

Selectivity Improvement for Drug Urinalysis using FAIMS and H-SRM on the Finnigan TSQ Quantum Ultra

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Key Words

- Finnigan™ TSQ Quantum Ultra™
- Background reduction
- Drug discovery
- Increased selectivity

Goal

To improve the performance of an LC-MS/MS assay by utilizing the combined selectivity of high-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) and Highly Selective Reaction Monitoring (H-SRM).

Introduction

When developing LC-MS methods, reference standards are used to establish initial conditions. Applying these initial conditions to real-world biological samples presents significant challenges including reduced signal-to-noise ratio (S/N). Biological matrices add interferences and contribute to reduced method selectivity. Bioanalytical scientists have many tools to solve selectivity problems that arise during method development. The three common classes of tools include 1) Mass Spectrometry (MS) 2) Liquid Chromatography (LC), and 3) sample preparation.

The easiest approach to increasing selectivity is changing the mass spectrometer parameters. On triple quadrupole mass spectrometers, changing parameters such as the ion-entrance voltages or m/z transitions may result in reduced sensitivity without an increase in selectivity. The only MS parameter that offers increased selectivity without significant loss in sensitivity is highly-selective reaction monitoring (H-SRM). H-SRM is a unique feature resulting from the hyperbolic cross-section of the quadrupoles on the Quantum family of mass spectrometers.

The selectivity of the LC method may also be increased by changing the mobile phase (gradient, modifiers) and/or the stationary phase. These adjustments lead to the altering of all the associated method parameters (integration retention time window, column equilibration time, etc.) making this a time consuming endeavor. Modification of sample preparation procedures may include choosing between solid-phase extraction (stationary phase, wash and elute solvents), liquid-liquid extraction (extracting solvent, buffers) or simple solvent addition (protein precipitation, dilute-and-shoot). Revisiting sample preparation wastes time and resources because the samples are already prepared and available for analysis.

FAIMS is a new tool, available on the Finnigan TSQ Quantum Ultra, for increasing LC-MS method selectivity. This interface is installed in the atmospheric pressure region between the ion source and the mass spectrometer, allowing three complementary dimensions of separation to in the analysis: LC, FAIMS, and MS. Ions produced by the

H-ESI or APCI ionization source are sampled by the FAIMS device. These ions are then exposed to alternating high and low electric fields as they are pulled toward the mass spectrometer by a flow of gas. Behavior differences of the ions under the conditions of high and low electric field results in ion separation and an increase in selectivity.

This application note describes the advantages of using LC, FAIMS and H-SRM on the Finnigan TSQ Quantum Ultra to improve selectivity for bioanalysis, using norverapamil in human urine as an example.

Experimental Conditions

Chemicals and Reagents

Norverapamil hydrochloride and verapamil hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade methanol and formic acid were acquired from VWR (Mississauga, Canada). All chemicals were used as received.

Sample preparation: 25 mL of human urine was fortified with analyte and internal standard to a final concentration of 100 pg/mL. This stock solution was treated with 75 mL of 0.1% formic acid in water. The resulting solution (25 pg/mL) was analyzed directly.

Sample analysis: HPLC analyses were performed on a Finnigan Surveyor™ HPLC system (Thermo). Samples were injected onto a 2.1×50 mm embedded polar group reversed-phase column. The injection volume was 10 µL. The gradient LC method used mobile phases A (0.1% formic acid in water) and B (70:30:0.1 methanol/water/formic acid) at a flow rate of 0.4 mL/min. The gradient was as follows: (time, %B): (0, 10) (3, 100) (4, 100) (4.1, 10) (5, 10). The entire LC effluent from the sample injections was directed to the Ion Max source on a Finnigan TSQ Quantum Ultra.

MS Conditions

Ion source and polarity: H-ESI, positive ion mode
Spray voltage: +3000V
Vaporizer temperature: 350°C
Sheath gas: Nitrogen, 60 psi
Auxiliary gas: Nitrogen, 40 psi
Transfer tube temperature: 300°C
Transfer tube offset: 35V
Tube lens offset: 110V

The transitions monitored were m/z 441.25 \rightarrow m/z 165.10 for norverapamil and m/z 455.25 \rightarrow m/z 165.10 for verapamil, each at a collision energy of 26eV and scan time of 100 ms. The mass spectrometer selectivity was regulated by setting Q1 resolution to a peak width of either 0.7 or 0.2 u full-width half maximum (FWHM).

Additional gas-phase separation prior to entry of ions into the mass spectrometer was achieved by including FAIMS in the analysis.

FAIMS Conditions

- Dispersion Voltage: -4500V
- Outer Bias Voltage: 35V
- Compensation Voltage: -16V
- Tinner electrode: 40°C
- Touter electrode: 50°C
- Gas Flow Rate: 2.5 L/min
- Gas Composition: 50% He in N₂

Results and Discussion

A representative LC-SRM chromatogram for the analysis of norverapamil in human urine collected using unit mass resolution (0.7 u in both Q1 and Q3) is shown in Figure 1. Although LC-MS/MS is a selective technique, many isobaric interferences appear in the chromatogram. These isobaric

interferences increase the chemical background and may make reproducible integration of the analyte peak difficult. A representative LC – H-SRM chromatogram (0.2 u in Q1) is shown in Figure 2. H-SRM has removed most of the interferences.

An even further increase in selectivity is achieved by utilizing FAIMS and H-SRM in the LC-MS/MS analysis. Implementing FAIMS requires the establishment of conditions for the transmission of the desired analyte(s) through the interface. Stable conditions for ion transmission is expressed by the compensation voltage (CV). Figure 3 shows a CV scan during infusion of a norverapamil reference standard. The CV was ramped from 0 to -30 V in 1.5 min. The maximum response for norverapamil occurred at -16 V and indicated the appropriate CV for LC – FAIMS – H-SRM analysis. The structure of norverapamil is shown in the inset.

Figure 4 shows a representative LC – FAIMS – SRM chromatogram for norverapamil in human urine. For this chromatogram the FAIMS CV was set to -16V. Comparing this chromatogram with Figure 2, these two techniques eliminated different interferences. Next, the combined selectivity of FAIMS and H-SRM was explored.

To acquire an LC – FAIMS – H-SRM chromatogram for norverapamil in human urine, the FAIMS CV was set to -16V and Q1 peak width was set to 0.2 u. A representative LC – FAIMS – H-SRM chromatogram for the analysis

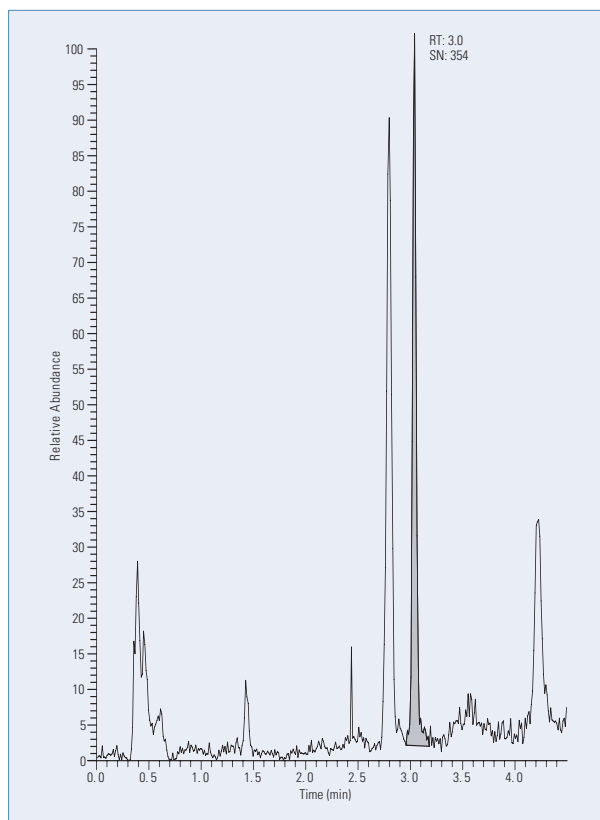


Figure 1: Representative LC – SRM chromatogram for norverapamil in human urine. Q1 peak width is 0.7 u FWHM

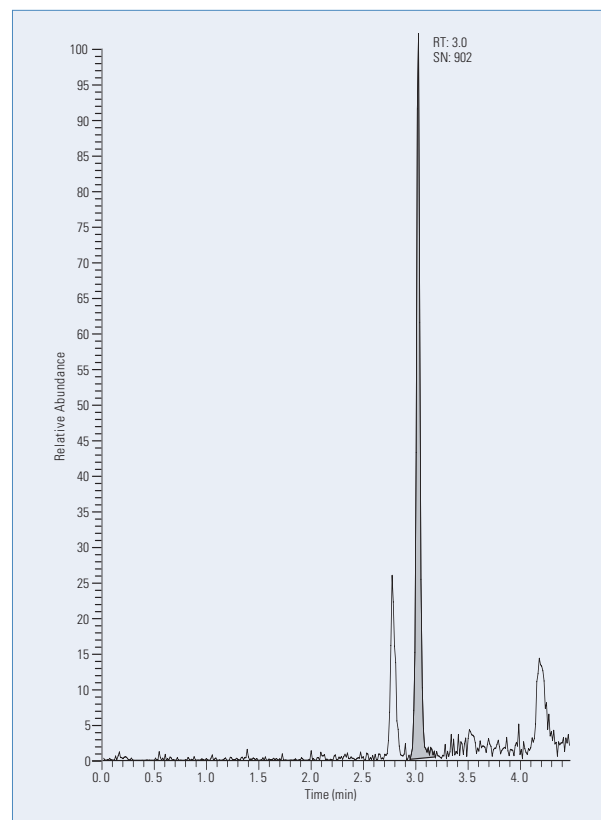


Figure 2: Using H-SRM. Representative LC – H-SRM chromatogram for norverapamil in human urine. Q1 peak width is 0.2u FWHM

of norverapamil in human urine is shown in Figure 5. The combined selectivity offered by FAIMS and H-SRM results in a cleaner chromatogram than the corresponding trace in Figures 1, 2, and 4. Very few interferences are

transmitted. Although a factor of three was lost in absolute signal, the “assay sensitivity,” as defined by signal-to-noise ratio, has increased five fold.

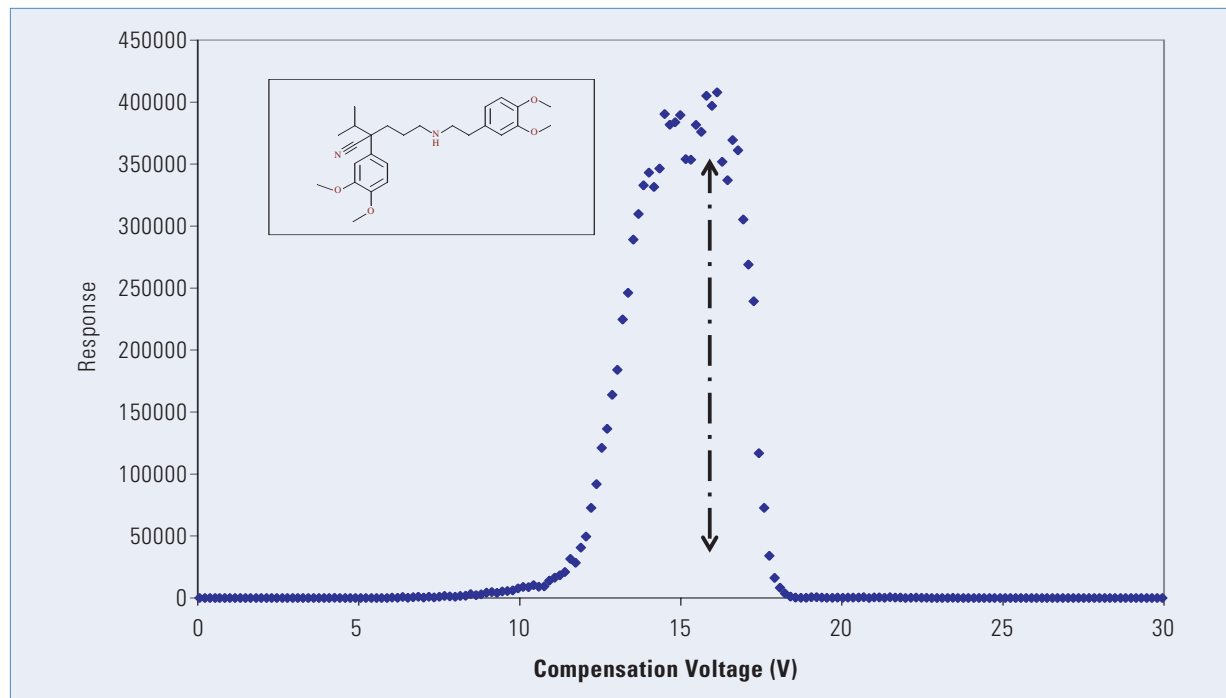


Figure 3: Compensation voltage scan for the infusion of norverapamil reference standard

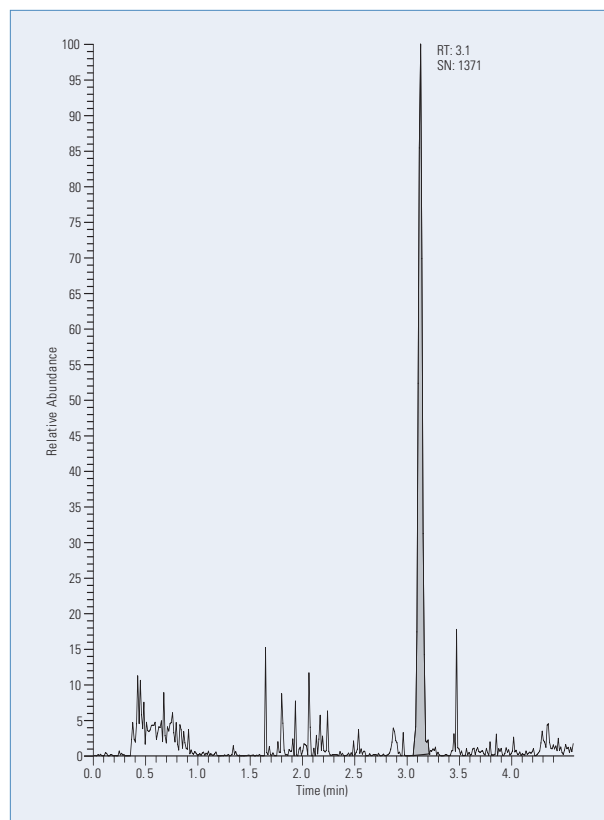


Figure 4: Using FAIMS. Representative LC – FAIMS – SRM chromatogram for norverapamil in human urine

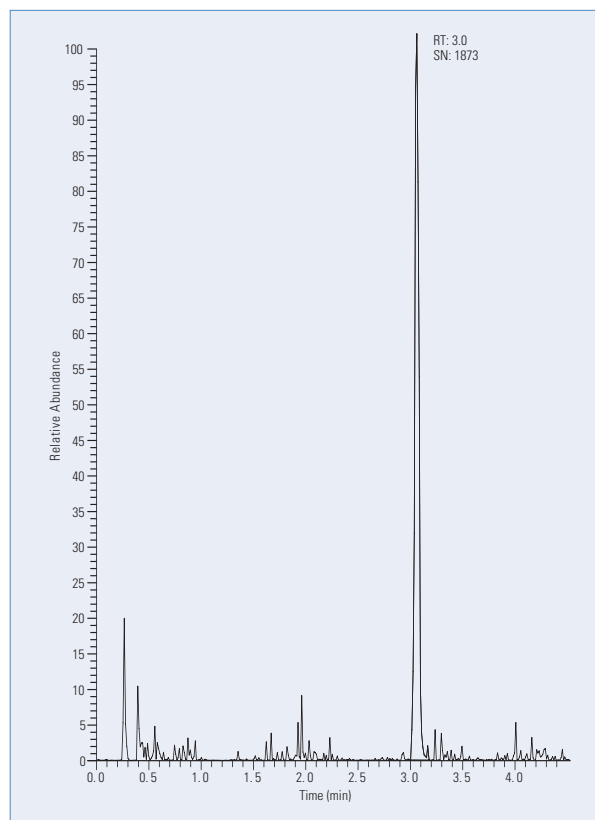


Figure 5: Using FAIMS and H-SRM. Representative LC – FAIMS – H-SRM chromatogram for norverapamil in human urine

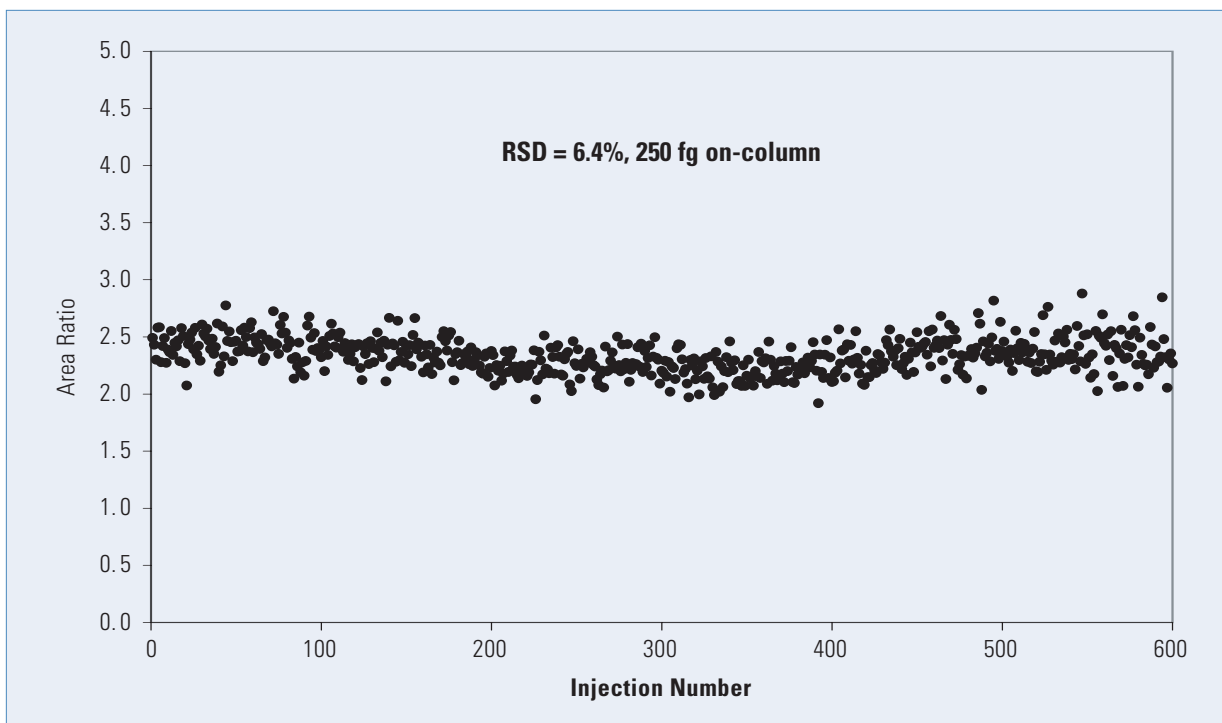


Figure 6: Robustness of FAIMS interface on Finnigan TSQ Quantum Ultra

Because complex biological samples are sprayed directly at the FAIMS interface, the robustness of the FAIMS-enabled system was evaluated. Figure 6 shows peak areas resulting from samples of norverapamil in urine injected every five minutes over a three-day time period. The %RSD for the analyses was 6.4%.

Conclusions

The increases in selectivity offered by the combination of FAIMS and H-SRM results in cleaner chromatograms and more easily and more reproducibly integrated chromatographic peaks. For the analysis of norverapamil in urine, the new method was developed without having to revert to modification of the LC method or sample preparation steps. The use of LC – FAIMS – H-SRM over LC – SRM reduced the chemical background and produced a five-fold increase in assay sensitivity.

References

Removal of metabolic interference during liquid chromatography/tandem mass spectrometry using high-field asymmetric waveform ion mobility spectrometry. Kapron et al. *Rapid Commun. Mass Spectrom.* 2005; 19: 1979-1983.

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