the full reaction was set up using the optimized parameters. The FAIMS system was set to scan a CF range of -4 to 0Td at 240Td DF. m-anisaldehyde and benzylamine were mixed in tetrahydrofuran at ambient temperature. The reaction proceeded for 30 minutes to allow build up of the imine intermediate, (E)-N-(3-methoxybenzylidene)-1-phenylmethanamine. 10 µL of solution was taken at 0, 10, 20 and 30 minute time points and analysed immediately.

Abundances of each ion were taken and plotted against time. Three repeats were made of each measurement. Benzylamine (*m/z* 108.08) abundance decreased as the intermediate (*m/z* 226.12) abundance increased, with steady state reached after approximately 20 minutes, in agreement with IR data (Figure 3).

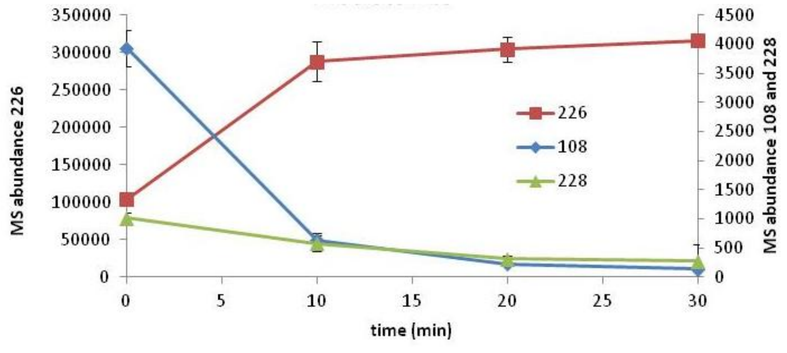


Figure 3 Time series experiments for conversion of m-anisaldehyde and benzylamine to the imine intermediate, (E)-N-(3-methoxybenzylidene)-1-phenylmethanamine.

After 30 minutes, sodium triacetoxyborohydride (STAB) was added to form the final amine product, N-benzyl-1-(3-methoxyphenyl)methanamine. 10 µL of the product solution was taken at 10 minute intervals between 0 and 60 minutes.

After addition of STAB, the abundance of the 226 ion immediately starts to rapidly decrease, with a corresponding increase in 228, as expected. Maximum abundance of the product ion (*m/z* 228.14) was reached about 20 minutes after STAB addition where it then stabilised (Figure 4).

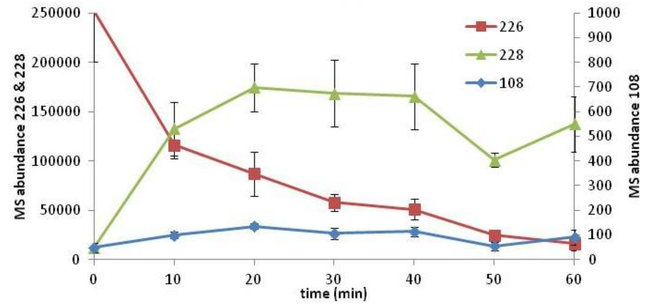


Figure 4 Time series experiments for conversion of the imine intermediate ,(E)-N-(3-methoxybenzylidene)-1-phenylmethanamine into N-benzyl-1-(3-methoxyphenyl)methanamine after STAB addition.

The plots below show the stability (left) and reproducibility (right) of the FAIMS peak CF positions. Over time, the average positions remain consistent (left plot). Repeats at each time point show some variation, although this is small compared with the separation of peaks and the peak widths. We believe this is due to instability in the modifier concentration and work is on-going to improve this part of the apparatus.

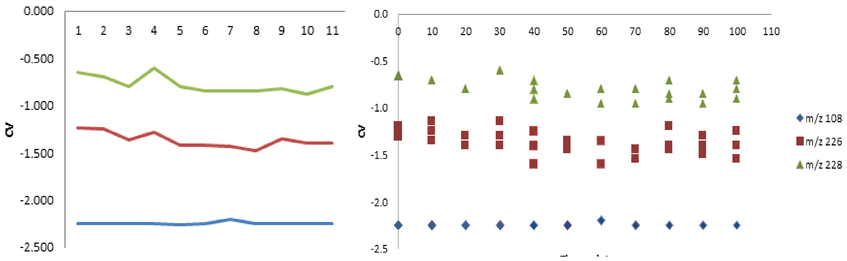


Figure 5 CF stability and reproducibility plots of benzylamine (m/z 108) (E)-N-(3-methoxybenzylidene)-1-phenylmethanamine (m/z 226) and N-benzyl-1-(3-methoxyphenyl)methanamine (m/z 228)

4. Conclusions

- Chip-based FAIMS-MS can be used to monitor reaction kinetics.
- The technique provides good separation of the structurally similar imine and amine ions, and allows monitoring of both ions over time, in contrast with prior UPLC work where the intermediate cannot be monitored.
- Analysis was much faster, at ~20secs/repeat, compared to 2-5 mins for UPLC-MS.

References

1. Shvartsburg AA, Tang K, Smith RD, Holden M, Rush M, Thompson A, Toutoungi D., *Anal.Chem.,* 2009; **81**, 8048
2. Brown, L.J., Smith, R.W., Reynolds, J.C., Toutoungi, D., Bristow, A.T., Ray, A., Sage, A., Wilson, I.D., Weston, D.J., Boyle, P., Creaser, C.S., *Anal Chem.*, 2012, **84**, 4095
3. Smith R.W., Toutoungi D.E., Reynolds J.C., Bristow A.W.T., Ray A., Sage A., Wilson I.D., Weston D.J., Boyle B., Creaser C.S., *J. Chrom. A.*, 2013, **1278**, 76

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