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Characterization of Loratadine API and Simultaneous Quantification of Seven Potential Genotoxic Nitrosamine Impurities in Single Method by LC-MS/MS in Loratadine API and its Dosage Forms

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Active pharmaceutical ingredients (APIs) of loratadine were characterized using spectroscopic methods such as infrared spectroscopy, mass spectroscopy, differential scanning calorimetry (DSC), ^{1}H NMR, 2D nuclear magnetic resonance (2D-NMR) and ^{13}C NMR. Nitrosamine impurities, which are considered under concern cohort according to the S2 FDA and ICH M7 guidelines, are highly genotoxic and their trace-level quantity must be controlled in drugs for safe human consumption. In this study, an ultra-sensitive, rapid and simple LC-MS/MS technique is developed to detect seven nitrosamine impurities (*N*-nitroso dimethylamine (NDMA), *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA), *N*-nitroso diethylamine (NDEA), *N*-nitroso ethyl isopropylamine (NEIPA), *N*-nitroso diisopropylamino (NDIPA), *N*-nitroso methyl phenylamine (NMPA) and *N*-nitroso dibutylamine (NDBA) in loratadine drug) with potential genotoxicity. Chromatographic separation was attained by employing the Zorbax SB C18 of 150 × 3 mm and a column of 3.5 µ with 0.1% formic acid in water and methanol as mobile phases A and B, respectively. At the total run time of 20 min, the flow rate was 0.3 mL/min in the gradient mode of elution. Through multiple reaction monitoring (MRM), all the seven nitrosamine impurities were successfully ionized and then quantified in the positive mode of atmospheric pressure chemical ionization (APCI). The method was validated according to the ICH guidelines by estimating quantification and detection limits. For all the seven nitrosamine impurities, the method provided excellent S/N ratios with a high linearity range of 0.8-5.30 ppm for loratadine sample concentrations with a regression coefficient of >0.99. The recovery for the method was established with a protocol of three-step sample preparation and was satisfactory within 15-115%. The proposed method can be employed for the routine detection of nitrosamines in loratadine APIs and its doses.

Keywords: Loratadine, Nitrosamines, LC-MS/MS, Method development.

INTRODUCTION

Loratadine is a receptor antagonist of the second generation histamine H1 and an azatadine derivative used to treat allergic urticaria and rhinitis. Different from classic antihistamines (histamine H1 antagonists), loratadine does not show central nervous system depressing influences, including drowsiness [1]. Its IUPAC name is ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate (*m.f.* $C_{22}H_{23}N_2O_2Cl$; *m.w.* 382.89 g/mol).

A pharmaceutical impurity is an undesired chemical formed during the synthesis of active pharmaceutical ingredients (APIs) or during drug degradation. This impurity can also result from storage, contaminations and excipient interactions. Nitrosamine impurities are a class of substances with a chemical structure having a nitroso group bonded to an amine. These impurities are highly genotoxic and classified as the category of concern cohort by the ICH M7 guidelines, and for human consumption, they must be determined in trace levels to ensure the removal of carcinogenic effect risk [2-6]. Recent trends

concerning the multiple recalls of various therapeutic drugs observed in the pharmaceutical industries achieved by various regulatory agencies such as European Medicines Agency (EMA) and United States Food and Drug Administration (USFDA), caused by the availability of different nitrosamine impurities have shown the significance of impurity removal for human safety. The screening of nitrosamine impurities is achieved on the basis of the synthesis route, solvents, starting materials and stability of drug products and drugs [7,8]. This study quantified the seven nitrosamine impurities with potential genotoxicity at trace levels in a loratadine drug substance.

Several LC-HR/MS and LC-MS/MS methods have been reported by the regulatory agencies for determining nitrosamines in irbesartan, losartan, telmisartan and valsartan drugs [9-11]. Several reports have presented the estimation of nitrosamines in the environment and food by using LC-HRMS and LC-MS/MS [12-14]. However, no approach is reported for the trace-level quantification of all the seven nitrosamine impurities present in loratadine by using LC-MS/MS so far.

Based on Control of Nitrosamine Impurities in Human Drugs requirements for seven nitrosamine impurities specifications calculated by the maximum daily dosage of loratadine drug. So all the seven nitrosamines concentration must be controlled in loratadine at concentration lower than 2.65 ppm. In this work, the Ultivo Triple Quadrupole LC-MS method development for the determination of all the seven potential genotoxic nitrosamine impurities namely *N*-nitroso dimethylamine (NDMA), *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA), *N*-nitroso diethylamine (NDEA), *N*-nitroso ethyl isopropylamine (NEIPA), *N*-nitroso diisopropylamino (NDIPA), *N*-nitroso methyl phenylamine (NMPA) and *N*-nitroso dibutylamine (NDBA) in loratadine drug (Fig. 1) is presented. The method was validated for its system precision, specificity, method precision, linearity, accuracy, limit of quantification (LOQ), limit of detection (LOD), robustness, ruggedness, and standard and sample solution stability according to the ICH guidelines [15].

EXPERIMENTAL

The LC-MS grade solvents and reagents were of highest purity >99.8%. Water and methanol were purchased from Honeywell (Charlotte, USA). Formic acid was procured from Bio solve. Loratadine and seven nitrosamine impurities were taken from GVK Biosciences Pvt. Ltd., Hyderabad, India.

LC-MS (Agilent Ultivo Triple Quadrupole LC-MS system with Mass Hunter software), Fourier transform infrared (Perkin-Elmer Spectrum Two with Spectrum software), differential scanning calorimetry (DSC Q 2000 with TA Instrument explorer software), and nuclear magnetic resonance (Bruker instrument with Top Spin software) were used for characterization of loratadine drug.

Chromatographic conditions: The chromatographic analysis was conducted using Agilent 1260 Infinity II HPLC provided with a multi sampler, a quaternary pump, and a diode array detector coupled with the APCI interface and an Agilent Ultivo Triple Quadrupole LC-MS/MS. A 3.5 µ column of Agilent Zorbax Eclipse plus C18 150 × 3 mm was employed to remove the seven nitrosamine impurities from loratadine. The gradient modes of elution with 0.1% formic acid in water and methanol were used as the mobile phases A and B, respectively, at the flow rate and run time of 0.3 mL/min and 20 min, respectively. Column oven and auto sampler temperatures were maintained at 40 and 12 ºC with the injection volume of 20 µL. The following gradient program was used (time in min/%B): 0.00/5, 3.00/5, 7.00/60, 11.00/95, 15.00/95, 15.1/5 and 20/5.

The mass spectrometric conditions were optimized in the APCI positive mode by using the MRM acquisition mode for all the seven nitrosamine impurities. The MRM transitions are represented in Table-1.

APCI source was operated with a capillary voltage 3000 V; Corona needle current 4 µA; drying gas temperature: 350 ºC, Drying gas flow: 6 L/min; nebulizer pressure: 55 psi; vaporization temperature: 350 ºC, respectively. Agilent Mass Hunter software version 10.1 was used to control all the parameters of LC and MS.

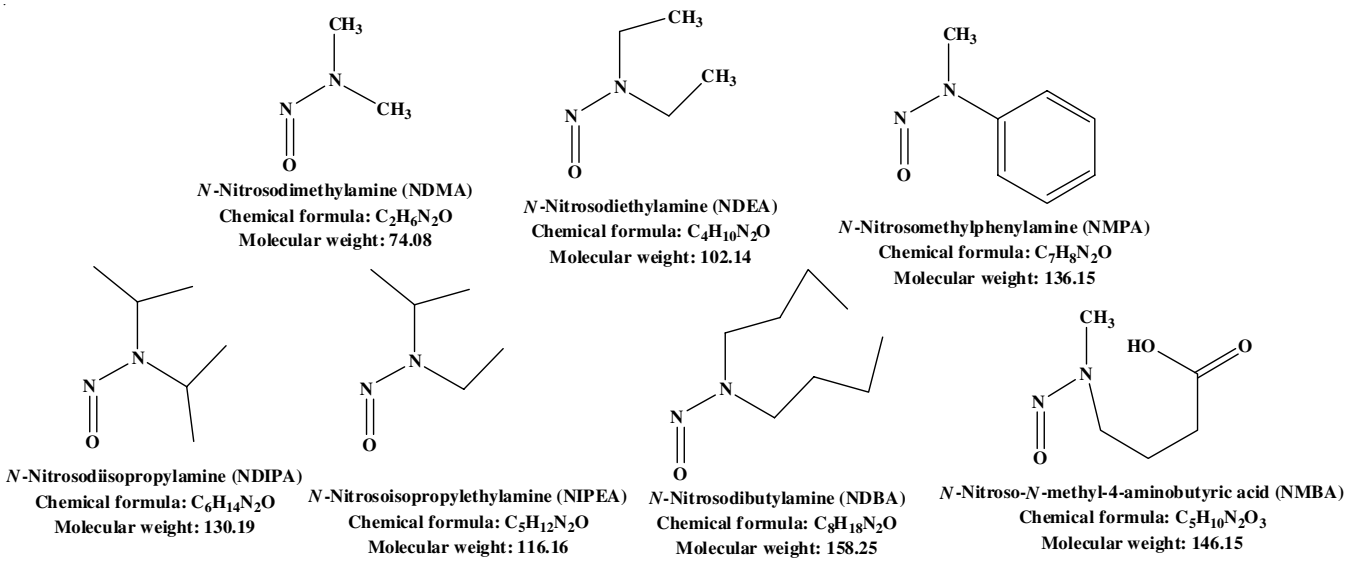

Fig. 1. Chemical structures of seven nitrosamines

TABLE-1
MRM TRANSITIONS

Compound name	Precursor ion (*m/z*)	MS1 resolution	Product ion (*m/z*)	MS2 resolution	Dwell (ms)	Fragment (v)	CE (eV)	Polarity
NDMA	75	Unit	43.1	Unit	100	110	16	+ve
NMBA	147	Unit	117	Unit	100	60	4	+ve
NDEA	103	Unit	75	Unit	100	78	12	+ve
NIPEA	117	Unit	74.9	Unit	100	82	8	+ve
NDIPA	131	Unit	89.1	Unit	100	80	5	+ve
NMPA	137	Unit	107	Unit	100	80	5	+ve
NDBA	159.1	Unit	57	Unit	100	86	12	+ve

Time table

Start time (min)	Type	Value
0	Diverter	To ms
15.6	Diverter	To waste

Specification calculation for nitrosamines: The specifications of all the seven nitrosamine impurities were calculated by the based on Accepted Intake limit of seven nitrosamines is divided by maximum daily dosage of loratadine. The calculated specifications are shown in Table-2.

TABLE-2
SPECIFICATIONS OF SEVEN NITROSAMINE IMPURITIES

Name of nitrosamines	Accepted ntake limit (ng/day)	Calculated limit as per API label claim 10 mg/day (ppm)	Taken limits (ppm)
NDMA	96	9.6	2.65
NMBA	96	9.6	2.65
NDEA	26.5	2.65	2.65
NIPEA	26.5	2.65	2.65
NDIPA	26.5	2.65	2.65
NMPA	26.5	2.65	2.65
NDBA	26.5	2.65	2.65

Note: Specification (ppm) = AI limit (ng/day)/MDD (mg/day)

Preparation of sample and impurity standard solutions

Diluent: Methanol was used as diluent for first impurity standard stock solution. And then premixed of water:methanol (95:5 v/v) solution was used as diluent further impurity standard and sample solutions.

Loratadine API sample preparation (20 mg/mL): Weighed and transferred 200.15 mg of loratadine API into 10 mL diluent. Sonicated about 3-5 min and filtered the solution through 0.45 µm syringe filtrate and preserved for the LC-MS analysis.

Preparation of impurity standard solution (2.65 ppm): Weighed and transferred about 20 mg of each nitrosamine impurity in 20 mL of volumetric flask and diluted to the volume with methanol and sonicated about 1 min (stock solution-1). Further transferred 2.5 mL of stock solution-1 in to 25 mL of volumetric flask and diluted with diluent (stock solution-2). Further transferred 0.5 mL above stock solution-2 in to 50 mL of volumetric flask and diluted with diluent (stock solution-3). Further transferred 2.65 mL above stock solution-3 in to 50 mL of volumetric flask and diluted with diluent (2.65 ppm with respect to loratadine 20mg/mL concentration).

Preparation of loratadine tablet solution: Loratadine tablets (no. 20) were taken for formulation analysis and grinded as fine powder. The amount was equivalent to 200 mg of loratadine was taken into 10 mL of volumetric flask, sonicated about 2-3 min and diluted to the mark with diluent and mixed well and then filtered through 0.45 µ syringe filter.

RESULTS AND DISCUSSION

Chromatographic method development: This study developed a highly selective and sensitive analytical technique for separation and trace-level quantification of the seven nitrosamine impurities with potential genotoxicity in loratadine APIs. Several gradient and mobile phase pH conditions were analyzed to obtain critical separation between loratadine and the seven nitrosamine impurities and excellent peak shapes. Finally, 0.1% formic acid in water and methanol was used as mobile phases A and B, respectively. Gradient elution presented good sensitivities and peak shapes. For separation between loratadine and impurities, few columns were tested. Co-elution between NDBA and critical pair loratadine was observed initially with a 3.5 µ column of hyper shill BDS C18 150 × 4.6 mm. After the evaluation of various column chemistries for critical separation between NDBA and loratadine, the 3.5 µ column of Zorbax Eclipse plus C18 150 × 3.0 mm was used to achieve separation in parallel with gradient method condition optimisation. Acetonitrile and methanol were tested as the mobile phase B, and 0.1% formic acid in methanol was selected because of relatively higher separation efficiency. Various flow rates were analysed, and the rate of 0.3 mL/min was selected. Column and sample cooler temperatures were set to 40 and 12 ºC after the analysing different temperatures for optimum separation. The injection volume was optimized to 20 µL. The retention times for *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA), *N*-nitroso dimethylamine (NDMA), *N*-nitroso diethylamine (NDEA), N-nitroso diisopropylamino (NDIPA), *N*-nitroso ethyl isopropylamine (NEIPA), *N*-nitroso methyl phenylamine (NMPA) and *N*-nitroso dibutylamine (NDBA) were 9.48, 4.97, 11.54, 13.38, 12.53, 13.50 and 15.36 min, respectively. Loratadine was eluted at 15.95 min.

Optimization of MS/MS parameters: The conditions of mass spectrometry were optimized to develop a selective, simple, robust, and highly sensitive method for determining seven nitrosamine impurities in loratadine APIs. An impurity mix solution of 1 µg/mL was used for MS/MS development.

The sensitivity of the positive mode ionization was higher than that of negative mode in the initial developmental stages because of the polarity of impurities. Compound-dependent parameters, such as gas temperature (ºC), capillary voltage (V), nebulizer pressure (psi) and vaporization temperature (ºC) were optimized for all the impurities to acquire desired response for the parent ion. Furthermore, the collision energies were optimized through cross-checking with various collison cell voltages to obtain reproducible and sensitive MRM transitions for all the seven impurities.

Characterization data of loratadine API: ^{1}H NMR and 2D-NMR (DMSO-d_6) δ ppm: 1.153-1.188 (t, 3H), 2.137-2.291 (m, 2H), 2.296-2.325 (m, 2H), 2.794-2.854 (m, 2H), 3.165-3.186 (d, 2H), 3.270-3.601 (m, 2H), 3.614-3.647 (m, 2H), 4.010-4.063 (m, 2H), 7.082-7.103 (d, 1H), 7.186-7.229 (m, 2H), 7.307-7.312 (s, 1H), 7.567-7.589 (m, 1H) and 8.336-8.352 (d, 1H). ^{13}C NMR δ ppm: 14.57, 30.13-30.92, 38.87-40.12, 44.28-44.36, 60.65, 122.35, 125.66, 128.92, 130.66, 131.58, 133.21-133.44, 136.44, 137.45-137.80, 140.14, 146.34, 154.50 and 156.77. From these NMR data, the proposed structure is confirmed as loratadine.

Mass, DSC and IR studies: Loratadine is also confirmed by mass, DSC and IR spectral data. The observed loratadine mass value is 383.55 *m/z* in positive ionization by mass spectroscopy. Loratadine gave a characteristic and sharp endothermic peak at 135.41 ºC by DSC, which is close to its melting point (134-136 ºC). Thus, it indicates the crystalline nature of the drug. Pure Loratadine spectrum showed a sharp characteristic IR peaks at 3441.00 cm^{-1} (N-H *str.*), 2925.45 cm^{-1} (C-H *str.* of methyl group) and 1703.39 cm^{-1} (C=O). From these, mass, melting point and characteristic IR frequencies support that the proposed structure is loratadine.

Interpretation by mass spectral data of seven nitrosamines: All the seven nitrosamine impurities were confirmed by mass analysis. The obtained masses of *N*-nitroso dimethylamine (NDMA) is 75.00 by +ve ionization; *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA) is 145.00 by –ve ionization; *N*-nitroso diethylamine (NDEA) is 103.10 +ve ionization; *N*-nitroso ethyl isopropylamine (NEIPA) is 117.00 +ve ionization, *N*-nitroso diisopropylamino (NDIPA) is 131.10 +ve ionization, *N*-nitroso methyl phenylamine (NMPA) is 137.00 +ve ionization and *N*-nitroso dibutylamine (NDBA) is 159.00 +ve ionization.

Method validation: The newly developed LC-MS/MS analytical method was validated for specificity, system precision, method precision, limit of quantification (LOQ), limit of detection (LOD), linearity, accuracy, recovery, robustness, Ruggedness and standard, sample solution stability according to the FDA and ICH guidelines [15] for the seven nitrosamine impurities in loratadine.

Specificity: A single solution of loratadine with seven impurity mixture was prepared at specification level. The spiked loratadine solution was then subjected to LC-MS/MS analysis. The obtained results showed that there is no interference of loratadine API with all the seven impurities. The data and chromatograms acquired was captured in Table-3 and Fig. 2.

TABLE-3
SPECIFICITY DATA

Name of nitrosamines	RT	Transition
NDMA	4.97	75.0 -> 43.1
NMBA	9.48	147.0 -> 117.0
NDEA	11.54	103.0 -> 75.0
NIPEA	12.53	117.0 -> 74.9
NDIPA	13.38	131.0 -> 89.1
NMPA	13.50	137.0 -> 107.0
NDBA	15.36	159.1 -> 57.0
Loratadine	15.95	383.1

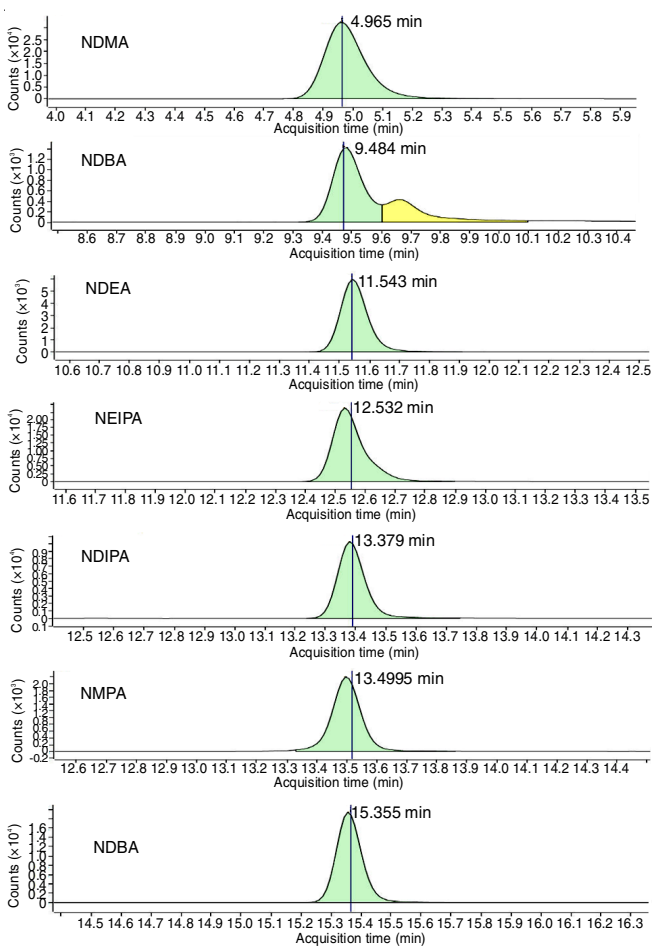

Fig. 2. Impurity standard chromatogram for seven impurities

System precision: The system precision of the method was evaluated at single level. Repeatability was checked by calculating the % RSD of six replicate determinations. Freshly prepared seven nitrosamine impurities standard solution at specification level was injected into LC-MS/MS. The % RSD of each nitrosamine impurity is not more than 15.0%. These results are represented in Table-4. This is confirmed an adequate precision of the developed method.

Method precision: Method precision was evaluated by preparing the six different preparations of seven nitrosamine impurities standard solution at specification level into the LC-MS/MS system as per the test method. The % RSD was calculated for the area of six preparations. The % RSD of each nitrosamine impurity is not more than 15.0% and the results are shown in Table-5.

TABLE-4
SYSTEM PRECISION DATA FOR SEVEN NITROSAMINE IMPURITIES

No. of injections	NDMA	NMBA	NDEA	NIPEA	NDIPA	NMPA	NDBA
1	284411	10575	40033	199898	66116	18939	121437
2	288140	9964	39129	185827	66768	17643	121836
3	288886	10484	40215	194264	65953	18064	121865
4	290237	10615	39357	196492	67235	18363	123592
5	289990	11245	40311	201808	67319	18725	123398
6	291448	10276	39338	182253	65834	17370	121514
ACVG	288852	10527	39731	193424	66538	18184	122274
STDV	2457	426	514	7808	658	611	963
% RSD	0.85	4.05	1.29	4.04	0.99	3.36	0.79

TABLE-5
METHOD PRECISION DATA FOR SEVEN NITROSAMINE IMPURITIES

No. of preparations	NDMA	NMBA	NDEA	NIPEA	NDIPA	NMPA	NDBA
1	321321	11364	38378	170195	64715	15129	117592
2	314159	11064	38378	178908	65157	15869	118022
3	315839	11011	38801	176565	65127	16005	120174
4	311566	9548	37687	162888	63045	14704	116471
5	309119	10608	38320	172096	65496	15416	117793
6	312209	10675	38554	175840	64386	14972	116644
ACVG	314036	10712	38353	172749	64654	15349	117783
STDV	4242	633	371	5766	878	512	1329
% RSD	1.35	5.91	0.97	3.34	1.36	3.34	1.13

Limit of detection (LOD) and limit of quantitation (LOQ): The LOQ and LOD values for all the seven impurities were estimated on the basis of the S/N ratios of 3.3 and 10, respectively, by injecting a known concentration of the standard. The results are presented in Table-6. For all the seven nitrosamine impurities, the S/N ratios were acquired using the Auto RMS algorithm in mass hunter software. The typical chromatograms of LOQ are presented in Fig. 3. At LOQ level, reproducibility was determined by using six injections. The % RSD of each nitrosamine impurity was not higher than 15% for reproducibility. The reproducibility data is shown in Table-7.

Linearity: For seven nitrosamine impurities, the linearity of the method was acquired from LOQ of 0.13 to 5.30 ppm (5-200%). The slope, regression coefficient and intercept values were obtained using the least squares linear regression analysis of impurity concentration versus average peak areas. A strong correlation (not lower than 0.99) between the concentrations and peak areas of seven impurities was observed (Table-8). The corresponding calibration curves are presented in Figs. 4 and 5. The results indicated that an excellent correlation existed between the peak areas and the concentration of the seven nitrosamine impurities.

TABLE-6
LOD AND LOQ CONCENTRATIONS FOR SEVEN NITROSAMINE IMPURITIES

Nitrosamines	LOD conc. (ppm)	LOQ conc. (ppm)	LOD area	LOQ area	LOD S/N	LOQ S/N
NDMA	0.04	0.13	4455	14813	1047	3450
NMBA	0.04	0.13	158	527	221	1493
NDEA	0.04	0.13	621	2071	425	817
NIPEA	0.04	0.13	2876	9585	1097	3578
NDIPA	0.04	0.13	1017	3390	561	5015
NMPA	0.04	0.13	265	884	387	1773
NDBA	0.04	0.13	1857	6189	1663	3638

TABLE-7
REPRODUCIBILITY AT LOQ FOR SEVEN NITROSAMINE IMPURITIES

No. of injections	NDMA	NMBA	NDEA	NIPEA	NDIPA	NMPA	NDBA
1	14813	527	2071	9585	3390	884	6189
2	15172	645	1992	9292	3306	759	5652
3	14413	526	1913	9208	3105	863	5941
4	14690	537	1986	9249	3322	865	5863
5	15680	523	2193	8987	3476	743	5885
6	14552	559	1986	9833	3158	881	6182
ACVG	14887	553	2024	9359	3293	833	5952
STDV	467	47	97	301	140	64	206
% RSD	3.14	8.51	4.79	3.22	4.24	7.67	3.46

TABLE-8
LINEARITY DATA FOR SEVEN NITROSAMINE IMPURITIES

Conc. (ppm)	NDMA (n = 2)	NMBA (n = 2)	NDEA (n = 2)	NIPEA (n = 2)	NDIPA (n = 2)	NMPA (n = 2)	NDBA (n = 2)
0.13	14930	539	2053	9552	3366	898	6122
0.27	29860	1076	4104	19104	6732	1795	12243
0.80	89717	3290	12228	57101	20118	5243	36973
1.33	148012	5620	20126	98078	33913	8877	62491
1.99	219732	8409	29930	148100	49921	13330	93681
2.65	292356	11592	40503	197464	66771	17722	124399
3.98	438967	17945	61898	311921	102741	27725	191752
5.30	571453	22059	79342	372529	129491	32935	238119
r2	1.000	0.998	1.000	0.997	0.999	0.997	0.999
Slope	108319	4292	15146	72940	24828	6438	45935
Intercept	2898	-9	129	1748	578	328	1268

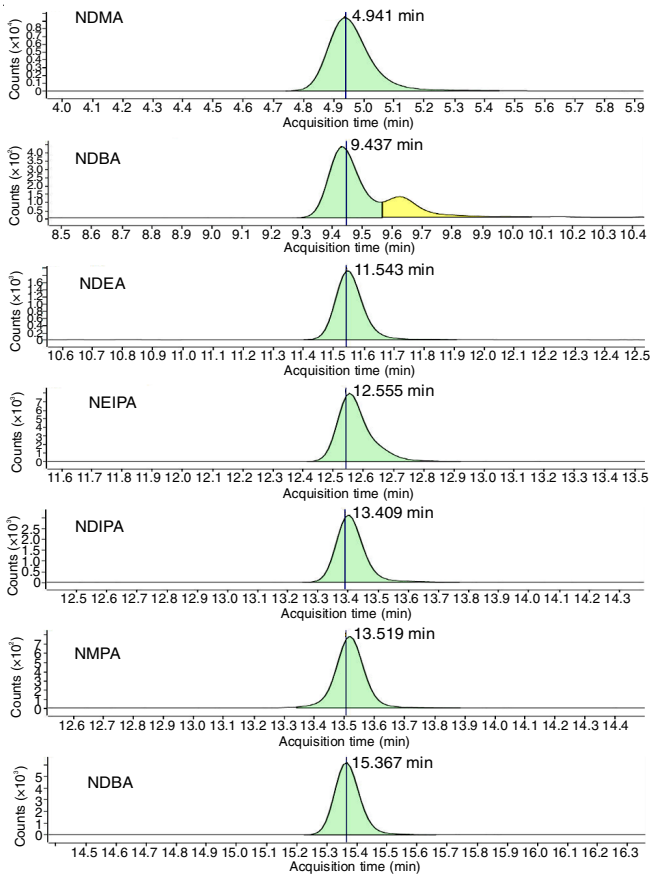

Fig. 3. LOQ chromatograms for seven nitrosamine impurities

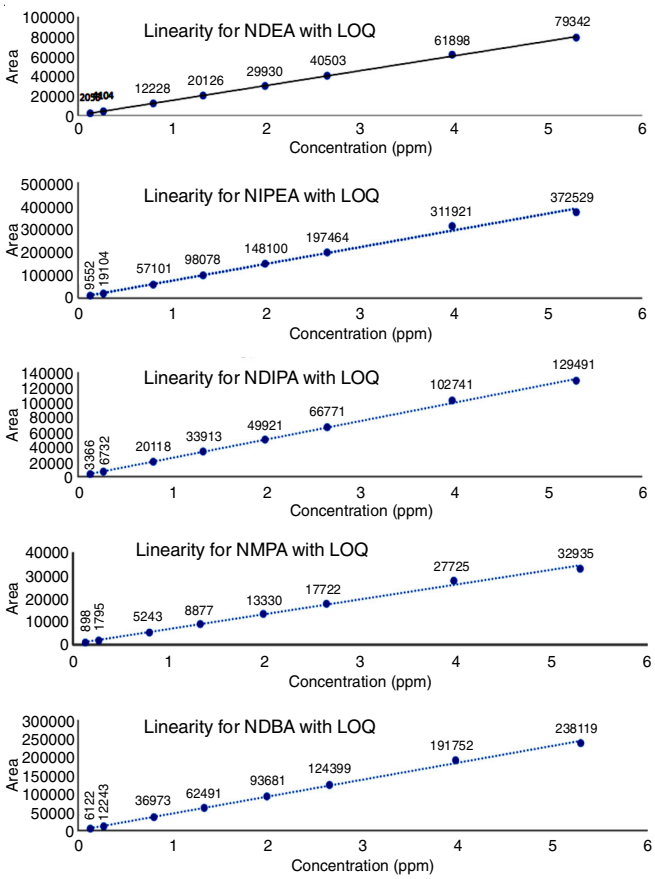

Fig. 5. Calibration curves for NDEA, NIPEA, NDIPA, NMPA and NDBA

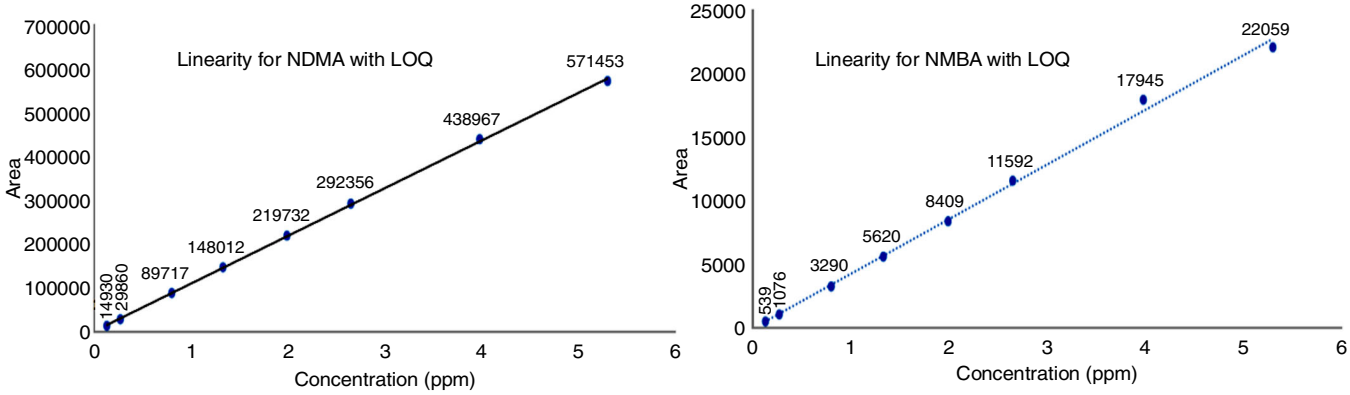

Fig. 4. Calibration curves for NDMA and NMBA

TABLE-9
RECOVERY DATA FOR SEVEN NITROSAMINE IMPURITIES

Recovery levels (%)	Name of nitrosamines						
	NDMA	NMBA	NDEA	NIPEA	NDIPA	NMPA	NDBA
LOQ	89.13	110.79	91.57	97.58	95.66	113.81	108.59
50	111.87	108.29	104.02	95.72	104.03	91.66	94.69
100	109.52	105.63	104.82	100.95	103.66	92.32	93.06
150	103.91	98.94	95.43	92.14	96.65	89.24	94.2

Recovery: Deviation from linearity, *i.e.* accuracy, which was approximately 5% (0.13 ppm) of the specified limit, was determined by injecting an impurity mixture from LOQ. Furthermore, recovery was evaluated through standard addition in triplicate for three concentrations of 50%, 100% and 150% (1.33, 2.65 and 3.98 ppm) levels in loratadine APIs. For accuracy, the acceptance criterion at LOQ was 80-120%. At 50%, 100%, and 150% accuracy was 85-115%. The percentage recoveries for each nitrosamine impurities are presented in Table-9.

Robustness: To evaluate method robustness, various method conditions, such as column oven temperatures and the flow rate of mobile phases, were used. The optimized mobile phase flow rate 0.3 mL/min was obtained after varying it from 0.27 to 0.33 mL/min. The influence of gas temperature on separation was analysed at 347 and 353 ºC. The % RSD was calculated for each parameter and should not be higher than 15%. No effect was observed on the chromatographic performance of the seven nitrosamine impurities because of the optimization, which proved method robustness. The robustness data are presented in Table-10.

TABLE-10
ROBUSTNESS DATA FOR SEVEN NITROSAMINE IMPURITIES

Name of nitrosamines	Flow rate (mL/min)		Gas temperature (°C)	
	0.27 mL/min (%RSD)	0.33 mL/min (%RSD)	347 °C (%RSD)	353 °C (%RSD)
NDMA	2.88	4.14	2.07	3.81
NMBA	5.83	5.6	5.63	6.48
NDEA	2.27	2.43	1.45	1.03
NIPEA	2.3	3.23	3.88	4.78
NDIPA	1.33	1.49	1.33	1.14
NMPA	3.66	2.29	3.65	2.05
NDBA	2.25	4.35	2.17	1.60

Ruggedness: Ruggedness of the method was evaluated by performing the standard impurity solution at specification level in six replicates using different analysts on different days and the results are summarized in Table-11. The %RSD was calculated at different analyst and different days for each nitrosamine impurity. The results were found with in acceptance criteria (NMT 15.0%) and indicate that the method adopted is rugged.

Solution stability: The solution stability of seven impurities and loratadine drug was determined by storing the non-spiked sample solutions at 25 ºC for 6, 12, 24, 48, and 72 h and by comparing it against the freshly prepared initial sample and standard solutions. The %variation solution stability was 100 ± 10%. No considerable changes were observed in the seven nitrosamine impurities. Therefore, the stability of impurities in sample solution was confirmed to last for a minimum of 72 h. The corresponding data are presented in Table-12.

Tablet analysis: The proposed method was evaluated by the assay of commercially available loratadine drug tablet for quantification of seven nitrosamine impurities present in it. The prepared loratadine tablet solution was injected into LC-MS/MS system as per proposed method. The all seven nitrosamine impurities were obtained below LOD. So, it is consider as not detected in loratadine formulations. The data is shown in Table-13.

Conclusion

The IR, mass, ^{1}H & ^{13}C NMR and 2D-NMR and DSC were utilized to confirm and identify the structures of loratadine APIs. An ultra-sensitive LC-MS/MS technique is developed for the simultaneous estimation of seven nitrosamine impurities in loratadine drugs. This method is more sensitive than the existing methods for nitrosamines. Many LC-MS/MS methods are available for nitrosamines and various drugs, but no method is reported for the simultaneous quantification of seven nitrosamines in loratadine by using LC-MS/MS so far. The LC-MS/MS technique allows the quantification of the highest number of impurities compared with other detection methods, such as GC-MS/MS, where a limitation to ionized impurities, such as NDBA and NMBA, is presented. We validated the

TABLE-11
RUGGEDNESS DATA FOR SEVEN NITROSAMINE IMPURITIES

Different days and analysts		%RSD for NDMA	%RSD for NMBA	%RSD for NDEA	%RSD for NIPEA	%RSD for NDIPA	%RSD for NMPA	%RSD for NDBA
Day-1	Analyst-1	2.14	4.77	1.63	2.25	2.45	2.6	3.88
	Analyst-2	1.71	1.91	2.34	2.5	3.24	2.35	2.49
	Analyst-1 & 2	1.87	5.25	1.92	2.28	2.74	2.39	3.47
Day-2	Analyst-1	2.87	3.34	2.22	2.85	4.02	4.35	3.37
	Analyst-2	2.36	0.76	3.13	1.82	3.16	1.54	3.13
	Analyst-1 & 2	4.28	4.3	3.53	2.43	3.45	3.44	3.16
Analyst-1	Day-1 & 2	3.59	3.95	2.17	2.67	3.17	3.43	3.74
Analyst-2	Day-1 & 2	2.03	1.39	2.87	2.08	3.06	2.06	2.73

TABLE-12
SOLUTION STABILITY DATA FOR SEVEN NITROSAMINE IMPURITIES

Name of nitrosamines	Initial hours (% variation)	After 6 h (% variation)	After 12 h (% variation)	After 24 h (% variation)	After 48 h (% variation)	After 72 h (% variation)
NDMA	Not applicable	0.19	0.43	-2.59	1.33	-2.81
NMBA	Not applicable	0.86	1.34	0.04	-0.76	-1.92
NDEA	Not applicable	0.40	-3.78	2.01	2.90	3.73
NIPEA	Not applicable	-2.24	2.72	0.92	-2.7	0.92
NDIPA	Not applicable	1.62	0.84	2.59	1.13	2.50
NMPA	Not applicable	-0.58	0.59	0.27	0.59	1.15
NDBA	Not applicable	-1.79	-0.31	-1.49	-2.51	1.26
Loratadine API	Not applicable	Not detected	Not detected	Not detected	Not detected	Not detected

TABLE-13
TABLET ANALYSIS

Name of drug	Label claim (mg)	NDMA (ppm)	NMBA (ppm)	NDEA (ppm)	NIPEA (ppm)	NDIPA (ppm)	NMPA (ppm)	NDBA (ppm)
Loratadine	10	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

efficiency of the method through critical parameters. Compared with the reported methods, our LC-MS/MS technique is sensitive and novel. Moreover, the Ultivo LC/TQ technique presents highly advanced approach to pharma analyses. The determined LOD and LOQ values are considerably low, which confirms the sensitivity of the technique. This method can be utilised for the routine quantification of the seven nitrosamine impurities in loratadine and its dose forms to a concentration of 0.8 ppm (0.16 ppm with respect to the test). Thus, our proposed method is sensitive, simple, accurate, economical, precise, and rapid for quantifying the seven nitrosamine impurities in loratadine APIs and its dose forms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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