

# The Use of FAIMS to Separate Loperamide from PEG Prior to MS Analysis Using an LTQ XL

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## Key Words

- LTQ XL™
- Discovery PK
- FAIMS
- Impurity Analysis
- Ion Mobility

## Goal

To increase the sensitivity for a target compound in the presence of a high-level background impurities by removing the PEG dosing vehicle using a high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) gas phase separation prior to mass spectrometry analysis.

## Introduction

It is common practice in pharmaceutical development to conduct discovery phase pharmacokinetic (PK) studies. In these studies, dosing vehicles such as Tween 80, PEG 400, and methyl cellulose are used in high concentrations to dissolve the test compounds in dose formulations. Subsequent qualitative study and quantification of the test compounds must be carried out in the presence of the dosing vehicles, which can cause significant ion suppression and complicate full scan mass spectra. Conventional liquid chromatography may be used to separate the dosing vehicle, however this approach is generally less than 100% efficient, and LC/MS analyses still show the significant presence of polymers.

The effect of ion suppression combined with complicated full scan mass spectral data makes successful LC/MS analysis of targets difficult or impossible. Reducing or removing multiple chemical interferences may dramatically improve spectral quality and enhance full scan signal-to-noise for the analyte of interest. In this application note, we report the successful atmospheric pressure separation by FAIMS of a large excess of dosing vehicle (PEG) from a test compound (loperamide) **prior** to mass spectrometry analysis.

## Methods

HPLC: not used

Separation: Gas phase differential ion mobility: FAIMS

Gas Flow Rate: 1.5 L/min

Gas Composition: 50% He / 50% N<sub>2</sub>

CV Range: -40 to 0 V

CV Scan Time: 1.2 minutes

Sample Introduction: Infusion at 2  $\mu$ L/min

Mobile Phase: 50/50 ACN/H<sub>2</sub>O with 0.1% Formic Acid

MS: LTQ XL

Ionization: Positive NSI

Spray Voltage: 4000 V

Capillary Temperature: 200 °C

## Results

Full scan positive ion NSI mode mass spectra were acquired over the range from  $m/z$  300 to  $m/z$  1000 using an LTQ XL linear ion trap mass spectrometer equipped with FAIMS. The compensation (CV) voltage was scanned from -30 V to 0 V at an approximate rate of 30 V/min.

A full scan mass spectrum obtained using a standard Ion MAX™ source (no FAIMS separation) is shown on the left hand side of Figure 1. Without FAIMS separation, evidence of PEGs is found over the entire mass range of interest, including at the nominal mass of the target drug, loperamide. The mass range between 470 and 485 is expanded on the right hand side of Figure 1, which clearly shows that the <sup>13</sup>C isotope of the PEG at  $m/z$  476.42 coincides with the mono-isotopic peak for loperamide at  $m/z$  477.42. The typical PEG spectrum is characterized by a series of  $m/z$  species which are separated by 44 mass units across the mass range from 400 to 1000 in Figure 1. The presence of the PEG dosing vehicle in the sample makes isolation, clean fragmentation, and hence identification of the target drug difficult, particularly when the target analyte is at low concentrations.

Contrast the results in Figure 1 with FAIMS results shown in Figure 2 where the compensation voltage (CV) is scanned, and the PEG and loperamide are clearly separated. In the top panel of Figure 2 is an extracted ion trace for a 1.5 amu window centered at the nominal mass of singly charged, protonated loperamide ( $m/z$  477). The presence of two peaks in the trace indicates several possibilities, including the existence of two conformations of the same molecule or, as in this case, the presence of two molecules of the same nominal mass but with different structures.

In the middle left panel of Figure 2 is the full scan mass spectrum obtained at a CV centered on the blue peak, -21V; in the middle right panel of Figure 2 is the full mass spectrum obtained at a CV centered on the red peak, -12V. The full scan mass spectrum obtained at CV = -21V contains multiple contributions from the PEG dosing vehicle including the component at  $m/z$  476.42; the full scan mass spectrum obtained at CV = -12V contains a single component, loperamide, at  $m/z$  477.34. The full scan mass spectral signal-to-noise has been improved (c.f. Figure 1) by greater than an order of magnitude. Verification of the identity of each component is achieved by MS/MS analysis, the results of which are shown for the PEG in the bottom left panel of Figure 2 and for loperamide in the bottom right panel of Figure 2.

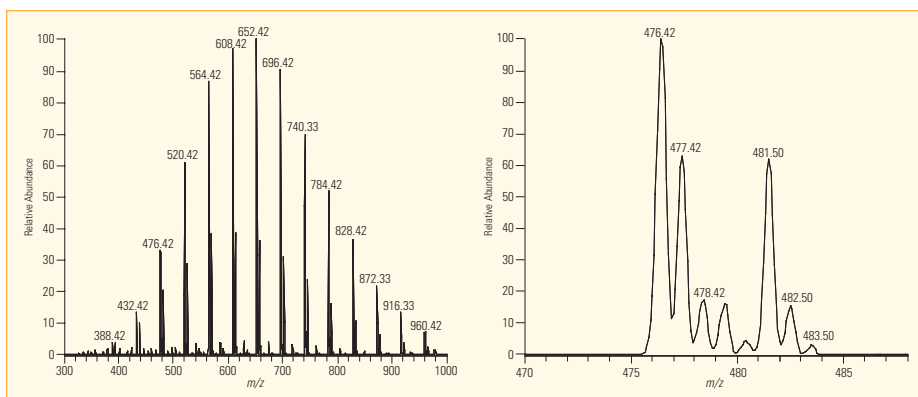


Figure 1

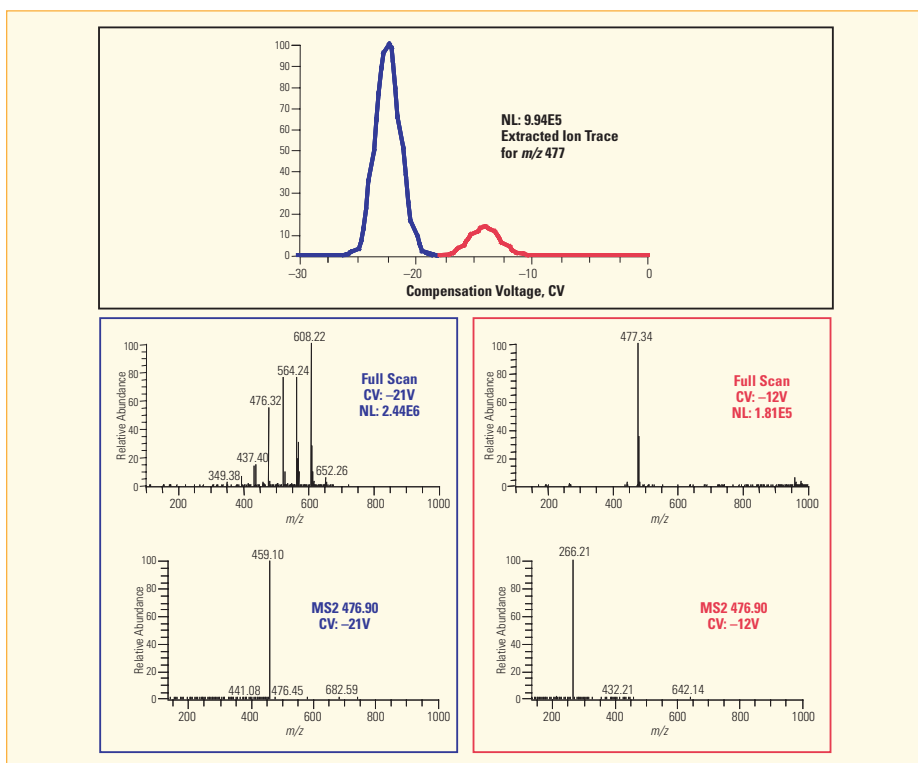


Figure 2

## Conclusion

In this document we report separation of loperamide from matrix interference at atmospheric pressure prior to entrance into the mass spectrometer using FAIMS. FAIMS in combination with the LTQ XL linear ion trap mass spectrometer made possible the detection and

identification of the target compound loperamide in the presence of a vast excess of the dosing vehicle PEG 600. The FAIMS-LTQ XL full scan mass spectrum signal to noise and sensitivity for the target compound was significantly increased.

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