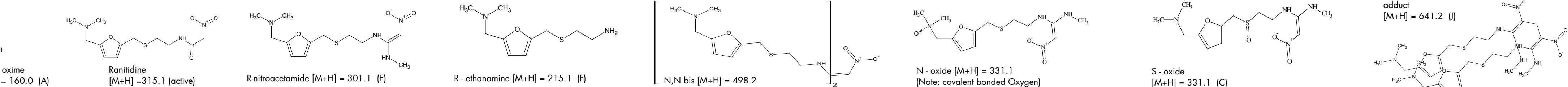


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## Introduction

Impurity profiling of pharmaceutical drug substances or dosage forms require methods involving high sensitivity and resolution for LC and MS alike as well as acceptable analysis time. FDA regulations require companies to develop methods of analysis to characterize not just the active pharmaceutical ingredient (API), but also the impurities and degradants that could arise from a particular synthesis process, drug substance provider, and/or storage conditions. To illustrate this methodology, ranitidine was selected for this study. Ranitidine is manufactured by numerous generic pharmaceutical companies. The histamine H<sub>2</sub>-receptor antagonist heals gastric and duodenal ulcers by reducing acid output as a result of H<sub>2</sub>-receptor blockage. A ranitidine assay and purity test are described in the USP, however a two stage process including thin layer chromatography (TLC) test and an HPLC assay is not the most efficient approach.<sup>1</sup> Other literature discussing a capillary electrophoresis (CE) method of analysis can be used for quantifying both ranitidine and the levels of the related substances with a 30 minute run time.<sup>2</sup> These analysis times are not acceptable for today's demanding pharmaceutical market.

In this poster, we will demonstrate the increased resolution and throughput achievable by "Ultra Performance Liquid Chromatography" (UPLC™) and show how the increased sensitivity combined with single quadrupole MS allows the identification of more impurities when compared to a conventional HPLC method that might be used today. Method development tools such as peak tracking by single quadrupole MS and usage of chromatography simulation software is applied to increase efficiency and confidence in the quality of the data collected. Although this technique may be application specific as proven with time, utilizing UPLC-PDA supported with MS data has the applicability for the fast tracking method development pace of today's pharmaceutical pipeline.

## Experimental

### Approach

The ideal approach would be to have authentic impurity standards and prepare standard solutions for use in the method development. In many situations, impurity standards are not readily available and forced degradation of the drug substance is an appropriate starting point to generate the degradants (from heating) or impurities (due from stress tests).

- Ranitidine (2mg/ml) which was investigated in this study, was forcefully degraded by refluxing at 85°C for 48 hours. Typically chemical stress tests are also performed as general practice, however the chemical stress tests that were applied showed no success of degradation.

LC method selection by traditional approaches of method scouting with four UPLC columns, various pH range, and temperature will be explored with a goal of maximum resolution and detection of maximum number of peaks while keeping in mind minimal run time.

Method optimization performed through assistance of method development simulation software (DryLab® 2000 plus)

Utilize development tools such as single quadrupole MS detection for peak identification, mass spectral and PDA-UV spectral analysis for peak tracking as well as dual acquisition to ensure maximum peak detection for any non-absorbent peaks in UV or MS alone

Compare to a typical HPLC method

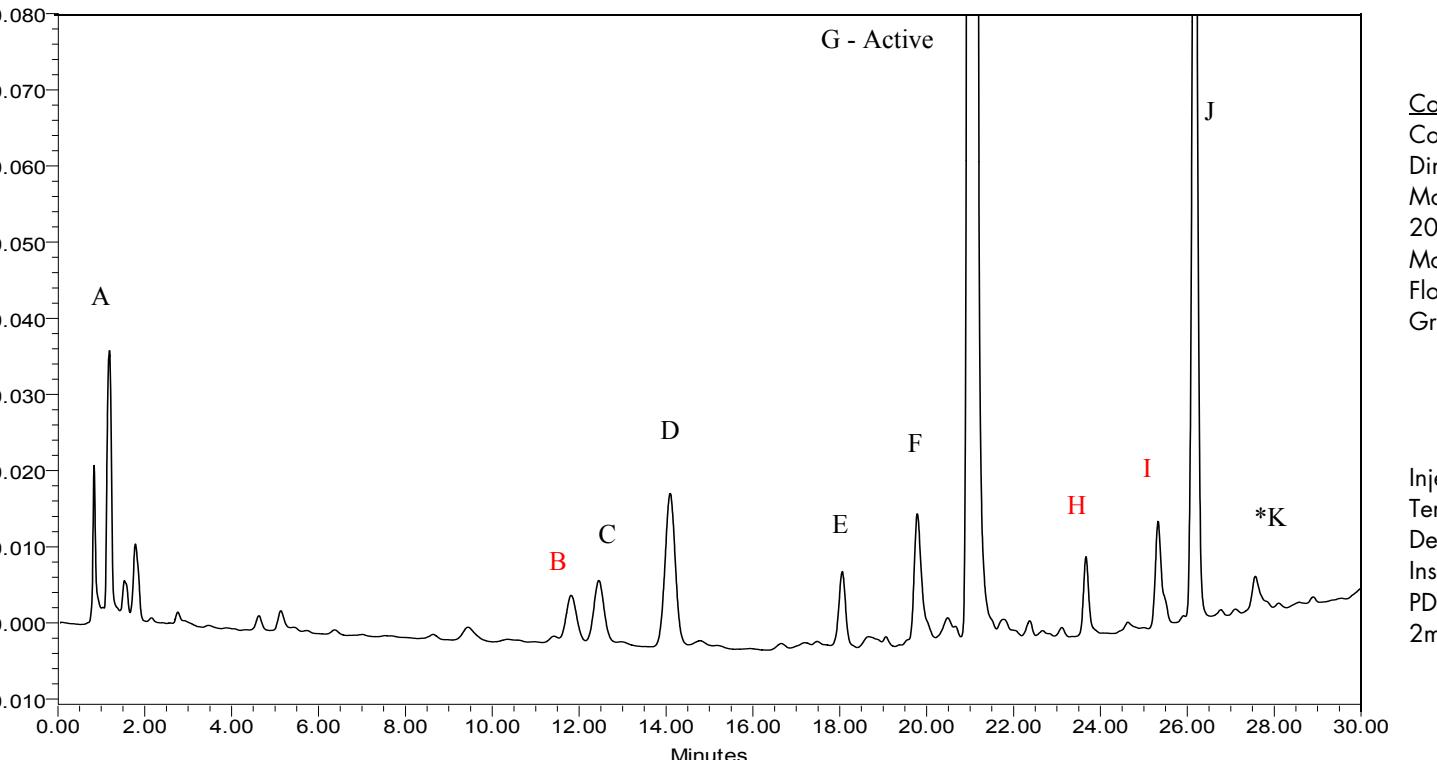
### Instrumentation

**UPLC Conditions**  
Column: ACQUITY UPLC™ BEH C<sub>18</sub>  
Software: Empower™ CDS  
Dimensions: 100 x 2.1mm, 1.7μm  
Mobile Phase A: 20mM Ammonium Bicarbonate  
Mobile Phase B: Methanol  
Weak Wash : 95:5 Water: MeOH 1200μL  
Strong Wash : 50:50 Water: MeOH 300μL  
Flow Rate: 0.45 mL/min  
Injection Volume: 1.0 μL  
Temperature: 50°C  
Detection: UV @ 230 nm  
Desolvation flow (L/Hr): 550

**Single Quad MS Conditions**  
Instrument: Waters® ZQ™ 2000  
Software: Empower™ CDS

**Tune Page Parameters:**

ES capillary (kV): 3.4	LM Resolution: 14
Cone (V): 15	HM resolution: 15
Extractor (V): 3	Multiplier: 650
RF Lens (V): 0	
Source Temp (°C): 350	Scan time: 0.15s
Cone Temp (°C): 120	Inter-scan delay: 0.05s
Cone gas flow (L/Hr): 30	Probe: ES+
Desolvation flow (L/Hr): 550	



**Conditions**  
Column: Xterra® MS C<sub>18</sub>  
Dimensions: 150 x 3.9mm, 5μm  
Mobile Phase A: 20mM Ammonium Bicarbonate pH 9.0  
Mobile Phase B: Methanol  
Flow Rate: 1.5 mL/min  
Gradient: Time Profile  
[min] %A %B  
0.0 95.0 5.0  
14.0 86.0 14.0  
30.0 35.0 65.0  
Injection Volume: 5.0 μL  
Temperature: 50 °C  
Detection: UV @ 230 nm  
Instrument: Alliance® 2695 XE w/ 2996 PDA  
2mg/mL Ranitidine degraded sample

\*Unsure of retention time based on collected data  
\*Unknown components based on current data

## Method Development Tools

### Single Quad Utilization

A single quadrupole MS was configured in-line with the PDA for peak tracking and preliminary peak identification confirmation.

#### TIPS & TRICKS:

- It is visually easier to use the review window of the Empower CDS. Integration of the peak displays a m/z spectra in the spectral window (right). Match the peaks by m/z from injection to injection
- 0.005in diameter peek tubing was cut to minimal length to minimize band broadening. 0.0025in peek tubing would be ideal.
- Using 0.0025in peek tubing directly to the mass spectrometer without the PDA configured in-line would further increase the resolution.

