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Liquid Chromatograph Mass Spectrometer LCMS™-8045

Application News

Quantitation of 8 Nitrosamines in Thiocolchicoside API by LCMS-8045 system

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User Benefits

- A simple and robust LC/MS method for the determination of nitrosamines in Thiocolchicoside API
- Simultaneous analysis of eight nitrosamines without the need of complex sample pre-treatment

■ Introduction

Thiocolchicoside: Thiocolchicoside is a muscle relaxant that is commonly used to treat back pain and other pains caused by vertebral column or spinal cord disorders, as well as to relieve pain after surgery. It is a natural derivated product from colchicine and a semi-synthetic derivative of colchicoside. This medication functions by interacting with specific receptors in the central nervous system. Figure 1 depicts the structure of thiocolchicoside.

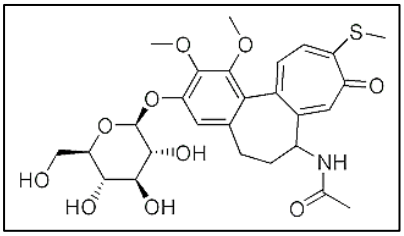

Fig. 1 Structure of Thiocolchicoside

Overview : The Drug Regulatory Authorities first noticed the presence of the nitrosamine (NSA) impurity, N-nitrosodimethylamine (NDMA) in products containing valsartan in July 2018. The discovery of nitrosamines in some types of drug products led the authorities to consider their existence in other APIs and drug products due to use of vulnerable processes and materials that may produce nitrosamine impurities. Therefore, the FDA published a guidance for industry; the recommendations in which apply to all chemically synthesized APIs.

Occurrence of Nitrosamines: Formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions. Under these conditions, nitrite salts may form nitrous acid, which can react with an amine to form a nitrosamine. Apart from these there are other routes such as; vendor-sourced starting materials and raw materials; recovered solvents, catalysts and reagents; cross contamination from common manufacturing facility; quenching process using nitrous acid; and packing/storage; which may result in nitrosamines formation or contamination.

Toxicity/ Regulation/ Methods: NDMA and N-nitrosodiethylamine (NDEA) have been classified as probable human carcinogens. Hence, United state Food and Drug Administration (USFDA) recommends the following acceptable intake (AI) limits for NDMA, N-nitroso-N-methyl-4-aminobutyric acid (NMBA), NDEA, N-nitrosoethyl isopropylamine (NEIPA), N-nitrosomethyl phenylamine (NMPA) and N-nitrosodiisopropylamine (NDIPA) (Table 1). These limits are applicable only if a drug product contains a single nitrosamine, and lowest of which is 0.03 ppm for drug substances with Maximum daily dose (MDD) of 880 mg/day. If more than one nitrosamine impurity is identified in the same drug substance the limit for total nitrosamines listed in table 1 is still not more than 26.5 ng/day or 0.03 ppm.
Hence, it is imperative to detect above mentioned nitrosamines with Limit of Quantitation (LOQ) as low as possible to be sure that not just single nitrosamine impurity is below 0.03 ppm, but also total nitrosamine impurities are below 0.03 ppm.

Table 1 . Acceptable Intake (AI) Limits for nitrosamines

Nitrosamine	AI Limit (ng/day)	Limit in ppm for MDD 880 mg/day
NDMA	96.0	0.109
NMBA	96.0	0.109
NDEA	26.5	0.030
NEIPA	26.5	0.030
NDIPA	26.5	0.030
NMPA	26.5	0.030

■ Experimental

Eight nitrosamines namely NDMA, NMBA, NDEA, NEIPA, NDIPA, N-nitrosodipropylamine (NDPA), NMPA, and N-nitrosodibutylamine (NDBA) were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera™ XS coupled with LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Figure 2).

LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

All eight nitrosamines are mid polar compounds. They were easily ionized by Atmospheric Pressure Chemical Ionization (APCI) interface.

Fig. 2 Nexera™ XS with LCMS™-8045 system

■ Method

The MRM transitions of 8 nitrosamines and 4 internal standards are given in table 2 and analytical conditions in table 3.

Table 2 MRM transitions of nitrosamines

MRM Transitions				
Nitrosamine Impurity	Type	ISTD Group	MRM (Quantifier)	MRM (Qualifier)
NDMA	Target	1	75>43	75>58
NDMA-d6	ISTD	1	81>46	-
NMBA	Target	2	147>117	147>44
NMBA-d3	ISTD	2	150>120	150>87
NDEA	Target	3	103>29	103>75
NDEA-d10	ISTD	3	113>34	-
NEIPA	Target	3	117>75	117>27
NDIPA	Target	4	131>89	131>43
NDPA	Target	4	131>89	131>43
NMPA	Target	4	137>66	137>107
NDBA	Target	4	159>41	159>29
NDBA-d18	ISTD	4	177>66	177>46

Table 3 Analytical conditions

HPLC System	: Nexera XS
Column	: Shim-pack AQ C18 (100 mm x 4.6 mm x 3 µm) (p/n: 227-30724-05)
Column Oven	: 40 ℃
Mobile Phases	: A-0.1% Formic acid in Water B-0.1% Formic acid in Methanol
Flow Rate	: 0.7 mL/min
Gradient program (B%)	: 0-8 min → 30(%); 8-14 min → 30-55(%); 14-20 min → 55-80 (%); 20-20.1 min→ 80-30 (%); 25 min → STOP
Injection Volume	: 20 µL
LCMS System	: LCMS-8045 : APCI
Temperature	: Interface: 350 ℃ Desolvation Line: 200 ℃ Heater Block: 200 ℃
Gas Flow	: Nebulizing Gas: 3 L/min Drying Gas: 5 L/min

■ Linearity of the nitrosamines

An MRM chromatogram for nitrosamines with overlayed UV chromatogram of Thiocolchicoside API is given in figure 3. A divert valve was employed to direct the API peak to waste and to protect the mass spectrometer from contamination.

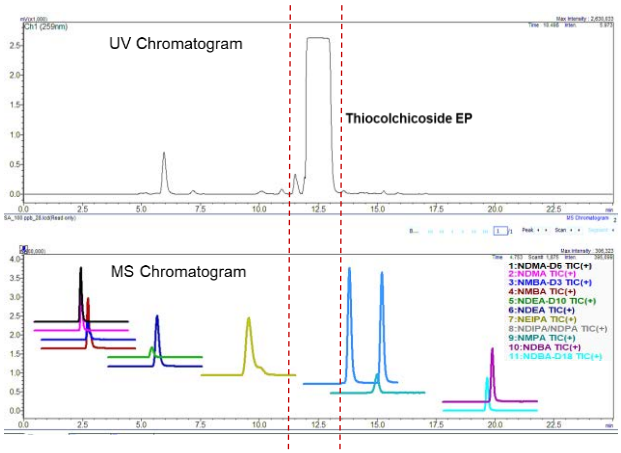

Fig. 3 Representative MRM chromatogram of 8 nitrosamines and its overlapping with UV chromatogram

Eight-points calibration curves for all 8 nitrosamines with 4 ISTDs were prepared in water and analyzed using the conditions described in Table 3. The figures 4 to 10 depicts the calibration curves, overlay of linearity standards & LOQ level chromatogram for NDMA, NMBA, NDEA, NEIPA, NDIPA, NDPA, NMPA, & NDBA, respectively.

NDMA

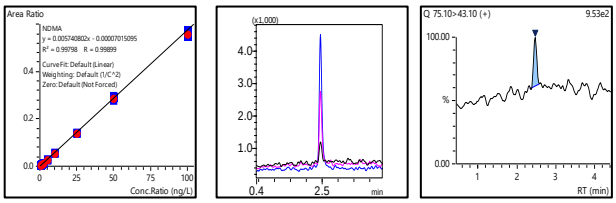

Fig.3 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NDMA

NMBA

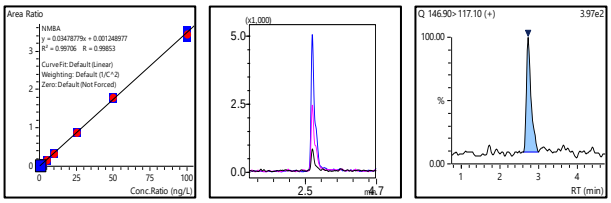

Fig.4 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NMBA

NDEA

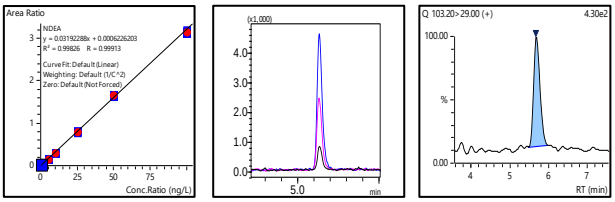

Fig.5 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NDEA

NEIPA

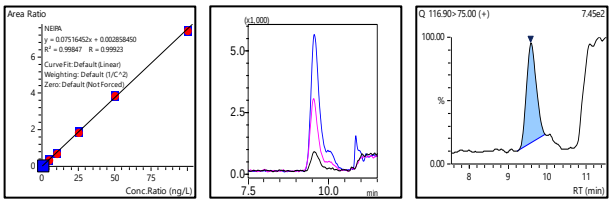

Fig.6 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NEIPA

NDIPA

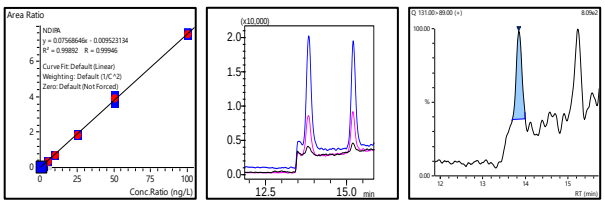

Fig.7 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NDIPA

NDPA

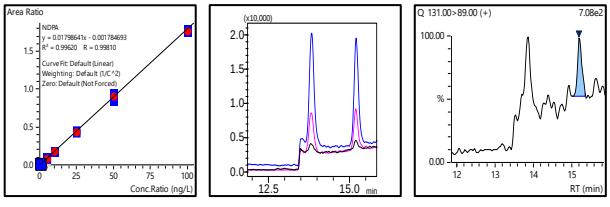

Fig.8 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NDPA

NMPA

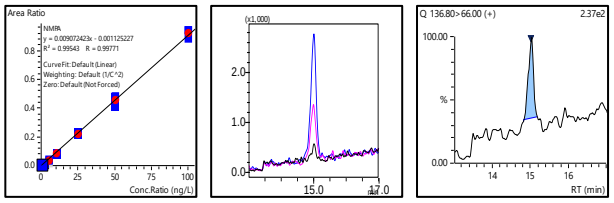

Fig.9 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NMPA

NDBA

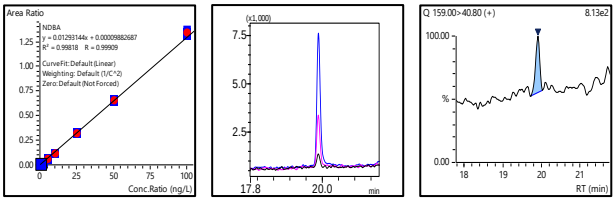

Fig.10 Calibration Curve, Overlay of Linearity Standards & LOQ level chromatogram for NDBA

The range for calibration curves, LOQ established from S/N and % RSD at LOQ are shown in table 4.

Table 4 Summary of calibration curves

Comp.	CC range (ppb)	R^2	LOQ		
			Conc. (ppb)	%RSD (n=6)	S/N
NDMA	0.5 to 100	0.997	0.5	9.70	6.63
NMBA	0.5 to 100	0.997	0.5	8.77	39.64
NDEA	0.5 to 100	0.998	0.5	4.28	54.64
NEIPA	0.5 to 100	0.998	0.5	1.40	4.35
NDIPA	0.5 to 100	0.998	0.5	6.42	0.87
NDPA	0.5 to 100	0.996	0.5	8.85	2.13
NMPA	0.5 to 100	0.995	0.5	7.84	5.55
NDBA	0.5 to 100	0.998	0.5	12.94	6.13

■ Sample Analysis

Weigh 100 mg of sample drug substance in 2 mL centrifuge tube, then add 970 µL of diluent and 30 µL of internal standard mixture. Vortex for 8 min. Filter the sample through 0.22 µm nylon syringe filter. Inject the sample for LC-MS/MS analysis.

The overall concentration was 100 mg/mL. The results of the sample spiked study (recovery study) for the Thiocolchicoside API at 0.5 ppb, 1 ppb and 3 ppb levels are given in table 5.

Table 5 The sample spiked study for the 8 nitrosamines

% Recovery			
Nitrosamine Impurity	0.5 ppb Spiked	1 ppb Spiked	3 ppb Spiked
NDMA	82.9	90.6	95.5
NMBA	94.7	100.7	104.0
NDEA	75.6	74.7	83.5
NEIPA	91.8	90.4	103.6
NDIPA	70.9	71.1	92.9
NDPA	93.9	101.7	97.7
NMPA	110.9	109.1	106.1
NDBA	81.5	101.6	99.8

■ Conclusion

- A single LC-MS/MS quantification method for eight nitrosamines in Thiocolchicoside API has been successfully developed on the Shimadzu LCMS-8045 system.
- Eight levels of linearity study was performed for all the eight nitrosamines by internal standard method.
- Correlation coefficient was greater than 0.99 for all the eight nitrosamines.
- The repeatability (n=6) at LOQ level was found to be less than 15% RSD.
- Recovery analysis was performed at LOQ level and found to be within acceptance criteria of 70 to 130 %.

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