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Simultaneous quantitation of three potential genotoxic impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide in sumatriptan succinate by liquid chromatography mass spectrometry

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Abstract

A quantitative method was developed and validated for potential genotoxic impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide present in sumatriptan succinate drug substance. With this isocratic method involving LC-MS with electrospray ionization, all the three impurities were separated on a hypersil silica column (250 x 4.6mm, 5µm) and quantified by selective ion monitoring mode. The method has very high sensitivity (LOQ - 4.5 ppm) with correlation coefficient, precision and accuracy well within acceptable ICH limits. This method can be used for routine quality control of potential genotoxic impurities in sumatriptan drug substance.

1.INTRODUCTION

Genotoxic impurities are a specific group of impurities which have recently been a subject of increased attention. These impurities are residues from the synthesis process of the drug substance, from the production of the drug formulation, and/or can result from degradation of the Active Pharmaceutical Ingredient (API) alone or in presence of excipients. Genotoxic substances are potentially mutagenic or carcinogenic causing genetic mutation and may contribute to the development of tumors [1] because of their predicted ability to combine with DNA. Hence it is important to identify the presence of genotoxic impurities in drug substances followed by monitoring and controlling in very low levels to ensure safety.

Sumatriptan succinate is a triptan sulfa drug containing a sulfonamide group. It is a 5 HT agonist and is used for the treatment of migraine. The synthetic intermediates 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide are present in small amounts as process impurities in API. These intermediates are identified as potential genotoxic impurities because of the presence of nitro and sulphonyl groups and hence should be controlled to ppm level in the final product. The chemical structure of sumatriptan succinate and these impurities are shown in Fig.1, 2, 3 and 4.

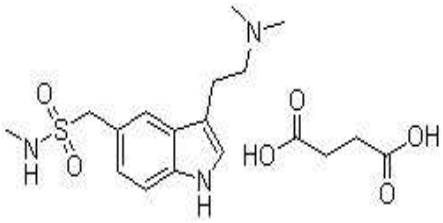

Fig.1: Sumatriptan succinate

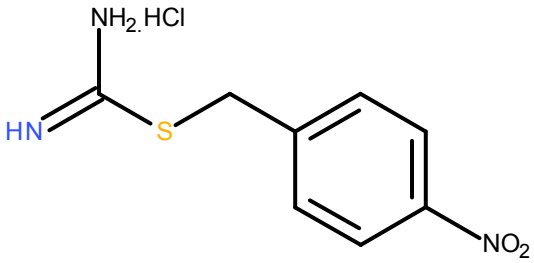

Fig.2: 4-Nitrobenzyl thiouronium hydrochloride

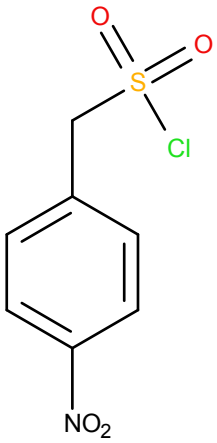

Fig.3: 4-Nitrobenzene methanesulfonyl chloride

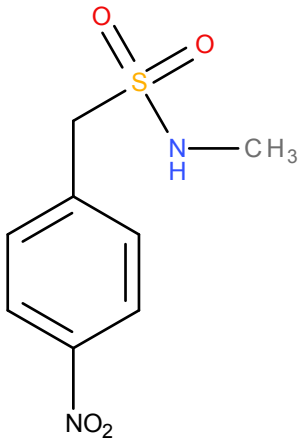

Fig.4: N-methyl-4-nitrobenzene methanesulfonamide

Complete elimination of the genotoxic impurities from the drug substance is often unachievable. So we must find out the acceptable risk level. A Threshold of Toxicological Concern (TTC) has been developed to define a common exposure level for any unstudied chemical that will not pose a significant risk of carcinogenicity or other toxic effects [2-13]. This TTC value was estimated to be 1.5 µg/person/day. Hence specification limit for impurities in ppm = 1.5 µg per maximum daily dose of the drug substance in grams. For example a drug dosed at 1g/day the genotoxic impurity level would be 1.5ppm. Based on the maximum daily dose of 100 mg of sumatriptan, its genotoxic impurities are required to be controlled at a limit of 15ug/g (15ppm).

Determination of these impurities at very low levels requires highly sensitive analytical methods. A tremendous analytical challenge associated with these genotoxic impurities is the resolution of the low level impurity from the matrix interferences of the relatively large amount of the API. The hyphenated technique of high-performance liquid chromatography-mass spectroscopy provides an efficient separation capability, good selectivity as well as sensitivity.

Quantification of a genotoxic impurity 4-chloro-2-hydroxybutanesulphonic acid sodium salt in sumatriptan succinate by LC/MS/MS using MRM mode was reported by Narayana et.al[14]. But there is no simultaneous method reported for the very low level quantification of all the three potential genotoxic impurities in sumatriptan succinate. Hence the present study was undertaken to develop a very sensitive, specific and accurate LC-MS method to quantify these three potential genotoxic impurities in sumatriptan succinate in a single run.

2.MATERIALS AND METHODS

Samples of sumatriptan succinate and its potential genotoxic impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide were obtained from Natco Pharma Ltd, Natco Research Centre, Hyderabad, India. AR grade ammonium acetate and acetic acid were purchased from Merck (Mumbai, India). HPLC grade acetonitrile was purchased from JT Baker (Mumbai,India). Purified water collected through Milli-Q Plus water purification system (Millipore,USA)

2.1 Instrumentation

The LC-MS method development and validation was done using Agilent 1200 series HPLC system connected with Agilent mass spectrometer G6120B Single Quad, equipped with electrospray ionization probe.

2.2 LC-MS chromatographic conditions

The analysis was carried out using hypersil silica 250x4.6 mm column with 5µm particle diameter with a flow rate of 1.0 ml/min. The mobile phase used was a a mixure of 10mM ammonium acetate with 0.1% acetic acid and acetonitrile using a isocratic composition in the ratio of 20 : 80 v/v respectively. The column temperature was maintained at 35°C and the injection volume was 20µl. Mass spectrometer was operated in electrospray ionization with both positive ion mode and negative ion mode with a capillary voltage of 3000V. The fragmentor was set at 70V, the drying gas flow was 13 L/min with a temperature of 350° C and nebulizer pressure was 60 psig. Under these conditions impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide in sumatriptan drug substance were quantified by SIM mode. 4-nitrobenzyl thioronium hydrochloride was monitored at m/z 212.1(protonated). 4-nitrobenzene methanesulfonyl chloride when dissolved in diluent readily converts into 4-nitrobenzene methanesulfonic acid and was monitored at m/z 216.1(deprotanated). N-methyl-4-nitrobenzene methanesulfonamide was monitored at m/z 229.1(deprotanated).

2.3 Preparation of standards and test sample solutions

The standard stock solutions of 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide were prepared approximately at 0.1 mg/ml in mobile phase. The API test samples were typically prepared at 1mg/ml in mobile phase.

3.0 RESULTS AND DISCUSSION

3.1 Method development

As the actual goal of the present work was to develop a method that could successfully resolve and estimate all the three potential genotoxic impurities in sumatriptan active pharmaceutical ingredient in a single run, different stationary phases and mobile phases were used and finally the desired chromatographic separation was achieved on a hypersil silica 250x4.6 mm column with 5µm particle size in isocratic mode using 10mM ammonium acetate with 0.1% acetic acid and acetonitrile in the ratio of 20 : 80 v/v respectively with a flow rate of 1.0ml/min.

3.2 Method validation:

The method has been validated for the quantification of potential genotoxic impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide in sumatriptan active pharmaceutical ingredient to ensure that the performance characteristics of the method meet the requirements for its intended analytical applications. During the method validation the assessed parameters were specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, precision and accuracy.

3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated with signal to noise ratios of 3:1 & 10:1 respectively and by injecting a dilute solution having known concentrations of impurities and established the minimum level at which the impurities can be reliably detected. Precision was also carried out at LOQ level by injecting the LOQ solution six times. The LOD & LOQ and precision at LOQ results obtained for impurities are listed in Table.1 and 2. The representative LOQ chromatogram is shown in figure.5.

Table 1: LOD and LOQ values

Impurity	LOD		LOQ	
	Conc.(ppm)	S/N ratio	Conc.(ppm)	S/N ratio
4-nitrobenzyl thiouronium hydrochloride	1.5	4.1 : 1	4.59	10.2 : 1
4-nitrobenzene methanesulfonyl chloride	1.5	3.1 : 1	4.57	10.1 : 1
N-methyl-4-nitrobenzene methanesulfonamide	1.5	6.0 : 1	4.61	11.6 : 1

Table 2: Summary of peak areas for precision at LOQ

S.No	4-nitrobenzyl thioronium hydrochloride peak area	4-nitrobenzene methanesulfonyl chloride peak area	N-methyl-4-nitrobenzene methanesulfonamide peak area
1	10589.130	5125.777	1111.778
2	11523.209	4805.417	1263.298
3	12108.528	4668.955	1106.096
4	11422.007	4754.904	1198.902
5	11323.477	4882.717	1060.644
6	11246.301	5060.322	1004.294
Mean	11369	4883	1124
% RSD	**4.31**	**3.65**	**8.33**

3.4 Specificity

The specificity of the optimized method was performed by injecting stock solutions of individual impurities to check resolution among the impurities and drug substance under the same conditions mentioned in LC-MS chromatographic conditions. The specificity is represented in the figure.5. Summary of retention time and m/z value for sumatriptan and its impurities are mentioned in the given table.

Table 3: Summary of retention time and m/z values

Compound	Retention Time(Minutes)	Mass(m/z) value
4-nitrobenzyl thiouronium hydrochloride	10.4	212.1 (+ve)
4-nitrobenzene methanesulfonyl chloride	2.2	216.1 (-ve)
N-methyl-4-nitrobenzene methanesulfonamide	3.0	229.1(-ve)

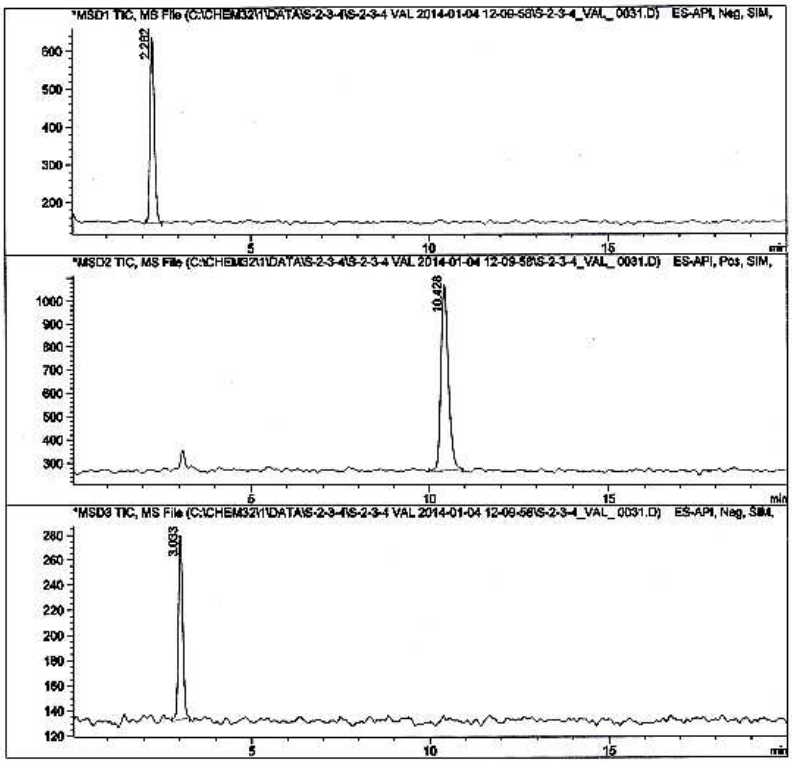

Fig.5: **Specificity and LOQ hromatogram**

3.5 Linearity

Linearity of the method was checked by preparing the solutions at 6 concentration levels from LOQ to 150% of specification limit (4.5, 7.5, 12.25, 15.0, 18.0, 22.5 ng/ml). The mean responses recorded for each impurity were plotted against concentration. The correlation coefficient of linear regression was found to be greater than 0.99 for each impurity, indicating good linearity. Corresponding linearity graphs are shown in figure 6, 7, 8 and data is represented in the table 4.

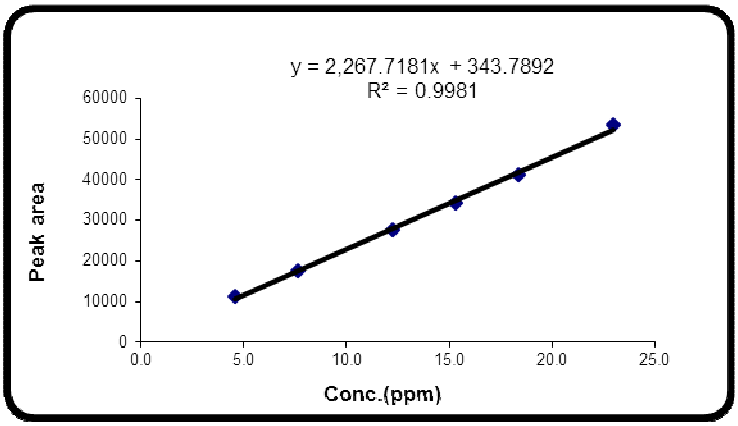

Fig.6: Linearity graph for 4-nitrobenzyl thiuoronium hydrochloride

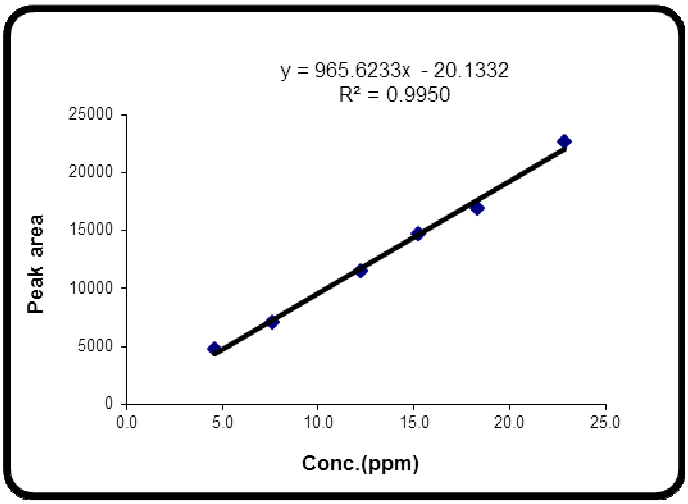

Fig.7: Linearity graph for 4-nitrobenzene methanesulfonyl chloride

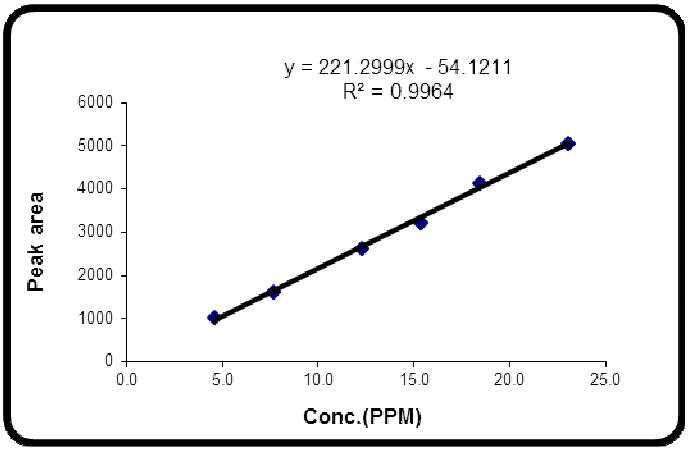

Fig.8: Linearity graph for N-methyl-4-nitrobenzene methanesulfonamide

Table 4: Linearity data

LEVEL	4-nitrobenzyl thiouronium hydrochloride peak area	4-nitrobenzene methanesulfonyl chloride peak area	N-methyl-4-nitrobenzene methanesulfonamide peak area
LOQ	11416	4839	1032
50%	17593	7146	1628
80%	27835	11485	2632
100%	34405	14727	3208
120%	41333	16975	4150
150%	53370	22633	5059
Slope	2267.7181	965.6233	221.2999
R^2	**0.9981**	**0.9950**	**0.9964**

3.6 Accuracy

Accuracy of the method was evaluated by using four solutions containing sumatriptan base spiked with the impurities at LOQ, 50%, 100% and 150% of the specification limit (15 ppm). Each concentration level was prepared in triplicate. The percentage recovery results obtained for the three impurities are listed in table.5, 6 and 7. A representative chromatogram of sumatriptan succinate sample spiked at LOQ level (4.5ppm) is shown in figure 9.

Table 5: Summary of % recoveries for 4-nitrobenzyl thiouronium hydrochloride

Level	% w.r.t Specification limit	Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	% Recovery	% RSD
LOQ	30.6	0.00000459	0.00000433	94.3	1.17
	30.6	0.00000459	0.00000428	93.2	
	30.6	0.00000459	0.00000438	95.4	
50 %	51.0	0.00000765	0.00000767	100.3	0.61
	51.0	0.00000765	0.00000768	100.4	
	51.0	0.00000765	0.00000760	99.3	
100 %	102.0	0.00001530	0.00001541	100.7	0.78
	102.0	0.00001530	0.00001534	100.3	
	102.0	0.00001530	0.00001518	99.2	
150 %	153.0	0.00002295	0.00002338	101.9	0.99
	153.0	0.00002295	0.00002320	101.1	
	153.0	0.00002295	0.00002365	103.1	

3.7 System precision and system suitability

The precision and system suitability was performed by injecting six replicates of the working standard solution (15ppm of the three impurites). The %RSD for the peak areas obtained was calculated. The data presented in the table 8 establishes system precision

Table 6: Summary of % recoveries for 4-nitrobenzene methanesulfonyl chloride

Level	*% w.r.t Specification limit*	*Theoretical Conc. (mg/mL)*	*Measured Conc. (mg/mL)*	*% Recovery*	*% RSD*
LOQ	30.5	0.00000457	0.00000522	114.2	10.04
	30.5	0.00000457	0.00000436	95.4	
	30.5	0.00000457	0.00000446	97.6	
50 %	50.7	0.00000761	0.00000773	101.6	3.64
	50.7	0.00000761	0.00000817	107.4	
	50.7	0.00000761	0.00000829	108.9	
100 %	101.5	0.00001523	0.00001532	100.6	3.72
	101.5	0.00001523	0.00001594	104.7	
	101.5	0.00001523	0.00001481	97.2	
150 %	152.3	0.00002284	0.00002219	97.2	2.48
	152.3	0.00002284	0.00002310	101.1	
	152.3	0.00002284	0.00002324	101.8	

Table 7: Summary of % recoveries for N-methyl-4-nitrobenzene methanesulfonamide

Level	% w.r.t Specification limit	Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	% Recovery	% RSD
LOQ	30.7	0.00000461	0.00000437	94.8	4.44
	30.7	0.00000461	0.00000408	88.5	
	30.7	0.00000461	0.00000444	96.3	
50 %	51.3	0.00000769	0.00000824	107.2	1.83
	51.3	0.00000769	0.00000829	107.8	
	51.3	0.00000769	0.00000853	110.9	
100 %	102.5	0.00001538	0.00001696	110.3	1.07
	102.5	0.00001538	0.00001707	111.0	
	102.5	0.00001538	0.00001672	108.7	
150 %	153.7	0.00002306	0.00002620	113.6	*1.54*
	153.7	0.00002306	0.00002592	112.4	
	153.7	0.00002306	0.00002541	110.2	

Table 8: **System suitability and system precision**

S. No.	peak area		
	4-nitrobenzyl thiouronium hydrochloride	4-nitrobenzene methanesulfonyl chloride	N-methyl-4-nitrobenzene methanesulfonamide
1	32702.352	12010.915	2751.035
2	33014.395	11932.209	2801.226
3	33406.875	12003.866	2786.074
4	33720.199	11821.132	2787.021
5	33048.160	12809.793	2732.095
6	32084.246	12397.184	2577.734
Mean	32996	12163	2739
% RSD	**1.72**	**3.06**	**3.04**

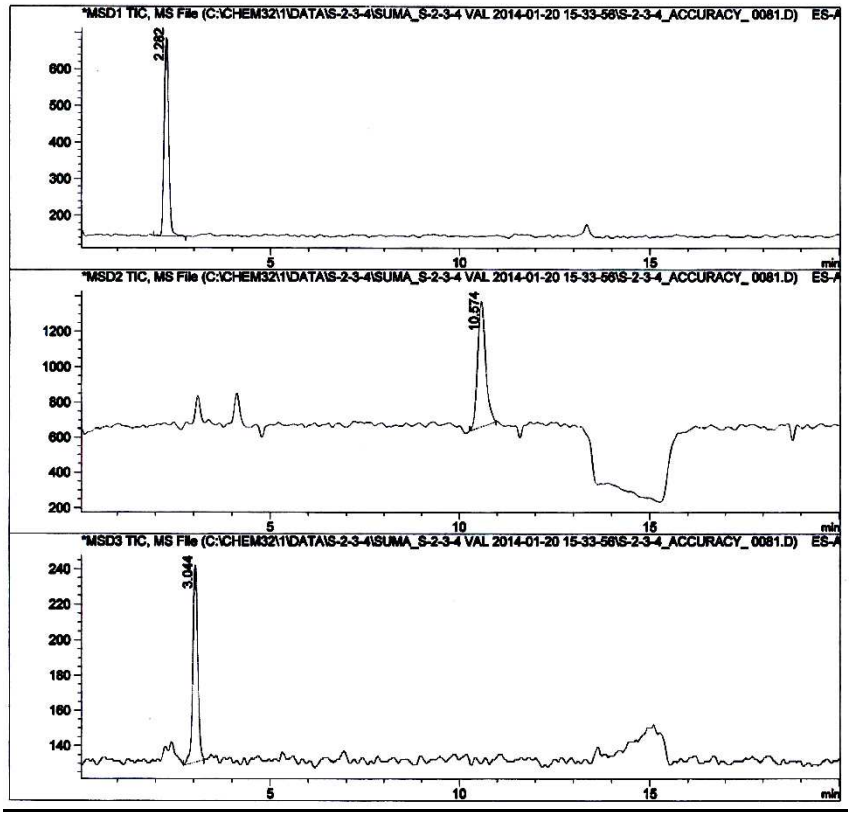

Fig.9: Representative chromatogram of sumatriptan succinate spiked at LOQ level

4.0 CONCLUSION

The proposed LCMS method is simple, sensitive and accurate to quantify simultaneously all the three impurities 4-nitrobenzyl thiouronium hydrochloride, 4-nitrobenzene methanesulfonyl chloride and N-methyl-4-nitrobenzene methanesulfonamide at ppm level present in sumatriptan succinate. The validated parameters are well within the limits and this method is suitable for routine quality control test for sumatriptan succinate drug substance.

5.0 ACKNOWLEDGEMENT

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