Helium Conservation in LC-FAIMS-MS Analysis

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Overview

Purpose: To explore methods by which helium consumption in Thermo Scientific FAIMS (high-Field Asymmetric waveform Ion Mobility Spectrometry) technology could be reduced during the analysis of samples. Helium was only used for 9% of the total run time resulting in conservation of this natural resource.

Method: Rat plasma extracts, from four different source animals, containing minoxidil and internal standard were injected onto a C18 column. The compounds were eluted using a standard gradient into the FAIMS (standard conditions) and monitored by SRM in positive heated electrospray ion mode. Helium in the FAIMS cell (helium:nitrogen 1:1 mixture) was used only just before and after peak elution (0.5 min per sample).

Result: LC-FAIMS-MS/MS significantly reduced interferences. At low concentration, the signal-to-noise ratio (S/N) was improved three times. At higher concentration, S/N did not improve but FAIMS provided increased precision. Interferences in the chromatograms due to variability of the rat plasma extract resulted in variable S/N. FAIMS chromatograms have greatly reduced interferences and S/N variability is reduced from 17% to 8% RSD.

Novel aspect: Helium was used only during chromatographic peak elution resulting in approximately ninety percent savings of helium.

Introduction

Bioanalytical LC-MS/MS doesn't always show interferences because LC-MS/MS is very selective. In cases of low selectivity, it is usually the complex matrix that causes interferences. Interferences may cause variable baselines which prevent precise characterization of the chromatographic peaks. Co-eluting interferences may cause bias which affects the accuracy of an assay. FAIMS is a selectivity technique that helps remove these interferences. One of the chief considerations during FAIMS analysis is the consumption of helium which is frequently between 1.5 and 2 litres per minute. One bottle of helium may be consumed within three days at this rate. Considering that helium supplies are limited due to natural deposits, and that this gas is expensive and not readily available in some countries, it was important to explore methods by which helium consumption could be reduced during the analysis of samples.

There are three ways to reduce helium use. The first is to develop modified FAIMS hardware that provides the same sensitivity and selectivity but does not use helium. Current FAIMS devices may operate with nitrogen-only (0.1 – 1% transmission), but benefit with helium relative to sensitivity (30 – 50% transmission).(1, 2) The second helium reduction method is to use less than the optimal 50% helium in nitrogen proportion.(3) The third way is to use helium only when necessary. In the case of targeted analysis, the helium could be turned on 0.25 min before compound elution. After the compound elutes the helium may be turned off.

This poster shows how to reduce helium consumption by using it only when necessary. The work will also demonstrate cost and resource savings through the use of this method.

Methods

Chemicals and Reagents
Minoxidil, lyophilized rat plasma, acetonitrile, water, formic acid.

Sample Preparation
Plasma from four different source animals was reconstituted and to each was added three volumes of acetonitrile. After centrifugation, the supernatant was used to prepare solutions of minoxidil (5ng/mL) and testosterone.

LC – FAIMS – MS/MS Conditions
A Thermo Scientific Surveyor MS pump was used. Injection volume was 10uL and run time was 7 minutes. The column was a Thermo Scientific Hypersil GOLD C18 3µm, 2.1x50mm. Mobile phase A – water with 0.1% formic acid; Mobile phase B – acetonitrile with 0.1% formic acid. All injections were made with a gradient beginning at 10%B holding for 1 min. Then B was ramped to 90% over 3 min and held for 0.8 min.

The FAIMS system was used with standard conditions. FAIMS was attached to the MS (Thermo Scientific TSQ Vantage) using source conditions: spray voltage 4kV, vaporizer temperature 500°C, sheath and auxilliary gases 40 and 10, respectively. Capillary temperature was 270°C.

MS transitions for minoxidil (***m/z*** 210 > 164 @ 24eV and **m/z** 210 > 193 @ 14eV) were combined for S/N and abundance determinations.

FIGURE 1. FAIMS is an ion filter between the LC effluent and the MS inlet. Ions with unstable trajectory are neutralised and swept away. Selected ions are transferred to the MS and contribute to the ion current profile.

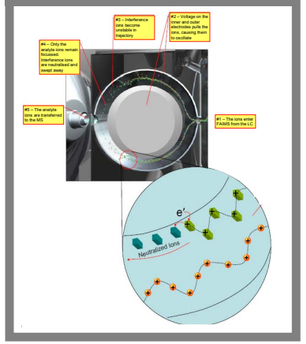
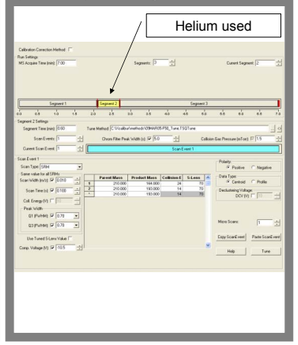


FIGURE 2. The triple quadrupole MS was set to contain three time segments. From 0 to 2.1 min a method using no helium was used. From 2.1 until 2.6 min, the method used 50% He. After peak elution, the third segment used no helium.



Results

Using FAIMS increases S/N at low concentration if interferences are present. Figure 3 shows the analysis of stanozolol in horse urine both by LC-MS/MS and LC-FAIMS-MS/MS.

FIGURE 3. Representative chromatograms from LC-MS/MS and LC-FAIMS-MS/MS for the analysis of stanozolol in horse urine demonstrating S/N increase at low concentration. Without FAIMS (black, left side, upper trace) the interferences overshadow the analyte peak and S/N is low. The ion filter successfully removed the interferences allowing better chromatographic peak identification, definition and high S/N (red, left side, lower trace).

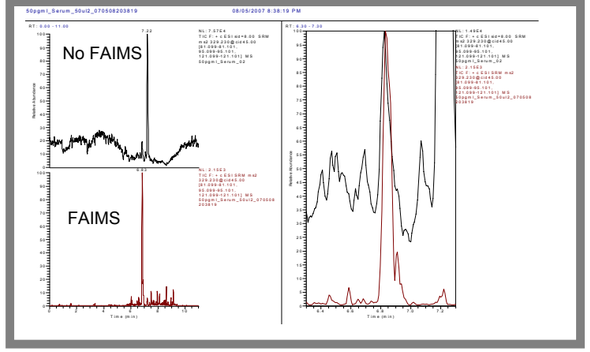
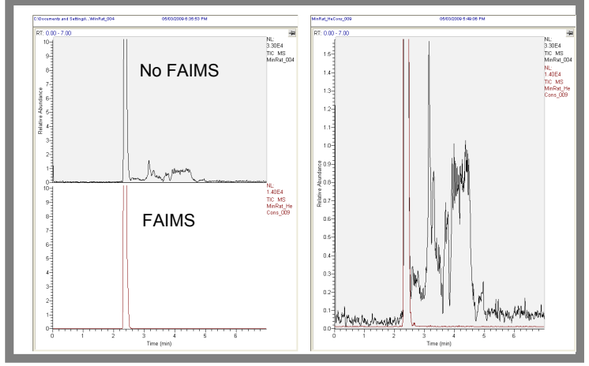


FIGURE 4. Representative chromatograms showing the analysis of minoxidil in rat plasma extract. Without FAIMS there are numerous interferences (left side, upper trace). Close inspection shows the chromatographic baseline to be rising during compound elution (right side, black trace). The FAIMS analysis is using 50% helium in nitrogen. Helium is used beginning 0.25 min before elution and ending 0.25 min after elution. Interferences are removed (red traces) demonstrating greater confidence in the peak integration.



In cases where analysis shows interferences, FAIMS may increase accuracy and precision particularly at the lower level of quantitation (LLOQ).(4) In order to examine only the precision of the analysis, samples in the higher concentration range were examined (5 ng/mL). Figure 4 shows samples of rat plasma extracts containing minoxidil. The minoxidil retention time is 2.35 min. Various sources of rat plasma give identical retention times, but varying levels and characteristics of interferences. The interferences elute at and after minoxidil.

FIGURE 5. Table of chromatographic peak areas and signal-to-noise ratios for minoxidil. The percent transmission with FAIMS is 45% relative to LC-MS/MS. The relative standard deviation (RSD) of the chromatographic peak area is identical for both analyses. Using helium conservation FAIMS, the signal-to-noise ratio is greatly improved (from 17% to 9% RSD). In this case, the benefit of using FAIMS arises from the precision of the S/N. The trade off in signal demonstrates more reliable S/N.

	Area		S/N	
Replicate	He Conservation	No FAIMS	He Conservation	No FAIMS
1	42149	111900	4190	4613
2	45546	90493	4204	3113
3	41475	97772	4005	3521
4	49467	102762	4734	3906
Avg	44659	100732	4283	3788
RSD	8%	9%	7%	17%
Area attenuation	44%			

At this high concentration, the level of interferences in these sources of rat plasma do not cause low area counts. The problem is not high interference – it is variable interference among plasma lots. This variation causes imprecise results as demonstrated by the high RSD in Figure 6. Analysis of minoxidil in four sources of rat plasma by LC-MS/MS provides acceptable precision on the chromatographic peak area (9% RSD), but S/N is very high (17% RSD). In comparison, with FAIMS the precision of the area and S/N is 8% and 7%, respectively. This demonstrates the effect of chromatographic baseline on precision. Note that the area counts have been reduced with FAIMS due to typical transmission efficiency on the order of 30 – 50%.

Figure 6 shows the calculated number of samples that may be analyzed using a standard helium tank. The helium cost per sample is only 4 euro cents.

FIGURE 7. Helium conservation results in considerable savings. For a five-minute run time, using helium only during compound elution (36 sec) results in saving 22 600 Euros ($30 000 US).

	Helium Conservation		
	Always on	36s / sample	Units
He use per sample	8.75	1.05	L
Samples per tank	954	7 952	Samples
He cost per sample	0,37 €	0,04 €	
Number of tanks required per year	73	9	Tanks
Cost per year	25 700 €	3 080 €	
Savings per sample	n/a	0,32 €	
Savings per year	n/a	22 593 €	
Assumptions:			
Samples per year	70 000		
He tank volume	8 350 L		
Rate of He use (when on)	1.75 L/min		
Helium cost per tank	350,— €		

Conclusions

FAIMS removes interferences. At low concentration, the analytical results show increased accuracy and precision. At high concentration, the analytical results show increased precision of S/N. Using helium only during compound elution results in an eight-fold savings in helium consumption from 1 000 to 8 000 samples per tank. This results in cost and resource savings.

References

(1) Mabrouki, R., et al. Improving FAIMS Sensitivity Using a Planar Geometry with Slit Interfaces. *J. Am. Soc. Mass Spectrom.* **2009**, in press.
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(3) Chen, Chung-Yu; Lee, Maw-rong. *Analysis of ketamine and its metabolites in urine by LC-FAIMS-MS.* National Chung-Hsing University, Taichung, Taiwan. ASMS 2009, #TPM324.
(4) Xia, Y.Q., et al. LC-FAIMS-MS/MS for quantification of a peptide in plasma and evaluation of FAIMS global selectivity from plasma components. *Anal. Chem.* **2008,** 80 (18), 7137-7143.

Acknowledgements

Keeley Murphy, Louis Maljers, and John Nemmers

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