Exploring Impurity Profiling Techniques: UPLC coupled with oa-Tof Mass Spectroscopy for Method Development and Structural Characterization of Impurities in a Pharmaceutical Drug Substance

Waters

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Introduction

Purity 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. The FDA regulations require companies to develop methods for their analysis and characterization of the active pharmaceutical ingredient (API), as well as the impurities/degradants that could arise from the synthesis process, raw material provider, and/or storage conditions. Ultra Performance LC™ exploits the chromatographic potential of sub 2µm stationary phases to generate high resolution, high sensitivity, rapid separations. This increased performance makes UPLC™ the ideal tool for purity profiling.

Ranitidine, the histamine H_2-receptor antagonist heals gastric and duodenal ulcers by reducing acid output as a result of H_2-receptor blockage, it is manufactured by numerous generic pharmaceutical companies. The assay and purity test as described in the USP, is a two stage process including thin layer chromatography (TLC) test and an HPLC assay.[2,4] The original TLC method had an analysis time of 30 minutes to achieve limits of detection of 0.05% (a/a) (in respect to % area of the main peak).[2,4] The HPLC method was only used for ranitidine separation from the impurities with an analysis time of 10 minutes without optimization of the impurities separation.[2] A capillary electrophoresis (CE) method facilitates quantification of ranitidine and related substances analysis with a 30 minute run time.[1] This throughput is not acceptable for today's demanding pharmaceutical market.

In this application, we demonstrate the increased resolution and throughput achievable by "Ultra Performance Liquid Chromatography" and show how the increased sensitivity combined with oa-Tof MS allows the detection and identification of more impurities in comparison to traditional HPLC. This "proof of concept" approach utilizing UPLC supported with oa-Tof MS/MS data for exact mass and structural characterization will provide insight to a peak's origin whether it is related to the parent as a degradant or a possible impurity due from another source. (i.e. catalysts, synthesis reagents, or solvents) The UPLC coupled with oa-Tof techniques for purity profiling increase efficiency and confidence in the quality of the data collected.

Experimental

The described approach illustrates the benefits of UPLC purity analysis combined with oa-Tof MS for exact mass and structural confirmation. Collision induced dissociation MS/MS was performed to determine the structural identity of the related substances relative to the pharmaceutical active ingredient (API) as well as to assist identification of any unknown impurity substances.

Instrumentation

UPLC Conditions
Column: ACQUITY UPLC™ BEH C_{18}
Software: Empower™ CDS
Masslynx™ (w/oa-Tof MS)
Dimensions: 100 x 2.1mm, 1.7µm
Mobile Phase A: 20mM Ammonium Bicarbonate pH 9.0
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
Instrument: ACQUITY UPLC w/ 2996 PDA

oa-Tof MS/MS Conditions
Instrument: Waters Micromass® Q-Tof MS
Software: Masslynx 4.0 SP4

Tune Page Parameters:
Source: ES+
Capillary (V): 3200
Sample Cone (V): 35 for reference
15 for analyte
Extraction Cone (V): 1.0

Gas Flow
Cone (L/Hr): 20
Desolvation (L/Hr): 550

Tof Settings
Acquisition
Range: 100 - 750Da
Scan Time: 0.28s
Interscan delay: 0.1s
Lock mass: 1.2pmol leucine/enkephalin @ ~60µL/min
Desolvation Temp (°C): 250.0
Source Temp (°C): 120.0

oa-Tof MS Structural Confirmation

Exact Mass Results

Using LC/MS in purity profiling experiments aids in peak tracking during method development and facilitates a high level of confidence with known analyte identification when exact mass is employed. ACS requires < 5ppm mass accuracy for patent submission and publication. By coupling exact mass with tools like elemental composition, it is possible to predict molecular formulas for the unknown analytes.

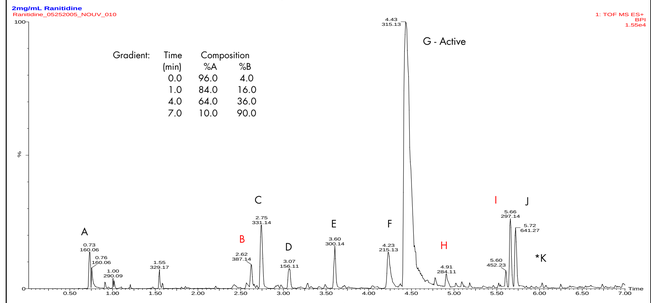


Figure 1: Base peak intensity chromatogram (UPLC - Q-Tof micro) of ranitidine sample, the labeled peaks in black are the known impurities of the API. [1,2] The peaks labeled in Red are the unknowns.

Compound	Formula	[M+H]	Result	ppm/mDa
One-Oxime (A)	C5H9N3OS	160.0544	160.0551	-4.4/0.7
S-Oxide (C)	C13H22N4O4S	331.1440	331.1445	-1.5/0.5
{5[(dimethylamino)methyl]-2-furyl} methanol (D)	C8H13NO2	156.1024	156.1031	-4.5/0.7
Furanmethamine (F)	C10H18N2OS	215.1218	215.1212	2.8/-0.6
Ranitidine (active)	C13H22N4O3S	315.1491	315.1490	0.3/-0.1
Adduct/dimer (J)	C27H44N8O3S2	641.2903	641.2898	0.8/-0.5
N,N-Bis (*K)	C13H22N4O3S	498.2208	498.2190	3.6/-1.8

Table 1: oa-Tof Exact Mass Error Table. The data in Table 1 illustrates the derived elemental composition and mass errors generated by the UPLC - Q-Tof micro system. A minimum combined average of five scans was used to determine the exact mass of each of the known impurities. The reported ppm values are single measurement not RMS (root mean square) calculations.

MS/MS Structural Characterization

The application of MS/MS provides insight into the structure of an unknown chemical moiety. When combined with the exact mass data generated by using the Waters Micromass Q-Tof micro mass spectrometer, a peak identity hypothesis can be confirmed or rejected based upon the structural fragmentation pattern of the MS/MS data and the derived elemental composition. As an example, the API compound (ranitidine) was studied by using MS/MS. The resulting MS/MS fragmentation pattern together with the exact mass information for the [M+H] = 315.1440 mass are consistent with this analyte being ranitidine

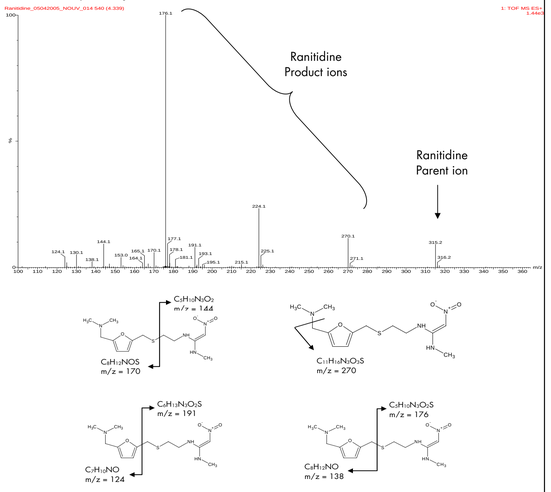


Figure 3: MS/MS spectrum of ranitidine. The structures above represent examples of the cleavage points producing the ranitidine product ions. The 224 amu fragment ID is currently under investigation, however it is hypothesized to be a loss of NO_2 from the 270 amu fragment via a rearrangement.

MS Structural Elucidation

Unknown Components: Impurity or Degradant?

The technique of exact mass MS/MS was applied to each degradant and impurity. MS/MS fragmentation of the unknown peak (m/z =387.1302) yields a similar fragmentation pattern as the MS/MS of ranitidine, thus confirming that the impurity is related to ranitidine. The MassLynx "Elemental Composition Calculator" supports the hypothesis that the fragment series are the same (Figure 4). Subsequent evaluation of the MS-Tof spectra and the fragmentation patterns of the MS/MS spectra yields information about the analyte composition (as explained below).

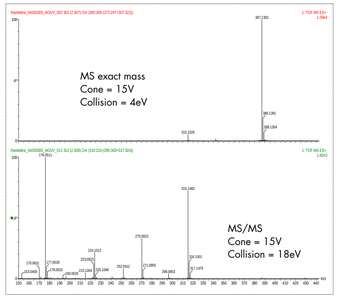


Figure 4: Exact mass combined spectra of 387.1302 amu and MS/MS combined spectrum of 387 amu.

Single Mass Analysis
Tolerance = 15.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
387.1302	387.1213	8.9	23.1	7.0	1	C14 H21 N5 O6 S
	387.1451	-14.9	-38.4	6.5	2	C14 H23 N6 O5 S
	387.1338	-3.6	-9.4	6.5	3	C15 H23 N4 O6 S
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**Score**	**Formula**
71.9887	71.9847	4.0	55.0	3.0	n/a	C2 O3
	71.9960	-7.3	-101.1	3.0	n/a	C N2 O2
	72.0000	-11.3	-157.0	7.0	n/a	C6
	72.0072	-18.5	-257.1	3.0	n/a	N4 O
	72.0086	-19.9	-275.8	2.5	n/a	C2 H2 N O2

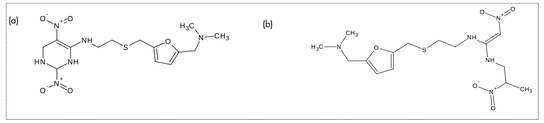
Figure 5: Elemental Composition Report

In MassLynx, the elemental composition (Figure 5) of 387.1302 suggests a chemical formula of $C_{14}H_{23}N_6O_5S$. The differences between the exact masses of the API and the unknown 387.1302 amu equates to 71.9887 amu. Elemental composition of the 71.9887 fragment yields a list of potential formulas, of which the formula/components can be deduced as the added components to mass [M+H]=315.1491 ($C_{13}H_{23}N_4O_3S$) to yield unknown [M+H]=387.1302. The 71.9887 fragment is not so obvious, hence the formulation of the true structure may be due in part via rearrangements. Some preliminary structures are illustrated in figure 6. Elucidation of the fragmentation mechanism is not straight forward and could be aided by utilization of fractionation followed by ^{1}H-NMR analysis.

Unknown Components: Impurity or Degradant?

The fragmentation pattern eludes to a relationship to the ranitidine (API). The mass spectrum suggests that the 387 amu compound consists of an even number of nitrogens as the parent ranitidine does (Nitrogen Rule) and one sulphur isotopic pattern. Exact mass difference between the API and unknown = 71.9808 amu, however the 71.9 amu difference is not so obvious and is currently under investigation.

Figure 6: Proposed structures for unknown [M+H] = 387



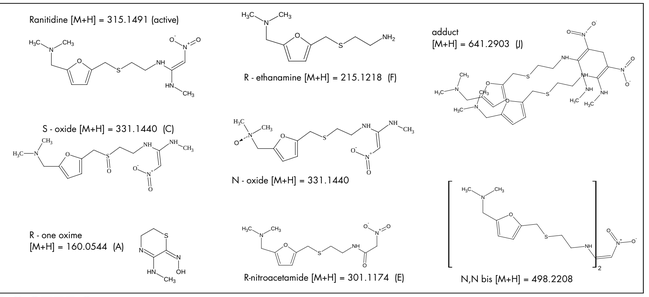
IUPAC

A. 3-(methylamino)-5,6-dihydro-2H-1,4-thiazin-2-one oxime
B. *Unknown m/z = 387.14
C. N-[2-[[[5-[[dimethylamino]methyl]-2-furyl]methyl]sulfinyl]ethyl]-N'-methyl-2-nitro-1,1-ethenediamine
D. {5-[[dimethylamino]methyl]-2-furyl]methanol (M+H=156.1024)
E. N-[2-[[[5-[[dimethylamino]methyl]-2-furyl]methyl]sulfanyl]ethyl]-2-nitroacetamide
F. 2-[[[5-[[dimethylamino]methyl]-2-furyl]methyl]sulfanyl] ethanamine
G. Ranitidine (active)
H. *Unknown m/z = 284.11
I. *Unknown m/z = 297.16
J. Dimmer (reported as Formaldehyde adduct)[2]
A. N,N'-bis[2-[[[5-[[dimethylamino]methyl]-2-furanyl]methyl]thio] ethyl]-2-nitro-1,1-ethenediamine

† N-[2-[[[5-[[dimethylamino]methyl]-2-furanyl]thio]ethyl]-N'-methyl-2-nitro-2,2-ethenediamine (N-oxide)

† Retention time not determined, but is a published degradant[1,2]

*Unknown components based on current MS data[3]



Discussion

The extra resolution and efficiency of the ACQUITY UPLC column allowed the analytical run time to be reduced to just 7 minutes, compared to 30 minutes for CE.[1,3]. Although not shown, integration comparisons of the HPLC chromatogram detected 34 impuritiy peaks with area percents greater than or equal to 0.05% area to that of the UPLC chromatograms detecting 45 peak with 0.05% area or greater. A total of 11 impurities of ranitidine with significant response were detected and identified with 3 of these being new unknowns. The UPLC peaks produced are very narrow, which therefore require high data sampling rates by the mass spectrometer. The acquisition speed of the Q-Tof micro mass spectrometer was balanced against UPLC peak widths to ensure that sufficient data points are collected across the LC peaks to allow high quality accurate mass data to be obtained. Appropriate sample dilutions need to be employed to prevent detector saturation. This presents an interesting problem in impurity analysis as loadability could become peak specific depending upon concentration (in respect to detector saturation of an active ingredient such that enough signal is detected to see the small impurities when using UV based detectors).

Conclusions

The extra resolution and throughput of UPLC makes it the ideal liquid chromatography tool for impurity and degradation analysis. Applying oa-Tof MS with UPLC techniques to assist with achieving exact mass allow for the confirmation of known or hypothesized degradant/impurity substances. Collision induced dissociation (MS/MS) helped define structural characterization of the API, known related substances and provided insight to some unknown impurity substances. The implementation of MS to impurity profiling provide added sensitivity in many cases. In summary, UPLC oa-Tof helps fast tracking method development of purity analysis methods.

References

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