

FAIMS for Drug Discovery and Development Using an Ion Trap and a Triple Quadrupole MS

Keeley Murphy, Kevin Cook, Julie Horner, James Kapron, Nicholas Duczak, Jr., Mark Harrison

Thermo Fisher Scientific, San Jose, CA



Overview

Purpose: The selective removal of interferences present in complex biological matrices such as plasma, urine and food, as well as interferences found in common drug vehicles, such as polymers, by using high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) technology coupled with a triple stage quadrupole or ion trap mass spectrometer.

Methods: Direct sample infusion, as well as liquid chromatography (LC) coupled with FAIMS and a triple quadrupole mass spectrometer, and LC coupled with FAIMS and an ion trap mass spectrometer.

Results: FAIMS effectively removed the interference peaks contained in the samples using both the ion trap and triple quadrupole mass spectrometers. Quantitative samples analyzed using FAIMS and a triple quadrupole improved the limits of detection (LOD) by 10X and limit of quantification (LOQ) by more than 5X.

Introduction

The drug discovery process is constantly evolving, requiring sample analysis with increasingly selective (specific) and sensitive analytical techniques. Such analyses are often complicated by the presence of interferences in biological sample matrices as well as drug dosing vehicles. The removal of these interferences is critical for accurate and reliable sample analysis. Relying entirely on extensive sample preparation to remove such interferences before the analysis often results in expensive and time-consuming methods leading to an unacceptable loss in physical sample (low recovery). Patented Thermo Scientific FAIMS technology coupled with MS analysis provides improved selectivity and increased limits of detection, without the need for extensive sample preparation.

FAIMS technology removes interferences by using an asymmetric waveform to guide the ions of interest between temperature-controlled electrodes before they enter into the mass spectrometer. The electrodes are physically located in front of the entrance to the mass spectrometer, allowing FAIMS to act as a filter, removing the unwanted ions, including those with the same m/z as the analyte of interest. A compensation voltage is applied to control the specific ions that are allowed to pass through FAIMS and enter the mass spectrometer.

Samples were introduced to the FAIMS-MS system using Heated Electrospray Ionization (HESI). Analytes were detected using Selected Reaction Monitoring (SRM) on the triple quadrupole, and a selected mass scan range on the ion trap.

Methods

Chemicals and Reagents

Reserpine, Minoxidil, Lyophilized Rat Plasma, Methanol, Acetonitrile, Water, Formic Acid, PEG-600, Ammonium Acetate.

Sample Prep

A stock solution of Minoxidil was prepared from powder and diluted to 50 pg/mL using a 50:50 Water:Acetonitrile solution. Lyophilized Rat Plasma was reconstituted in water and then precipitated by the addition of Acetonitrile at a 3:1 ratio and centrifugation at 5000 rpm for 5 minutes. The supernatant was then diluted by 50% by the addition of Water. A five-point calibration curve from 0.5 pg/mL to 50 pg/mL was then made using the 50 pg/mL Minoxidil stock solution and the diluted Rat Plasma supernatant.

A solution containing 10 ng/mL of Reserpine, 100 ng/mL PEG 600, and 10 mM Ammonium Acetate was made using commercially available bottled reagents.

Mass Spec Conditions

Standard mass spectrometer (Thermo Scientific TSQ Vantage, Thermo Scientific LTQ Orbitrap XL) conditions were used including the following: Polarity Positive mode; Capillary Temperature: 350 °C; Spray Voltage: 4000V; Q2 gas pressure: 1.5 mTorr; Vaporizer Temperature: 500 °C; Scan Width: 1.0u; Sheath Gas Pressure: 45; AUX Gas 35; Scan Time: 20 milliseconds.

LC Conditions

Injection Volume: 5 μ L, Run Time: 2.5 min; Column: Thermo Scientific Hypersil GOLD C18 3 μ m, 2.1x50; Mobile A – Water with 0.1% formic acid; Mobile B – Methanol with 0.1% formic acid. All injections were made using a standard gradient ramping Mobile B from 5% to 95%.

FIGURE 1. Compensation voltage optimization of Reserpine at m/z 609.2

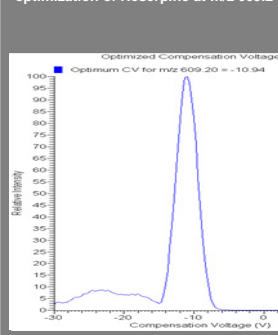
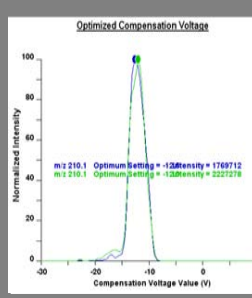


FIGURE 2. Compensation voltage optimization of Minoxidil using two transitions, m/z 210 \rightarrow m/z 193 and m/z 210 \rightarrow m/z 164



FAIMS Conditions

Standard FAIMS conditions were used: -5000V dispersion voltage; carrier stream of 4 L/min (equimolar nitrogen and helium); inner and outer electrode temperatures 70 °C and 90 °C, respectively. The compensation voltage parameter was determined at -12V (Reserpine) and -10V (Minoxidil).

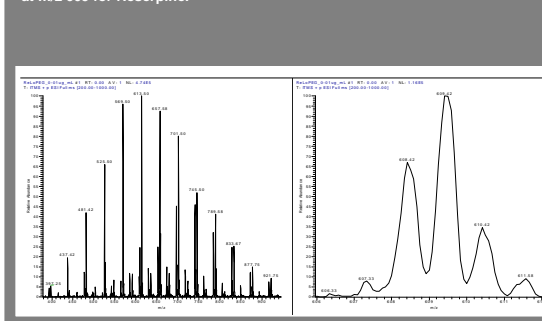
The Minoxidil calibration curve was analyzed on a triple quadrupole both with and without the FAIMS technique. Each calibration point was injected and analyzed using both LC-MS-MS and LC-FAIMS-MS-MS. The Reserpine/PEG-600 solution was infused directly into the ion trap and the sample was analyzed using full scan with and without FAIMS.

The compensation voltage for Minoxidil and Reserpine were both determined using automated software included with FAIMS (Figures 1 and 2).

Results

In the experiments using the ion trap and the triple quadrupole mass spectrometers, FAIMS effectively removed the interference peaks contained in both the samples and mobile phase that could not be removed using standard MS experiments or LC-MS-MS alone. The removal of these interferences enhanced the quality of the data by lowering the limit of detection and by removing overlapping peaks, thereby eliminating uncertain assignments of signal contribution, improving the confidence and reliability of the experimental results.

FIGURE 3. Reserpine/PEG-600 solution, 10 ng/mL conc., direct infusion at 5 μ L/min, analyzed without FAIMS. Right side displays expanded mass range at m/z 609 for Reserpine.



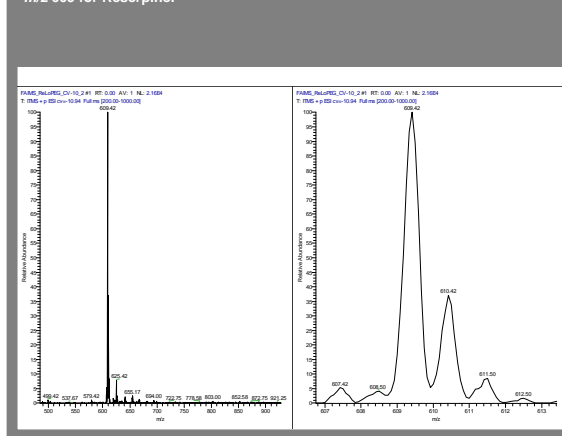
Analysis of the Reserpine/PEG-600 solution using the ion trap without FAIMS resulted in the presence of multiple ion intensities, characteristic of those in a PEG-600 envelope, which clearly overlapped with Reserpine ions. The presence of these overlapping interferences from the PEG-600 mask the isotope ratios of Reserpine and make qualitative analysis difficult (Figure 3).

Using FAIMS, analysis of the Reserpine/PEG-600 solution prevented the interfering ions from entering the trap. The ion signal contributed by the PEG-600 component of the sample solution was selectively removed resulting in isotopic abundances that more closely reflect the true nature of the analyte of interest (Figure 4).

The FAIMS technique, which allows only the analyte of interest to enter the trap, offers several benefits in drug discovery. First, space-charge effects in ion trap MS systems are reduced, enabling detection at lower concentration levels. Second, compound identification is more reliable due to the cleaner spectrum, regardless of trap resolution. Third, instrument sensitivity is further improved by permitting a longer injection time.

This experiment can also be used to selectively trigger a data-dependent MSn scan. In the case without FAIMS, the analyte MS peak is lost in the baseline noise. In contrast, with FAIMS the analyte forms the base peak and will readily be selected as the most intense ion for a data-triggered MS analysis.

FIGURE 4. Reserpine/PEG-600 solution, 10 ng/mL conc., direct infusion at 5 μ L/min, analyzed with FAIMS. Right side displays expanded mass range at m/z 609 for Reserpine.



Analysis of the Minoxidil standard curve samples using the triple quadrupole without FAIMS resulted in a high level of baseline noise, which prevented reliable peak integration and quantification at the low end of the curve (Figure 5). An increase in baseline noise can be seen at the retention time of Minoxidil due to interferences in the mobile phase that cannot be removed by LC-MS-MS alone. Using FAIMS, the overall intensity of a blank injection at its highest level is decrease by fifteen times when compared to the same injection made without FAIMS (Figures 5 and 6).

The removal of interferences in the baseline that are contributed by unknown components in the mobile phase with the same mass transitions allowed for an improvement in the limit of detection of ten times and an improvement in the limit of quantification of more than five times. Furthermore, peak integration at the lower end of the calibration curve was much more reliable due to the elimination of the baseline noise, adding a higher level of confidence and reliability to the experimental results (Figure 7).

FIGURE 6. Minoxidil/Rat Plasma solution 5 μ L injection, without FAIMS

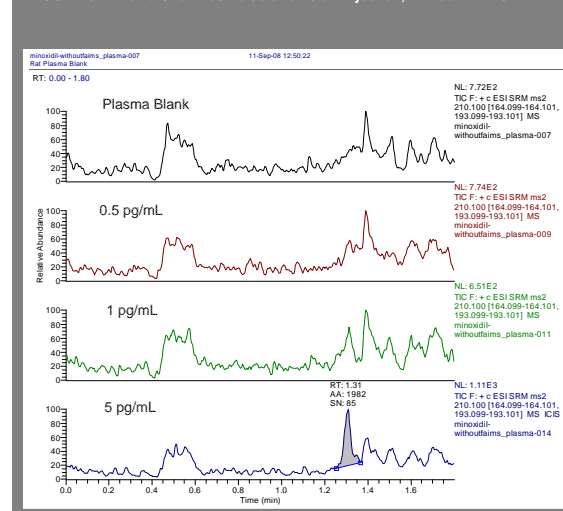


FIGURE 6. Minoxidil/Rat Plasma solution 5 μ L injection, with FAIMS

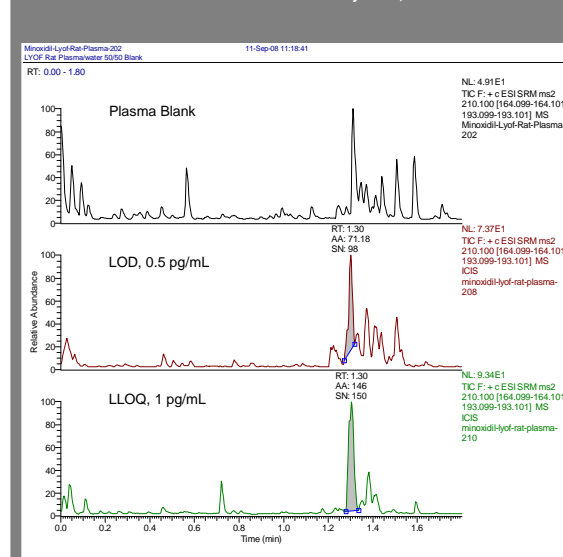
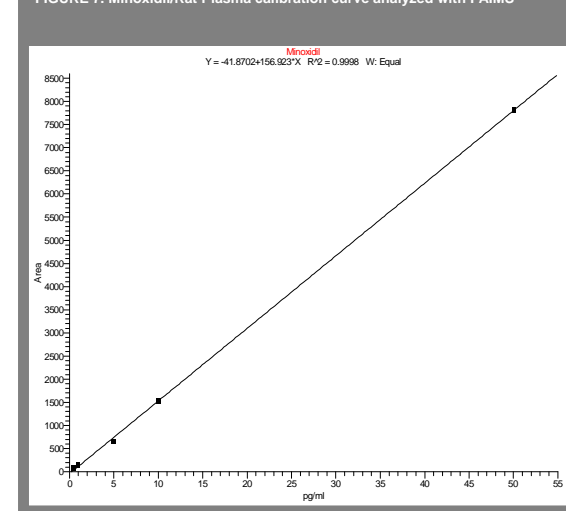


FIGURE 7. Minoxidil/Rat Plasma calibration curve analyzed with FAIMS



Conclusions

The FAIMS technique allows for the easy removal of interference peaks on both triple quadrupole and ion trap mass spectrometers without the need for additional sample clean up or preparation. For ion trap experiments, both selectivity and sensitivity were increased. This enables more complex sample analysis with a higher level of certainty and confidence in the results. For triple quadrupole experiments, FAIMS was utilized to improve the signal-to-noise ratio for the analyte peak, resulting in significantly lower limits of detection.

All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.