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Determination and validation of Monomethylamine content by Ion Chromatography method in pharmaceutical drug substances

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Abstract

A simple and sensitive ion chromatography method was developed, optimized and validated for the determination of Monomethylamine (MMA) in various drug substances, which is the process impurity. The validation of analytic method was realized through specificity, linearity, LOD, LOQ, precision and accuracy parameters. A model compound, Tadalafil drug substance was chosen for this study and limit of detection (LOD) and limit of quantification (LOQ) values were 0.09 µg/mL and 0.30 µg/mL respectively. The average accuracy value is 101.6%. And also determination of MMA in different drug substances like Alfuzosin hydrochloride, Sumatriptan succinate, Sertraline hydrochloride and Didanosine with slight modifications in methodology were discussed in this work.

Key words: Ion chromatography, Gas chromatography, Monomethylamine, Tadalafil, Validation.

INTRODUCTION

Tadalafil is a phosphodiesterase type 5- inhibitor, used in the management of Erectile Dysfunction (ED) or impotence [1,2]. ED, inability to achieve a penile erection sufficient for satisfactory sexual performance, is estimated to affect many men world wide[3-5]. ED is more common in advanced age and related to hypertension or diabetes mellitus or use of certain pharmacological agents e.g. antihypertensives [3]. Tadalafil is a secondary messenger for the smooth muscle relaxing effects of nitric oxide, which plays an important role in the vasodilatation of erectile tissues[6-8].

Chemically Tadalafil name is cis-(6R,12aR)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1',2':1,6] pyrido- [3,4-b]indole-1,4-dione.The chemical structure of Tadalafil was given in Fig.1.

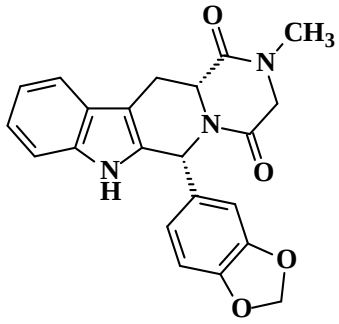


Fig .1 Chemical structure of Tadalafil.

In the process of Tadalafil, MMA was used as one of the process reagents to convert N-Chloroacetyl-β-Carboline to Tadalafil. Similarly, MMA was used in the various stages of preparation of various drug substances like Alfuzosin hydrochloride, Sumatriptan succinate, Sertraline hydrochloride and Didanosine. Consequently it may be retained or may not be retained in final stage of drug substance. And there is no human or animal information about carcinogenicity, teratogenicity, embryotoxicity, reproductive toxicity and mutagenicity[9]. But in the aspect of regulatory agencies requirements, it's median lethal dose, 50% (LD_{50}) in mouse is 2400 mg/m^3 was reported, so it is controlled as a List 1 substance [10] by the United States Drug Enforcement Agency (DEA). Hence, control of this impurity is required in the drug substances. Determination of monomethylamine was reported in literature by using following analytical techniques in different samples. MMA detection in forensic examination of explosive residues by using capillary electrophoresis technique [11], analysis of MMA by solid-phase microextraction by HPLC after on-fibre derivatization with 9-fluorenylmethyl chloroformate [12], a colorimetric method for the analysis of methylamine in urine samples [13], determination of volatile amines in sediment and water samples by Gas chromatographic method [GC] [14] and volatile amines like dimethylamine, trimethylamine and MMA in fish samples by GC [15]. To the best of our knowledge no report has been published on the analysis of MMA in Tadalafil and for various drug substances in literature.

MATERIALS AND METHODS

Chemicals, reagents and samples

Monomethylamine hydrochloride and aqueous methylamine solution (40%) were procured from Fluka.

L-tartaric acid, dipicolinic acid (Pyridine-2,6-dicarboxylic acid) ,N,N-dimethylformamide, ethyl acetate and dimethylsulfoxide were procured from E.Merck; India. Tadalafil and its related substances were prepared at Aurobindo Pharma Limited Research Centre, India. Highly pure milli-Q water was used with the help of millipore purification system.

Ion chromatography
An Ion chromatography system Metrohm 761 Compact IC with conductometric detector, peristatic pump and Metrohm 750 auto sampler with 20µl loop, equipped with the Metrohm 761 Compact IC software data handling system was used. Sartorious analytical, microbalances and Research centrifuge C24 was used for this experiment.

The mobile phase was a mixture of 4 m mole of tartaric acid and 1 m mole of dipicolinic acid in one litre of water. The analysis was carried out on Metrosep Cation 1-2, 125 mm long, 4.0 mm i.d., 7µm particle diameter column, maintained at ambient conditions. Mobile phase was flushed through the column at a flow rate of 0.8ml/min.The run time for the standard and sample were 20min. The injection volume was 20µl. The retention time of methylamine is about 5.0 min. As Tadalafil is practically insoluble in water, so the sample was first dissolved in N, N-Dimethylformamide and then water is added to make the solution aqueous and shake vigorously for 1min, centrifuge and filter.

Standard Solution
Accurately weigh 65 mg of methylamine hydrochloride into a 100 ml volumetric flask, bring to volume with water and mix to prepare a final methylamine concentration of 3.0µg/ml.

Sample Solution
Accurately weigh 100 mg of Tadalafil into centrifuge tube and dissolve in 2 ml of N,N-Dimethylformamide and add 8 ml of water to prepare a concentration of 10000µg/ml and shake vigorously for 1 minute and finally centrifuge for 5 min at 5000 rpm and use the filtrate solution for analysis.

Gas Chromatography
A Gas chromatography system Shimadzu GC2010 equipped with split injector, a Flame ionization detector, auto sampler Shimadzu AOC-20i long with data handling system GC Solutions, versions 2.30.00 SU6 was used. The analysis was carried out by using fused silica capillary column, 30m long ; 0.53 mm internal diameter coated with 5% diphenyl and 95% dimethyl polysiloxane stationary phase of 5.0 µm film thickness (Rtx-5 Make:Restek). The injector temperature was 180°C and detector temperature was 260°C. The GC oven temperature was maintained at 40°C for 5 min then programmed 20°C per minute to a final temperature of 220°C which was held for 11 minutes. Helium was used as carrier gas at a column pressure of 30KPa, the split ratio was 1:1 and injection volume was 1.0µl.

Internal standard solution
Accurately dispense and weigh 28 mg of Ethyl acetate into a 10 ml volumetric flask, bring to volume with Dimethylsulfoxide and mix to prepare a final Ethyl acetate concentration of 0.014 mg/ml.

Standard solution
Accurately dispense and weigh 56 mg of 40% aqueous methylamine solution into a 25 ml volumetric flask, bring to volume with internal standard solution and further 1 ml of this solution diluted to 50 ml with internal standard solution.

Sample Solution
Accurately weigh 300 mg of Tadalafil into a 5 ml volumetric flask, bring to volume with internal standard solution.

RESULTS AND DISCUSSION

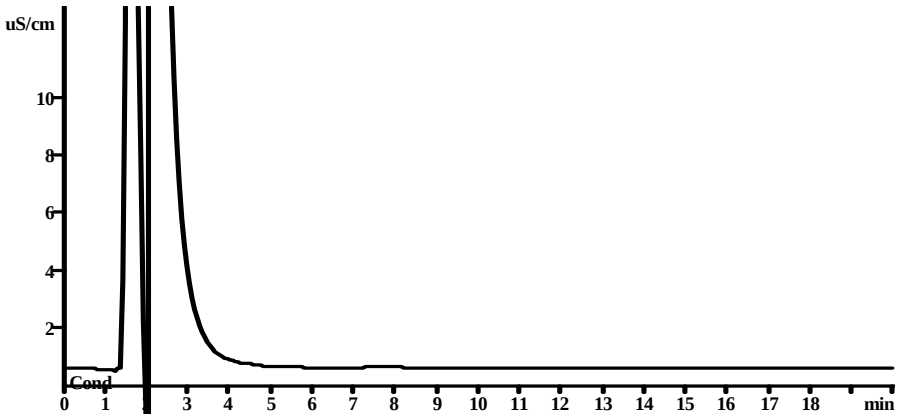
Method optimization
The objective of this work is to determine ppm level concentrations of low molecular weight amines in presence of strongly retained in drugs by using ion chromatography. During direct injection to IC, where the late elution of strongly retained drugs requires organic modifier like acetone and acetonitrile for fast elution of target amines as well as drug matrix. During method development and optimization, solubility of drug(s) and extraction of targeted analyte was taken for consideration. As tadalafil was practically insoluble in water and freely soluble in N,N-Dimethylformamide, subsequently sample was first dissolved in 2 ml of N,N-Dimethylformamide and added 8 ml of water , this solution was injected into IC, less recovery was observed. For better recovery of our interest of analyte, sample solution was shaken vigorously and centrifuged for 5 min at 5000 rpm and filtered. For optimizing method for tadalafil, modified the eluent compositions by using tartaric acid, dipicolinic acid and nitric acid, changing in the eluent flow-rates and used different stationary phases and satisfactory results were achieved in Metrosep Cation 1-2, 125 mm long, 4.0 mm i.d., 7µm particle diameter column with flow rate 0.8ml/min. Our area of interest, same methodology was employed for few of the drug substances like Alfuzosin hydrochloride, Sumatriptan succinate, Sertraline hydrochloride and Didanosine. In the case of Sumatriptan succinate, we observed the interference due to drug matrix, this was optimized by using gradient flow rate. In Didanosine, we modified the eluent by using tartaric acid and organic modifier acetone for better peak shape and fast elution of analyte.

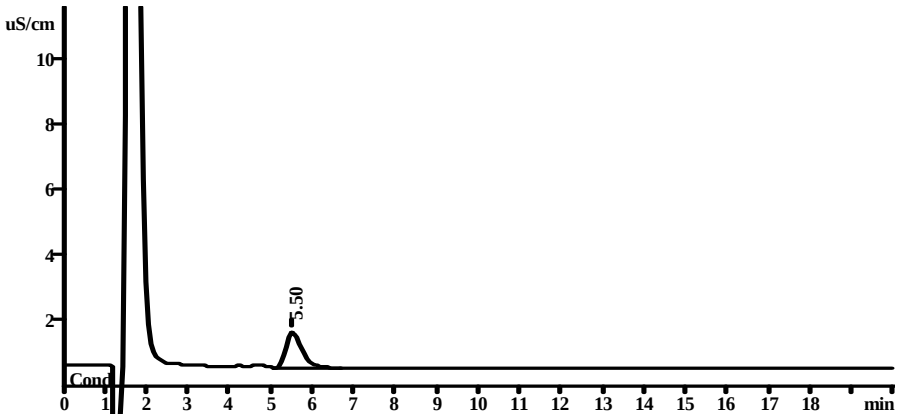
Method validation on Ion Chromatography
In order to determine the monomethylamine in Tadalafil drug substance, the method was validated as per the ICH guidelines [16]. Individually in terms of specificity, LOD, LOQ, linearity, accuracy, precision and stability of sample solution.

Specificity
Specificity is the ability of the method to measure the analyte response in the presence of all impurities related to drug substances, as well as many common cations like lithium, sodium, ammonium, calcium and magnesium and other amines like dimethylamine, triethylamine. For specificity determination, checking the interference of blank, monomethylamine spiked to drug substance at known concentration level and all known related substances of Tadalafil including monomethylamine with known concentration level were spiked to Tadalafil drug substance. The solutions were prepared and injected separately into triplicate and determined the monomethylamine content. The % difference between mean of Monomethylamine content in spiked individually and spiked with known related substances was determined, it was observed that the blank peaks and other related substances peaks did not interfere with our interest of monomethylamine peak. In conjunction, Fig. 2 depicts an overlay chromatogram of blank solution, Monomethylamine standard solution, Tadalafil spiked with Monomethylamine along with related substances of Tadalafil and specificity data given in Table 1.

Table 1: Specificity data of monomethylamine in Tadalafil

S.No	Without spiking of the related substances	Spiked with related substances
	Monomethylamine content (%w/w)	
1	0.014	0.013
2	0.013	0.013
3	0.014	0.013
Average	0.014	0.013
SD	0.0006	0.0000
%RSD	4.2	0.0
% difference between spiked & unspiked	7.1	





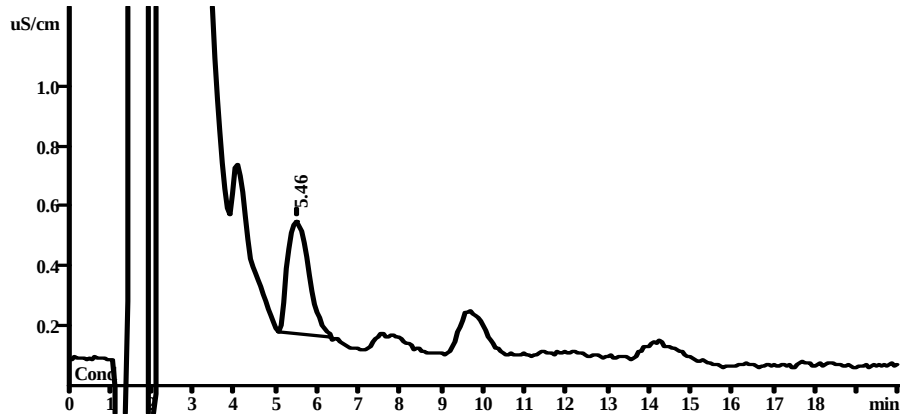


Fig.2 An overlay of typical ion chromatograms of Blank, Monomethylamine Standard and Tadalafil drug substance spiked with its related substances along with monomethylamine.

LOD and LOQ

For determining the limit of detection (LOD) and limit of quantification (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted. Injected the standard solution to ion chromatograph from lower concentration to higher concentration range (0.1 -3.6 µg/ml). A plot of peak area(µS/cm*sec) versus concentration(µg/ml) was drawn and LOD/LOQ values were predicted by using residual standard on deviation response(SD) and slope(S) method by using the formula 3.3 x SD/S for LOD and 10 x SD/S for LOQ. LOQ value was predicted as 0.3 µg/ml and LOD value was predicted as 0.1µg/ml.The LOD and LOQ solutions were prepared at about predicted concentration levels and analyzed six times for checking the precision.

Linearity

The linearity of the method was determined by taking the same linearity data obtained in LOD/LOQ prediction. The Linearity of conductometric detector response to different concentrations of monomethylamine was studied in the range from 0.3-3.6 µg/mL. The data was subjected to statistical analysis using a linear-regression model. The statistical evaluations like slope, intercept and correlation coefficient values of linearity data and LOD/LOQ values were given in Table 2.

Accuracy

Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries of I, II and III were determined by spiking monomethylamine at three different levels ranging from 1.2µg/ml to 4.2µg/ml into Tadalafil drug substance. These samples were prepared as per the procedure and analyzed in triplicate and the percentage recoveries were calculated. The recovery values for monomethylamine ranged from 98.4% to 105.2% and the average recovery of three levels (nine determinations) were 101.6%. The fully validated accuracy results were shown in Table 3.

Table 2: Statistical data of linearity, LOD/LOQ for monomethylamine in Tadalafil

Statistical parameters	**Results**
Correlation coefficient	0.9997
Concentration range (µg/ml)	0.3 - 3.6
Intercept	0.364
Slope	9.561
Limit of detection(µg/ml)	0.09
Limit of quantification(µg/ml)	0.30
Precision for Limit Of Detection (%R.S.D)	12.1
Precision for Limit Of Quantification (%R.S.D)	7.2

Table 3: Recovery (%) values for monomethylamine in Tadalafil

Accuracy (Average of 3 replicates)	**Level-I (1.2µg/ml)**	**Level-II (2.4µg/ml)**	**Level-III (4.2µg/ml)**
Added (µg/ml)	1.192	2.384	4.172
Recovered (µg/ml)	1.196	2.392	4.350
Recovery (%)	100.3	100.3	104.3
R.S.D(%)	1.4	1.7	1.0

Precision

The precision of the method was studies using repeatability and reproducibility (ruggedness) .The performance of the method was evaluated with replicate injections of standard and sample solutions. Monomethylamine standard solution was analyzed six times for checking the performance of the ion chromatographic instrument under the chromatographic conditions on the day tested (system precision). Repeatability was the intra-day variation (method precision) and the intermediate precision was the inter-day variation (ruggedness) in determination of monomethyl amine was evaluated by analyzing the six sample solutions separately by spiking monomethylamine at known concentration level. The ruggedness of the method was defined as the degree of reproducibility obtained by the analysis of the same sample under a variety of conditions at different lot of column, with different analyst on different day. Achieved results like %RSD and 95% Confidence interval for six determinations were 4.5 and ±0.0007 respectively for method precision and 6.9 and ±0.001 respectively for ruggedness.

Solution Stability

The sample solution was prepared by spiking monomethylamine at known concentration level to Tadalafil drug substance, and stability of the solution was tested as freshly prepared and at different intervals with the gap of every one hour and upto 15 hours at ambient conditions. The stability of solution was determined by comparing results with freshly prepared sample solution. The results indicating that sample solution was stable for 15 hours at ambient conditions.

Comparison of IC and GC Methods

Gas chromatographic method for the determination of monomethylamine content in Tadalafil was developed and validated. In these two methods, the specificity test demonstrated that there was no interference with any of the peaks. Hence it was concluded that both the methods were selective. And %recovery values were found between 95.0 and 105.2 for both methods. Where as in GC method, LOD and LOQ values were obtained 26µg/ml and 52µg/ml respectively, which are very high with respect to the regulatory requirements. Hence, alternatively we developed and validated this IC method. There was no significant difference between these two methods with respect to all the validation test parameters except sensitivity. Evaluation of comparative studies of both methods was given in Table 4.

Table 4: Comparative study between IC and GC methods

Validation Parameters	By Ion chromatography	By Gas chromatography
Specificity	No interference from related substances of Tadalafil.	No interference from other solvents which are used in the process of Tadalafil.
System Precision (%R.S.D) Repeatability (n=6, %R.S.D) Method Precision Intermediate precision	2.0 4.5 6.9	2.4 0.7 2.1
Linearity Concentration Range Correlation coefficient	0.3 - 3.6 (µg/ml) 0.9997	52.0-453.6 (µg/g) 0.9996
Accuracy Recovery (%)	98.4 - 105.2	95.0-104.5
LOD & LOQ LOD (µg/ml) LOQ (µg/ml)	 0.09 0.30	 25.7 51.5

Applications of the IC Method

This method has been used for the quantification of monomethylamine in other selected drug substances like Alfuzosin hydrochloride, Sumatriptan succinate, Sertraline hydrochloride and Didanosine with minor modifications in methodology. During the method development and optimization, different diluents for different drug substances were prefered for best recovery results. And depending upon drug solubility and fixation of specification of monomethylamine, standard and sample concentrations were proposed separately. These methods had been validated. Methodologies and validation data were given in Table 5 and Table 6 respectively.

Table 5: Summary of methodologies for determination of monomethylamine in various drug substances

	Alfuzosin hydrochloride	Sumatriptan succinate	Sertraline hydrochloride	Didanosine
Mobile phase	600mg of Tartaric acid and 167mg of Dipicolinic acid dissolved in 1000ml of water.	600mg of Tartaric acid and 167mg of Dipicolinic acid dissolved in 1000ml of water.	1600mg of Tartaric acid in 1000ml of water.	(1500mgof Tartaric acid in 1000ml of water) :Acetone 92:8 %v/v
Diluent	Water	1.0mM Hydrochloric acid solution	1%v/v solution of ethanol in water	water
Column	Metrosep cation 1-2, 7µm (125mm x 4.0mm)	Metrosep cation 1-2, 7µm (125mm x 4.0mm)	Metrosep cation 1-2, 7µm (125mm x 4.0mm)	Metrosep cation 1-2, 7µm (125mm x 4.0mm)
Flow Programme	0.8ml/min	0-12 min 0.8ml/min 12-25min 2.0ml/min 25-35min 0.8ml/min	0.8ml/min	0.7ml/min
Injection volume	20 µlitre	20 µlitre	20 µlitre	20 µlitre
Run time	20 min	35 min	20 min	20 min
Standard concentration (µg/ml)	2.5	10	2.5	3.0
Sample concentration (µg/ml)	2500	2000	2000	1500
Retention time(min) of methylamine peak	~ 5.0	~ 5.0	~ 5.0	~ 5.0

Table 6: Summary of method validation data for monomethylamine in various drug substances

Validation Parameter	Alfuzosin hydrochloride			Sumatriptan succinate			Sertraline hydrochloride			Didanosine		
Specificity	No interference from related substances of Alfuzosin hydrochloride			No interference from related substances of Sumatritptan succinate			No interference from related substances of Sertraline hydrochloride			No interference from related substances of Didanosine		
% Difference	2.0			4.7			4.3			1.8		
LOD – LOQ Precision at LOD (%R.S.D)	12.1						13.5			13.3		
Precision at LOQ (%R.S.D)	7.2						6.4			3.4		
LOD (µg/ml)	0.09						0.16			0.07		
LOQ (µg/ml)	0.30						0.48			0.20		
Linearity Concentration range (µg/mL)	0.3 - 3.6						1.0 - 3.0			0.2 - 5.0		
Calibration Points	9						5			9		
Slope	9.561						6.580			7.075		
Intercept	0.364						0.429			0.076		
Correlation coefficient	0.9997						0.9986			0.9999		
Accuracy (Average of 3 replicates)	Level-I 0.6µg/ml	Level-II 1.2µg/ml	Level-III 1.8µg/ml	Level-I 1.1µg/ml	Level-II 2.2µg/ml	Level-III 3.3µg/ml	Level-I 1.2µg/ml	Level-II 2.4µg/ml	Level-III 3.6µg/ml	Level-I 0.3µg/ml	Level-II 0.7µg/ml	Level-III 1.0µg/ml
Added (µg/ml)	0.630	1.261	1.891	1.105	2.209	3.314	1.263	2.526	3.790	0.358	0.716	1.075
Recovered (µg/ml)	0.633	1.270	1.975	1.054	2.076	3.191	1.257	2.544	3.727	0.353	0.689	1.042
Recovery (%)	100.5	100.7	104.4	95.4	94.0	96.3	99.5	100.7	98.3	98.6	96.2	96.9
R.S.D(%)	3.1	0.6	0.6	2.1	1.7	1.1	1.3	2.0	0.5	2.1	1.4	0.7

CONCLUSION

A sensitive ion chromatography method was developed, optimized and validated for the determination of monomethylamine and the results of various validation parameters demonstrated that the method is specific, linear, precise and accurate in various pharmaceutical drug substances like Tadalafil, Alfuzosin hydrochloride, Sumatriptan succinate, Sertraline hydrochloride and Didanosine.

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