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A SENSITIVE AND VALIDATED GC METHOD FOR THE DETERMINATION OF MONOISOPROPYLAMINE CONTENT IN CARISOPRODOL DRUG SUBSTANCE

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ABSTRACT
A specific GC method has been developed and validated for the determination of Monoisopropylamine (MIPA) content in Carisoprodol (CP) drug substance using Diethylamine (DEA) as internal standard (IS). Efficient chromatographic separation was achieved on Rtx-5 capillary column (30 m x 0.53 mm, 5.0 µm), consists of 5% diphenyl and 95% dimethylpolysiloxane as stationary phase and passing nitrogen as carrier gas. In this method, in presence of ethyl chloroformate, pyridine and dichloromethane (DCM) solvent medium monoisopropylamine and diethylamine were converted to monoisopropylamine derivative and diethylamine derivatives respectively. Therefore, monoisopropylamine is monitored by flame ionization detector (FID) and quantified as monoisopropylamine derivative. The performance of the method was assessed by evaluating specificity, linearity, sensitivity, precision and accuracy. The established limits of detection (LOD) and limits of quantification (LOQ) values for MIPA were 0.5297 µg mL^{-1} and 1.0594 µg mL^{-1} respectively. The correlation co-efficient value of linearity experiment was 0.9999 and the average recovery was 103.7%. The results proved that the validated method is suitable for the determination of monoisopropylamine in carisoprodol drug and method successfully applied for the quality control analysis of carisoprodol drug substance.

KEYWORDS: Gas chromatography, Carisoprodol, Development, Validation, Content.

1. INTRODUCTION
Carisoprodol (CP) is a member of the drug class skeletal muscle relaxants and is used to relieve skeletal muscle spasms and associated pain in acute musculoskeletal conditions and lower back pain.[1,2] Chemically, carisoprodol is known as N-Isopropyl-2-methyl-2-N-propyl-1,3-propandiol dicarbamate. The molecular formula of carisoprodol is $C_{12}H_{24}N_2O_4$ and the molecular weight is 260.33. Carisoprodol, marketed under the brand name of soma and other commercial forms, is a prescription drug marketed since 1959. In the synthesis process of carisoprodol drug substance, Monoisopropylamine (MIPA) was used as a reactant and MIPA is widely used in pharmaceutical industry for the synthesis of several drug substances. Further, this organic residual impuritity (MIPA) may come through the manufacturing process of CP drug substance. United States Food and Drug Administration (USFDA) and ICH Q3A/B issued the guidelines and draft guidance on the limitation of genotoxic impurities in pharmaceutical ingredients.[3,4] Based on the structural alert, MIPA come under toxic category. As per the toxicological threshold concern (TTC) approach, MIPA toxicity should be <100 µg g^{-1}. In present study, the structure of carisoprodol has monoisopropylamine moiety and it should be controlled in the carisoprodol drug. Any impurity other than active moiety is to be controlled with appropriate analytical methods and limits in the drug substance irrespective of its harmful nature as per international conference on harmonization guidelines (ICH) on impurities. To get the best quality of carisoprodol drug, monoisopropylamine impurity level must be monitored and controlled with appropriate methods. In the available literature, few analytical procedures have been reported for the estimation of MIPA content in environmental samples[5], different classes of many compounds[6], bonding agents[7] and in drug substances.[8,9]

A GC method was reported for the determination of amines in environmental samples by following derivatization reactions.[5] An advanced GC method was reported for the analysis of volatile amines, ammonia and alcohols in different classes of many compounds by using base-deactivated capillary column.[6] A

spectroscopy method was reported for the determination of primary amine content in tetraethylenepentamine (TEPA), a bonding agent used in composite solid propellants, was determined by near IR (NIR) spectroscopy. The band at 4930 cm^{-1}, which showed to be affected only by primary amine groups, was chosen as the analytical band.[7] Further, a simple ion chromatography method was reported for the estimation of residual isoproylamine in metroprolol succinate by using carboxylic acid groups cationic ion exchange resin as stationary phase and flushing acetone-water mixed solution of methanesulfonic acid as mobile phase with conductivity detector.[8] Finally, a simple and sensitive spectrophotometric method was reported for the determination of monoisopropylamine at very low levels in carisoprodol and isoproterenol hydrochloride drug substance. The method was based upon the observation, that characteristic colour results by the addition of colour reagent (which consists of Acetyl acetone, formaldehyde and pyridine) and the coloured complex shows maximum absorption at 420 nm. The reported Limit of detection (LOD) and limit of quantitation (LOQ) were 33 µg g^{-1} and 100 µg g^{-1} espectively.[9] Therefore, we made an attempt to develop an alternate method, which is simple, sensitive and accurate method for the determination of monoisopropylamine at very low levels in carisoprodol drug by following derivatization procedure with GC-FID.

2. MAERIALS AND METHODS

2.1 Instrumentation, reagents and chromatographic conditions

The analysis was carried on gas chromatograph system (Agilent 7890A model) equipped with Combipal CTC analytics auto sampler and data handling system with Empower-3. Restek Rtx-5 capillary column (30 m x 0.53 mm, 5.0 µm), consists of 5% diphenyl and 95% dimethylpolysiloxane material was used as stationary phase. High purity nitrogen gas was used as the carrier gas with the column flow 5.0 mL/min.

The temperature of column oven is initially 40°C is maintained for 5 min and then increased to 220°C at rate of 10°C/min, followed by holding at 220°C for 27 min. The run time was fixed as 50 min. The injection volume is 2.0 µL and a split ratio set at 5:1. The injector temperature and detector temperature were 180°C and 260°C respectively. High purity gases were used in the volumes of 30 mL/min (Hydrogen), 300 mL/min (Zero air) and 29 mL/min Nitrogen for the ignition of FID. The samples of Carisoprodol drug substance was procured from APL Research Centre-II (A division of Aurobindo Pharma Limited). Analytical reagents (AR grade), Monoisopropylamine, Diethylamine, Ethyl chloroformate, Pyridine, Toluene, Triethylamine, Benzene were obtained from Sigma-Aldrich Limited (India) and HPLC grade Dichloromethane was procured from Merck Limited (India). A typical representative structures of Carisoprodol, Monoisopropylamine and Diethylamine are shown in Fig. 1.

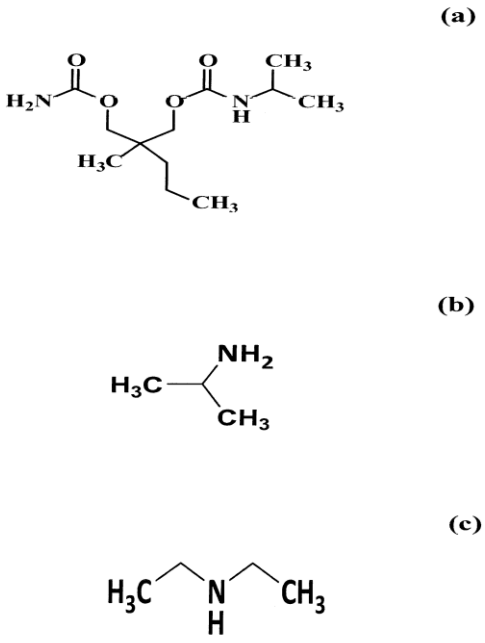

Fig. 1. The structures of (a) Carisoprodol (b) Monoisopropylamine and (c) Diethylamine

2.2 Preparation of standard and sample solutions

A diluent solution was prepared by adding 4.0 mL of Ethyl chloroformate and 0.5 mL of Pyridine to the 500 mL clean, dry volumetric flask containing about 100 mL of dichloromethane, mixed well and made upto the volume with dichloromethane. This solution was used as a diluent. An internal standard (IS) solution was prepared by appropriate weighing and respective dilutions of Diethylamine in the diluent solution to get a concentration of 18 µg mL^{-1}. This solution was used as a blank solution. A standard solution was prepared by appropriate weighing and respective dilutions of Monoisopropylamine in the internal standard solution to get a concentration of 10 µg mL^{-1} and used as standard solution. Further, 0.5 g of Carisoprodol sample was dissolved in 5 mL of internal standard solution to get a concentration of 100000 µg mL^{-1} and used for the analysis.

Warning : Chlorinated solvents are not "less toxic"; they are generally considered cancer suspect agents. Hence, while handling Dichloromethane solvent use safety precautions: wear protective clothing, always wear personal protective equipment such as chemical splash goggles and safety gloves. Work in preferably in an environment with a fume extraction system.

3. RESULTS

3.1 Method validation

In order to determine the content of Monoisopropylamine in Carisoprodol drug substance, the method was validated as per the ICH guidelines[10] individually in terms of specificity, limit of detection, limit of quantification, linearity, accuracy and precision (system precision and method precision).

3.1.1 Specificity

Specificity is the ability of the method to measure the analyte (Monoisopropylamine) response in presence of carisoprodol drug including all known residual solvents (toluene, triethylamine and benzene), which are used in the synthesis process of carisoprodol drug substance. For specificity determination, monoisopropylamine, diethylamine, dichloromethane, pyridine, ethyl chloroformate, toluene, triethylamine and benzene solutions were prepared individually and injected into GC, to confirm the retention times. Later on, solutions of blank, control sample (or) real sample (carisoprodol sample), spiked sample (carisoprodol sample spiked with monoisopropylamine) and all spiked sample (carisoprodol sample spiked with all known residual solvents including monoisopropylamine) were prepared and injected into GC, to confirm any interference of all known residual solvent peaks with monoisopropylamine derivative and Diethylamine derivative (internal standard) peaks. From the chromatograms of blank solution, individual solvent solutions, control sample, spiked sample and all spiked sample it is observed that monoisopropylamine derivative and diethylamine derivative (internal standard) peaks were well resolved from each other and there is no other interference (co-elution) from the sample matrix indicated that the method is selective and specific for the determination of monoisopropylamine content in carisoprodol drug substance. The overlaid chromatograms of control sample, spiked sample and all spiked sample chromatogram are shown Fig. 2.

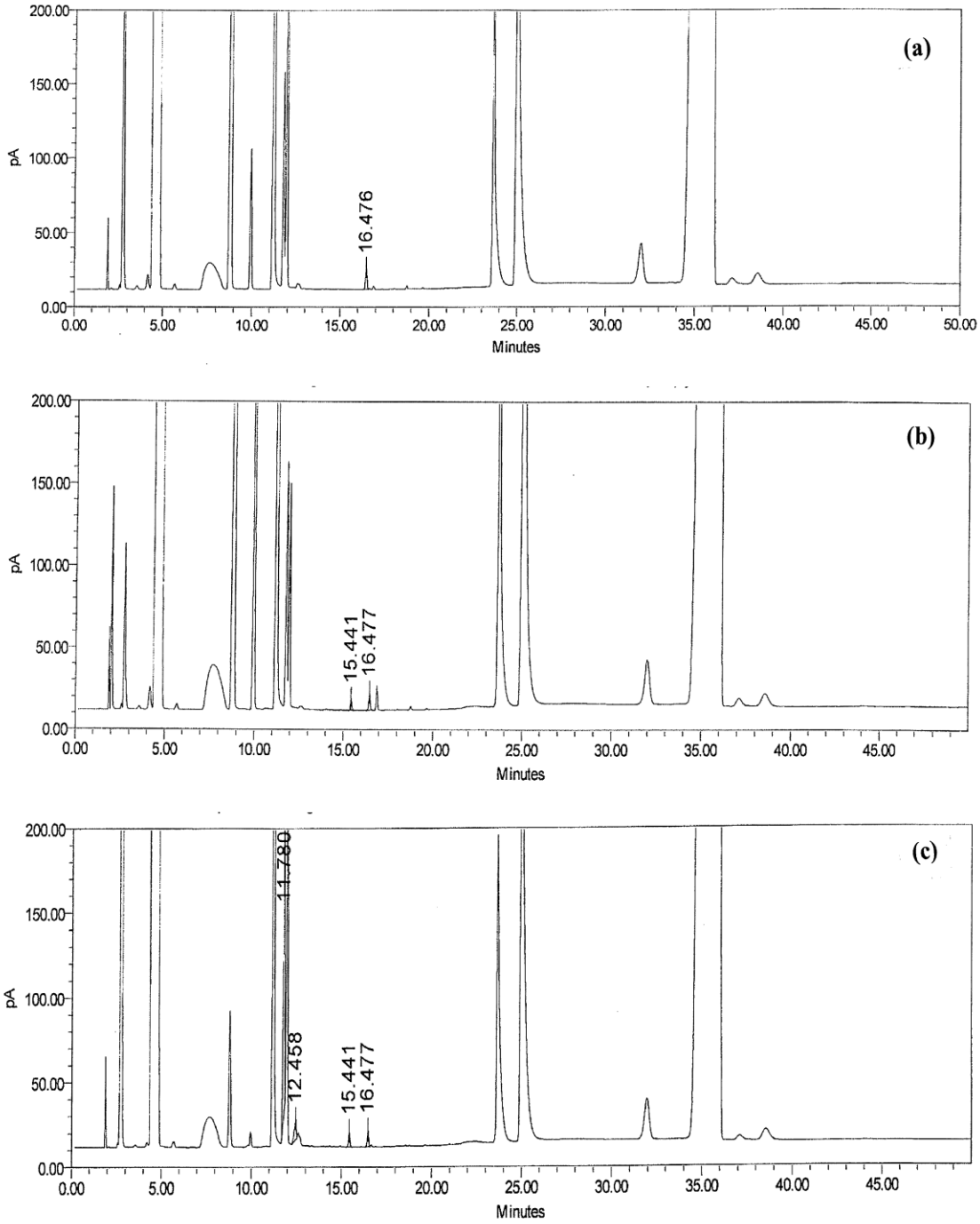

Fig. 2. Typical representative overlaid chromatograms of (a) Control sample (b) Spiked sample (c) All spiked sample

3.1.2 Limit of detection and limit of quantification (LOD and LOQ)

The LOD and LOQ values for Monoisopropylamine was predicted from Signal to Noise ratio (S/N ratio) method. LOD and LOQ values were predicted with concentration of standard solution (C) and standard solution S/N ratio, using the formula [3.3 x C / (S/N)] for LOD and [10 x C / (S/N)] for LOQ. Each predicted concentration was verified for precision by preparing the solutions containing Monoisopropylamine at about LOD and LOQ predicted concentrations and injected each solution six times into GC by following the test method conditions for LOD and LOQ precision. The relative standard deviation [% RSD (n=6)] for LOQ precision was 0.3; for

LOD precision 1.2. The overlaid chromatograms of LOD solution and LOQ solution are shown in Fig. 3. The detailed precised LOD and LOQ values are shown in Table 1.

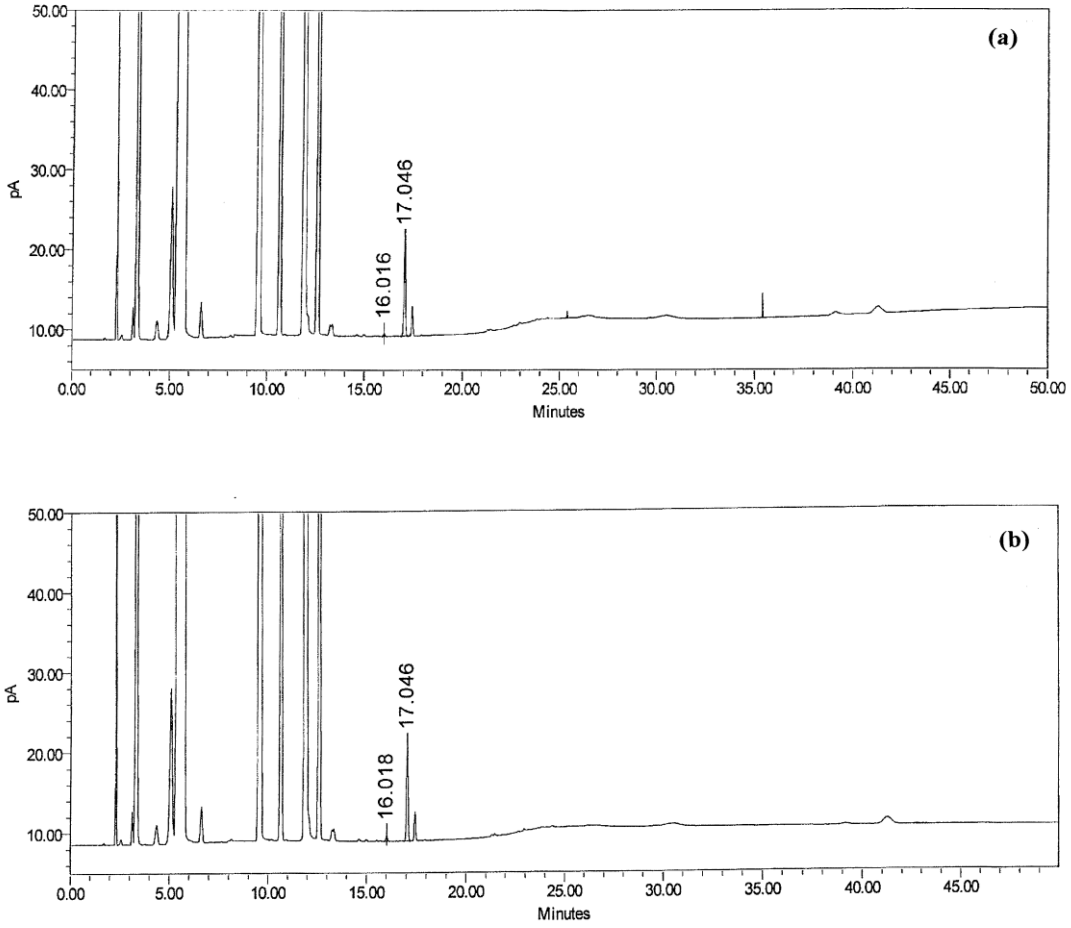

Fig. 3. Typical representative overlaid chromatograms of (a) LOD solution (b) LOQ solution

Table 1: Statistical data of linearity, LOD/LOQ for Monoisopropylamine.

Statistical parameters	Monoisopropylamine (MIPA)
Slope	0.0576
Intercept	0.0086
Residual standard on deviation response	0.0026
Correlation coefficient	0.9999
Concentration range (µg mL^{-1})	1.0594 - 15.6656
Limit of detection (µg mL^{-1})[a]	0.5297
Limit of quantification (µg mL^{-1})[a]	1.0594
Precision for Limit Of Detection (% RSD)	1.3
Precision for Limit Of Quantification (% RSD)	0.3

a: Precised LOD and LOQ values

3.1.3 Linearity

The linearity was evaluated by measuring the response of Monoisopropylamine (MIPA) about seven different concentrations were prepared across the range concentrations were studied in the range of about 1.0594 µg mL^{-1} to 15.6656 µg mL^{-1}. The seven concentrations of MIPA were 1.0594, 2.6109, 5.2219, 8.3550, 10.4437, 12.5325 and 15.6656 µg mL^{-1} level linearity solutions prepared and injected each in single injection into GC. The data was subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation and correlation coefficient values were calculated. The calculated statistical results were shown in Table 1.

3.1.4 Accuracy

Accuracy experiment was performed by spiking known amount of Monoisopropylamine at LOQ level, 50%, 100%, and 150% level (with respect to 100 µg/g limit) into carisoprodol drug substance. In the accuracy experiment, carisoprodol sample solutions [real sample (or) control sample] were prepared with out spiking Monoisopropylamine in triplicate and injected into GC. Further, carisoprodol sample solutions [spiked sample] were prepared in triplicate by spiking with Monoisopropylamine at LOQ level (10 µg g^{-1}), 50% level (50 µg g^{-1}), 100% level (100 µg g^{-1}) and 150% level (150 µg g^{-1}) and injected into GC. Control samples, spiked samples were analyzed and the percentage recoveries were calculated. The average % recovery values of four levels (LOQ, 50%, 100% and 150% levels) for twelve determinations for monoisopropylamine was 103.7%. The complete validation accuracy results were shown in Table 2.

Table 2: Accuracy data of Monoisopropylamine.

Identification	Monoisopropylamine (MIPA)			
	LOQ Level	Level-I (50% Level)	Level-II (100% Level)	Level-III (150% Level)
*Added (µg/g)	10.6	51.0	102.0	153.0
*Found (µg/g)	10.7	55.7	96.0	169.0
Recovery (%)	100.9	109.2	94.1	110.5
* % RSD	0.9	2.1	0.0	0.0

* Average of 3 replicates.

3.1.5 Precision

The precision was the study of the method using repeatability (system precision) and reproducibility (method precision). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the GC-FID system under test method conditions on the day tested (system precision). The % RSD result obtained for the system precision experiment was 1.0. The reproducibility of the method was studied by analyzing six sample solutions separately by adding Monoisopropylamine at about known concentration (100 µg g^{-1}) level and injected in to GC. The % RSD the content results of the method precision experiment was 0.4. The complete validated precision (System precision and Method precision) results are shown in Table 3. A typical representative overlaid chromatograms of blank solution and standard solution are shown Fig. 4.

Table 3: Statistical data of precision.

S.No.	*Repeatability (System precision)*	*Reproducibility (Method precision)*
	Area ratio = Monoisopropylamine derivative area counts / Diethylamine derivative area counts	***Monoisopropylamine derivative content (µg/g)***
1	0.5720	96
2	0.5786	96
3	0.5839	96
4	0.5865	97
5	0.5842	96
6	0.5881	96
Mean	0.5822	96
SD	0.0060	0.4
% RSD	1.0	0.4

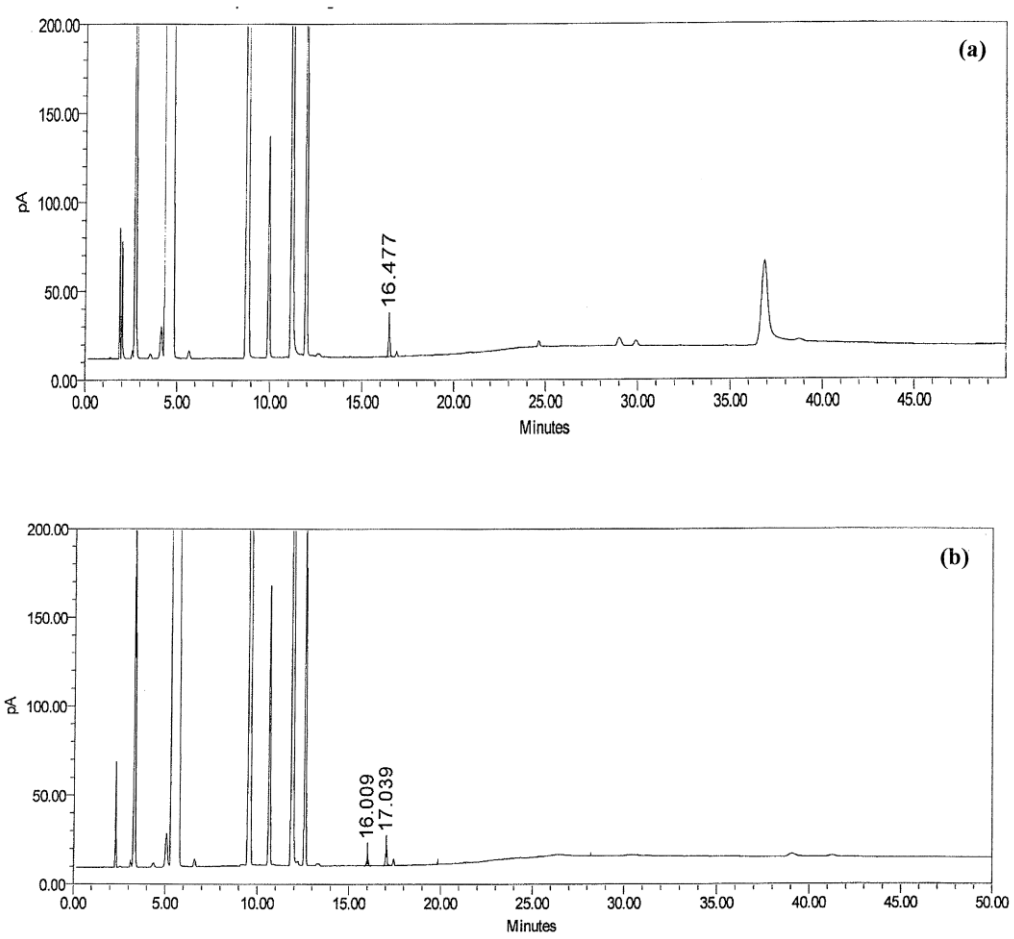

Fig. 4. Typical representative overlaid chromatograms of (a) Blank solution and (b) Standard solution

Tables

Table 1: Statistical data of linearity, LOD/LOQ for Monoisopropylamine.

Table 2: Accuracy data of Monoisopropylamine.

Table 3: Statistical data of precision.

Figures

Fig. 1: A typical representative structures of (a) Carisoprodol, (b) Monoisopropylamine and (c) Diethylamine.

Fig. 2: A typical representative overlaid chromatograms of (a) Control sample (b) Spiked sample and (c) All spiked sample.

Fig. 3: A typical representative overlaid chromatograms of (a) LOD solution and (b) LOQ solution.

Fig. 4: A typical representative overlaid chromatograms of (a) blank solution and (b) standard solution.

4. DISCUSSION

4.1 Method development and optimization

The objective of this work is, to evaluate a quantitative method for the determination of Monoisopropylamine (MIPA) in Carisoprodol (CP) drug substance. In the synthesis process of CP drug, MIPA was used as a reactant and this organic residual impurity (MIPA) may come through the manufacturing process of CP drug substance. Method development activity was initiated with solubility and miscibility studies of CP drug and MIPA. Amines are generally known to be very difficult to analyze due to their basic character. Because of this, amines will interact with any active site that may be present within the GC system with decreasing molecular size, the influence of the amine group becomes larger, which results in stronger adsorption characteristics. This effect is most critical with the primary amines. In addition, the amino group introduces a large dipole in the molecule. This dipole is responsible for a strong interaction with silanol groups and siloxane bridges, which often results in non-linear adsorption effects, appearing as strong tailing peaks in the chromatogram. The best way to prevent the interaction of the strong dipole is to derivatize the amine or to deactivate the column in such a way that the interaction is minimized. The advantage taken based on their (CP drug and MIPA) volatility nature to develop the analytical chromatography method by gas chromatograph (GC)

equipped with flame ionization detector (FID) by following derivatization procedure. In the derivatization process, monoisopropylamine and diethylamine (internal standard) are converted to monoisopropylamine derivative and diethylamine derivative respectively in presence of ethyl chloroformate, pyridine and dichloromethane (DCM) medium. Therefore, monoisopropylamine is quantified as monoisopropylamine derivative. We made few trials by changing different chromatographic conditions by GC with FID. Finally, a well resolved chromatographic GC method was achieved by using Restek Rtx-5 capillary column (30 m x 0.53 mm, 5.0 µm), consists of 5% diphenyl and 95% dimethylpolysiloxane material was used as stationary phase. High purity nitrogen gas was used as the carrier gas with the column flow 5.0 mL/min. The temperature of column oven is initially 40°C is maintained for 5 min and then increased to 220°C at the rate of 10°C/min, followed by holding at 220°C for 27 min. These chromatographic conditions create a satisfactory separation with better peak shapes were achieved and the method was used for validation study to evaluate its performance characteristics.

5. CONCLUSION

This study established, an alternate analytical method was developed for the determination of Monoisopropylamine (MIPA) by GC-FID at very low levels in Carisoprodol drug substance. Method validation data demonstrated that the developed method is a simple, sensitive, specific, precise, linear and accurate for the estimation of trace quantities of MIPA in Carisoprodol drug substance.

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