Degradation products Analysis of Pantoprazole using High resolution mass spectrometry

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INTRODUCTION

Pantoprazole sodium, chemically sodium 5-(difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]- 1*H*-benzimidazole is an oral pharmaceutically active compound having the promising anti-ulcer activities and belong to the class of 2-[(2-[yridyl)methyl]sulfinyl]-1H-benzimidazoles. The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug product. Degradation product analysis is an important area in pharmaceutical analysis, particularly during the product development and quality control. The safety of any drug product is dependent not only on the toxicological properties of the active drug substance itself, but on the impurities that it contains. Monitoring of the degradation products or impurities in new drug substances is a key component of the guideline issued by the International conference in Harmonization (ICH). Due to the complexity of the analysis of degradation products or impurity profiling, sufficient sensitivity and separation is difficult to achieve with HPLC. Using the new hybrid quadrupole time-of-flight mass spectrometer (AB SCIEX TripleTOF® 4600) with HPLC provides new dimension to the sensitivity, resolution and mass accuracy. Accurate mass and product ion spectra provide important information for the identification and structure confirmation of potential degradation products or impurities.

Therefore the objective of present study was: (I) to find out the major degradation product formed with the m-CPBA at different time points using generic information dependent acquisition (IDA) with novel on fly dynamic background subtraction (DBS) algorithm.

MATERIALS AND METHODS

Sample Preparation:

Commercially available Pantoprazole tablet was crushed, dissolved and sonicated in Methanol. The solution was centrifuged and supernatant was taken for study. We have used 1% m-CPBA in methanol for oxidation study. Pantoprazole and 1% m-CPBA (50/50 v/v) were mixed together in room temperature for reaction. Different time point samples were taken and diluted in mobile phase for HRMS analysis using generic IDA workflows and further confirmation by real time multiple neutral loss – IDA workflow. Synthetic standards of Pantoprazole (>99.9%) was accurately weighted and serially diluted in methanol for calibration curve. Different concentration points (1.0 – 2000.0 ng/ml) were prepared to generate the calibration curve using generic IDA workflow on Triple TOF 4600 system.

Chromatography: Shimadzu Prominence UFLC XR system was used to perform the LC separation of the degradation product formed during the stress condition.

Mobile Phase A: 10mM ammonium acetate in water (pH 4.0)
Mobile Phase B: Acetonitrile and methanol (60/40, v/v)

Column: Kinetics C18 (50 x 2.10mm, 1.7µ, Phenomenex USA)
Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Gradient: Described in the Table 1

Mass Spectrometry:

Sample analysis was performed on the AB SCIEX TripleTOF® 4600 system in positive electrospray mode using DuoSpray™ source. The generic information dependent Acquisition (IDA) method consisted of a TOF MS survey scan (m/z 100-1000) followed by 8 TOF MS/MS dependent scans (m/z 50-1000).

Time	%B
0.0	10
0.5	10
9.0	95
11.0	95
12.0	10
15.0	STOP

Table 1. UFLC Gradient Profile

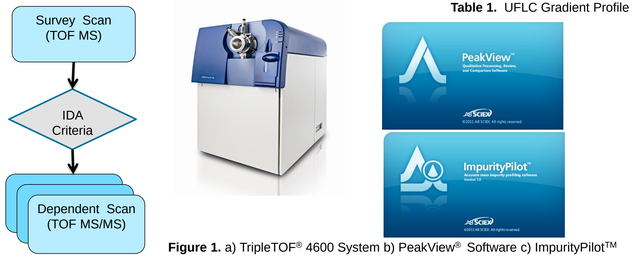

Figure 1. a) TripleTOF® 4600 System b) PeakView® Software c) ImpurityPilot™

Figure 2. Workflow for Information Dependent Acquisition (IDA)

RESULTS

Major Degradation Products (DPs)	MS RT (Min)	Exact Mass for major degradation products	Molecular Formula (RDB)	Error in ppm	Major Fragments Ion (MS/MS)
				TOF MS	
DP -1	6.10	400.0775	$C_{16}H_{15}N_3O_5F_2S$ (10)	0.40	152.0704,169.0735, 185.0522,202.0532, 216.0324
DP-2	6.26	416.0717	$C_{16}H_{15}N_3O_6F_2S$ (10)	-1.30	168.0651,185.0516, 232.0273
Pantoprazole	6.71	384.0824	C16H15N3O4F2S (10)	0.00	138.0544, 153.0078, 170.0808, 200.0366, 366.0708
DP-3	6.83	400.0778	C16H15N3O5F2S (10)	1.20	152.0702,216.0324, 336.1151

Table 2. Major degradation products (DPs) identified in using m-CPBA reagent with generic IDA –DBS workflow. Retention time in MS,TOF MS with their molecular formula, RDB, error in MS and MS/MS (ppm) and major fragments ion.

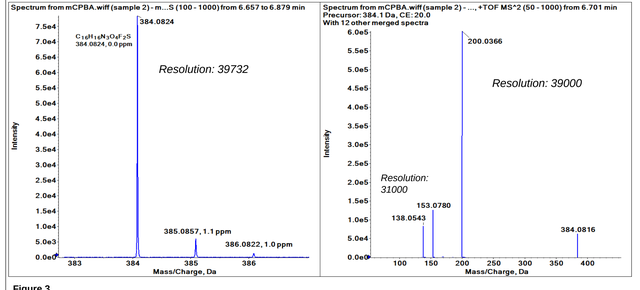

Figure 3.
(a) Accurate mass of pantoprazole m/z 384.0824 (b) product ion spectra (TOF MS/MS) of m/z 384.08

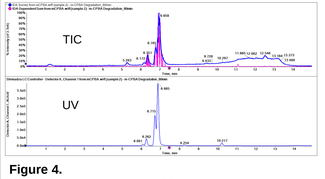

Figure 4.
IDA-UV chromatogram for 60 min sample

Figure 5.
ImpurityPilot™ Software showing different viewing option for the spectra after processing (a) List of impurities identified with for formula, accurate masses of impurities, mass accuracy (b) XICs of the impurities (c) Accurate mass of the DP-1 (m/z 400.0775) (d) Product Ion spectra of DP-1 and its matching Patten with pantoprazole

Index	Sample Name	Sample Type	Component Name	Retention Time	Conc. Units	Area	Actual Concentration	Used	Calculated Concentration	Accuracy
1	Blank	Blank	Pantoprazole	N/A	ng/ml	N/A	N/A	☑	N/A	N/A
2	STD A	Standard	Pantoprazole	6.78	ng/ml	697	1.00	☑	1.037	103.65
3	STD B	Standard	Pantoprazole	6.80	ng/ml	1801	2.00	☑	2.024	101.19
4	STD C	Standard	Pantoprazole	6.79	ng/ml	4218	5.00	☑	4.186	83.72
5	STD D	Standard	Pantoprazole	6.80	ng/ml	9452	10.00	☑	9.904	99.04
6	STD E	Standard	Pantoprazole	6.82	ng/ml	23925	20.00	☑	19.845	94.22
7	STD F	Standard	Pantoprazole	6.80	ng/ml	65210	50.00	☑	58.747	117.49
8	STD G	Standard	Pantoprazole	6.78	ng/ml	310265	250.00	☑	277.960	111.18
9	STD H	Standard	Pantoprazole	6.80	ng/ml	2224192	2000.00	☑	1990.059	99.50

Table 3. Linearity for pantoprazole

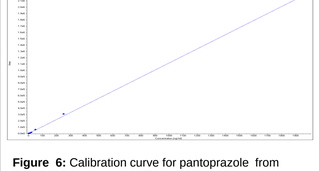

Figure 6: Calibration curve for pantoprazole from 1.0 - 2000.0 ng/ml using Triple TOF® 4600

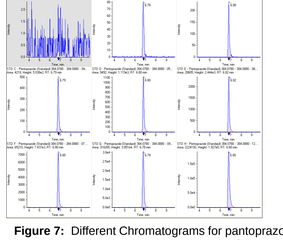

Figure 7: Different Chromatograms for pantoprazole From 1.0 - 2000.0 ng/ml using Triple TOF® 4600

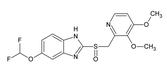

Fig 8a: Pantoprazole
C16H15F2N3O4S
[M+H]+ : 384.08241

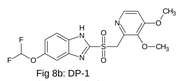

Fig 8b: DP-1
$C_{16}H_{15}F_2N_3O_5S$
$[M+H]^+$: 400.07733

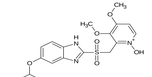

Fig 8c: DP-2
$C_{16}H_{15}F_2N_3O_6S$
$[M+H]^+$: 416.07224

CONCLUSIONS

- The new hybrid TripleTOF® 4600 systems has high sensitivity quadrupole and time of flight analyzer which enable rapid non targeted and targeted degradation product (DPs) analysis in single injection.
- High resolution and high mass accuracy TOF MS and MS/MS data will help to characterize and confirm the known and unknown degradation products masses and their elemental composition identified in the stressed sample.
- Generic information dependent acquisition (IDA) method with unique dynamic background subtraction (DBS) will help to trigger more number of MS/MS of the real precursor masses in this new hybrid mass spectrometer.
- The m/z 400.0775, 416.0717 and 400.0778 were major degradation product (DPs) identified in m-CPBA, stress condition.
- The resolution achived for these DPs were more than >35000 in TOF MS and > 25000 for TOFMS/MS mode.
- The mass accuracy were less than 2 ppm in TOF MS mode using IDA method for all the degradation products.
- HRMS quantitation were performed for pantoprazole. The linearity was found to be from 1.0 – 1000 ng/ml with > 3x linear dynamic range.

REFERENCES

1. Reddy. G.M, Bhkaskar. B.V, Reddy. P.P, Ashok.S, Sudhakar.P, Babu.M.J, Vyas.K, and Mukkanti. K, Structural identification and characterization of potential impurities of pantoprazole sodium. *J. Pharma. Biomed. Anal.* **2007**, 45, 201-210.
2. ICH, stability testing for new drug substances and products Q1A (R2) in: International conference on harmonization, IFPMA, Geneva, (2003).
3. G.L. Andrews, B.L. Simons, J.B. Young, A.M. Hawkridge, D.C. Muddiman. Performance characteristics of a new hybrid quadrupole time-of-flight tandem mass spectrometer. *Anal Chem.* **2011**, 83, 5442.
4. Ramagiri, S, Olsen.L, Impey G, Fox J, Accelerated Impurity Profling workflows for bulk drug and formulation using an accurate mass workhorse, AB SCIEX Technical Note – 09320114-01

TRADEMARKS/LICENSING