IJPSR (2016), Vol. 7, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 August, 2015; received in revised form, 16 October, 2015; accepted, 13 November, 2015; published 01 February, 2016

A SIMPLE AND SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF TRACE LEVEL MONO ISOPROPYLAMINE IN PHARMACEUTICAL DRUG SUBSTANCES

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Keywords:

UV Spectrophotometry, Mono isopropylamine, Carisoprodol, Isoproterenol hydrochloride, Method development, Validation.

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ABSTRACT: A simple and sensitive spectrophotometric method was developed and optimized for the determination of content of mono isopropylamine at very low levels in Carisoprodol and Isoproterenol hydrochloride. The method was based upon the observation, that characterstic colour results up on addition of colour reagent (consists of Acetyl acetone, formaldehyde and pyridine) and heat for 10 minutes on water bath to form coloured complex. This coloured complex is slightly yellow and its absorption maximum at 420 nm. The optimized method was validated to prove its performance characterstic by demonstrating selectivity, sensitivity(limit of detection and quantification), linearity, precision and accuracy. The established limit of detection and limit of quantification of mono isopropylamine was found to be 3.3µg/ml and 10µg/ml respectively. The present work was aimed to develop a visible spectrophotometric method, which is simple, sensitive, accurate and cost effective to evaluate the quality of the bulk drugs.

INTRODUCTION: Mono isopropylamine [1, 2, 3] (**Fig.1**) is widely used in pharmaceutical industry for the synthesis of drug substances. In the synthesis of carisoprodol and Isoproterenol hydrochloride, Mono isopropylamine is used as a reactant it should be controlled in the drug substances. Any impurity other than active moiety is to be controlled with suitable limits in the drug substance irrespective of its harmful nature as per international conference on harmonization(ICH) guidelines on impurities.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(2).834-39

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).834-39

However, the analysis of mono isopropyl amine presents numerous distinctive challenges due to its behaviour.

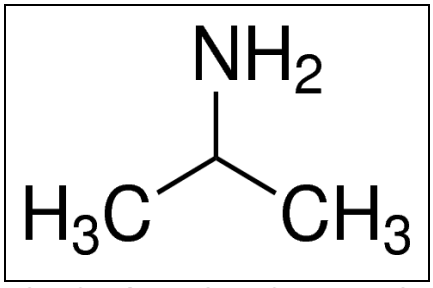

FIG.1: MOLECULAR STRUCTURE OF MONO ISOPROPYLAMINE

Carisoprodol [3-5] **(Fig.2)** is a dicarbamate, centrally acting, oral skeletal muscle relaxant whose chief application is in the treatment of acute muscular spasm associated with

craniomandibular disorder, lumbago, sciatica, and other lower back syndromes. Carisoprodol is a centrally acting skeletal muscle relaxant that does not directly relax tense skeletal muscles in man. The mode of action of carisoprodol in relieving acute muscle spasm of local origin has not been clearly identified, but may be related to its sedative properties. Carisoprodol is official in British Pharmacopoeia and United state pharmacopoeia but few analytical methods are available for Carisoprodol.

Isoproterenol [6, 7] (**Fig.3**) is a synthetic catechol compound and potent beta adrenergic agonist with peripheral vasodilator, bronchodilator, and cardiac stimulating properties. Isoproterenol exerts its effect on the beta-1 adrenergic receptors in the myocardium, thereby increasing heart rate and cardiac output. In addition, isoproterenol acts on beta-2 adrenergic receptors in bronchiolar and vascular smooth muscle, thereby causing smooth muscle relaxation.

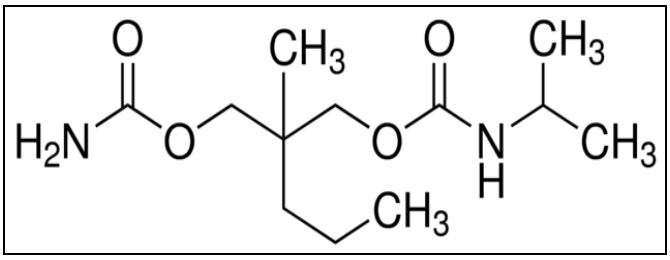

FIG. 2: MOLECULAR STRUCTURE OF CARISOPRODOL

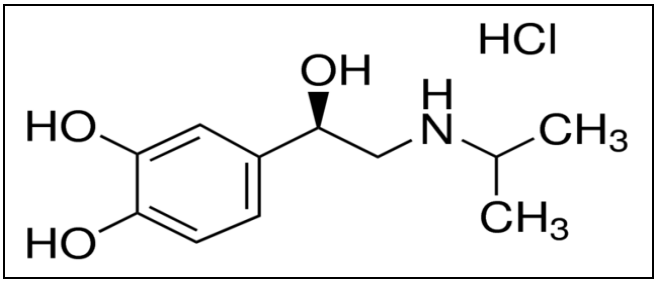

FIG.3: MOLECULAR STRUCTURE OF ISOPROTERENOL HYDROCHLORIDE

In present studies, the structure of carisoprodol and Isoproterenol hydrochloride drug substances have mono isopropylamine moiety. Therefore, an attempt was made to develop a simple and rapid derivatization approach of spectrophotometric method for the determination of mono isopropylamine in pharmaceutical drugs. Literature survey reveals many analytical methods include GC [8, 9], HPLC, ion-chromatography, and other derivatization method for the determination of biogenic amines in food and pharmaceutical drug samples. The optimized UV spectrophotometric method was validated according to ICH guidelines[10] to prove its suitability and reliability for the determination of mono isopropylamine in pharmaceutical drug substances during routine analysis.

MATERIALS AND METHODS:

Chemicals and reagents:

Analytical reagent grade of mono isopropyl amine, pyridine, formaldehyde, acetyl acetone from Merck chemicals, India. Water was distilled and purified with Merck Millipore system, Isoproterenol hydrochloride, carisoprodol drug substances provided by our APL Research Centre-II(A Division of Aurobindo pharma limited)

Instrument:

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance

Methods:

Preparation of Solutions:

Reagent solution: Mix 2 ml of Acetyl acetone with 1 ml of formaldehyde solution and 47 ml of pyridine (Prepare fresh solution)

Standard solution:

Transfer 0.1g accurately weighed isopropyl amine with the help of syringe into a 100 ml clean dry volumetric flask containing 20 ml of water, mix well and make up to the volume with water dilute 5 ml of this solution to 50 ml with water. Pipette out exactly 2 ml of the above solution into 10 ml clean, dry volumetric flask add, 4 ml water and 2 ml reagent solution. Heat the flask for 10 min on water bath. Cool and dilute to 10 ml with pyridine.

Sample preparation for carisoprodol drug substance: Transfer about 1.0 g of sample into a 10 ml volumetric flask add 4 ml of 96% ethanol and 2 ml colour reagent solution. Heat the flask for 10 min on water bath, cool and dilute to 10 ml with pyridine.

Sample preparation for Isoproterenol hydrochloride:

Transfer about 1.0 g of sample into a 10 ml volumetric flask add 6 ml of water and 2 ml colour reagent solution. Heat the flask for 10 min on water bath, cool and dilute to 10 ml with pyridine.

Blank solution:

Prepare blank solution separately for two drug substances as per above procedure without sample addition and make a necessary correction.

Procedure:

Scanned the yellow colour chromogen of standard and sample solution between 300 nm to 500nm with blank correction the absorbance maxima 420nm. (**Fig.4**)

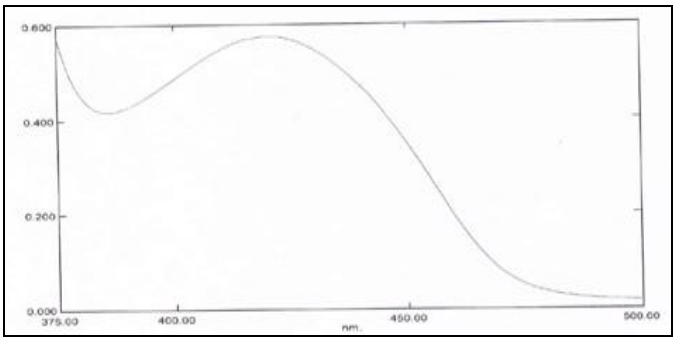

FIG.4: UV SPECTRUM OF DERIVATISED MONO ISOPROPYLAMINE

Spectral Characteristics:

Standard solutions of derivatized mono isorpropylamine at different concentrations level were prepared. Calibration curve was constructed by plotting the concentration level versus corresponding absorbance at 420nm (**Fig.5**). The results show an excellent correlation between absorbance and concentration level of Mono isopropylamine within the concentration range 5.0 to 60 µg/ml show good agreement with Beer's law.

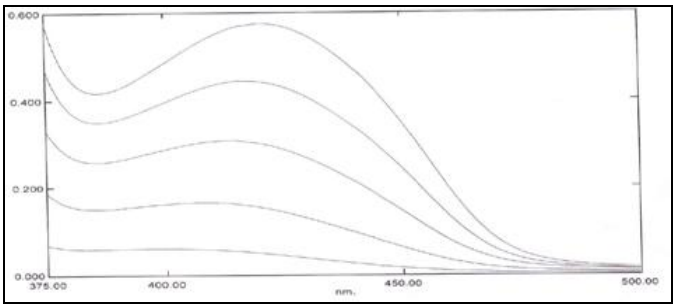

FIG.5: OVERLAY SPECTRUM OF DERIVATISED MONO ISOPROPYLAMINE AT DIFFERENT CONCENTRATIONS

RESULTS AND DISCUSSIONS:

Method development and optimization:

The most commonly used method for the determination of mono isopropylamine in pharmaceutical drug substances. The objective of this work is to determine trace level concentration of mono isopropylamine. During method development and optimization, solubility of drug(s) was taken consideration. As carisoprodol is soluble in 96% Ethanol, very slightly soluble in water, freely soluble in acetone, alcohol and methylene chloride. Isoproterenol hydrochloride is freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride. Subsequently carisoprodol was first dissolved in 4 ml of 96% Ethanol as Isoproterenol hydrochloride freely soluble in water sample first dissolved in 6 ml of water and proceeded with 2 ml colour reagent, heat the volumetric flask at 40°C$\pm$2 for 30 mins and cool the solution to room temperature and make up to the volume with pyridine then record the absorbance at 420 nm the intensitiy of colour is proportional to the isopropyl amine concentration.

This is the basis to determine the content of mono isopropyl amine. We measured mono isopropylamine content in drug substances on the basis of trial and error colour reagent is prepared.

Method validation:

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines [10] for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of all impurities related to drug substance, for specificity determination checking the interference of blank,

mono isopropylamine spiked to drug substance at known concentration level and all known related substances of drug substance including mono isopropylamine with known concentration level were prepared and analysed and record the absorbance into triplicate and determine the mono isopropylamine content .The percentage difference between mean of monoisopropyl amine content in spiked individually and spiked with known related substance was determined. Calculate the percentage difference between spiked and unspiked.

Precision:

System precision:

System precision was demonstrated by analyzing six replicates of standard solution at working concentration as per the procedure and record the absorbance. The percentage relative standard deviation of six replicates of monoisopropyl amine was found to be less than 1 concerning absorbance for the monoisopropyl amine .Which indicates the acceptable reproducibility and thereby the precision of the system

System precision results are tabulated in (**Table 1).**

Table 1: System precision results of Mono isopropylamine.

n	Absorance
1	0.228
2	0.228
3	0.222
4	0.231
5	0.227
6	0.232
Mean	0.228
SD^	0.004
%RSD*	1.75

^ Standard deviation

* Relative standard deviation

Method precision:

Method precision was demonstrated by preparing six separate sample solutions were prepared using single batch of each pharmaceutical drug substances spiking with known amount of mono isopropylamine spiked in sample solution and record the absorbance.

Method precision was performing content of mono isopropylamine by spiking with known concentration of mono isopropylamine in drug substances under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intra day precision):

Six consecutive recording of absorbance at 420 nm of the mono isopropylamine from the same homogeneous mixture at working concentration showed % RSD less than 1, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 2** and **Table 3**).

TABLE 2: INTRA DAY PRECISION RESULTS OF MONO ISOPROPYLAMINE (200µg/g) SPIKED IN CARISOPRODOL DRUG SUBSTANCE.

n	Mono isopropylamine content(µg/g)
1	200.08
2	200.63
3	199.00
4	197.74
5	200.63
6	196.95
Mean	199.62
SD	1.55
%RSD	0.8

TABLE 3: INTRA DAY PRECISION RESULTS OF MONO ISOPROPYLAMINE (200µg/g) SPIKED IN ISOPROTERENOL HYDROCHLORIDE DRUG SUBSTANCE.

n	Mono isopropylamine content(µg/g)
1	198.80
2	197.69
3	200.04
4	200.82
5	199.35
6	198.39
Mean	199.34
SD	1.14
%RSD	0.60

Intermediate Precision (Inter day precision/ Ruggedness):

Six consecutive recording of absorbance at 420 nm of the mono isopropylamine from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 1 within and between days, which indicate the method developed is inter day precise / rugged (**Table 4** and **Table 5**).

TABLE 4: INTER DAY PRECISION RESULTS OF MONOISOPROPYL AMINE (200µg/g) SPIKED IN CARISOPRODOL DRUG SUBSTANCE.

n	Mono isopropylamine content(µg/g)		
	Day 1	**Day 2**	**Day 3**
1	200.43	199.70	199.30
2	199.17	200.59	200.23
3	199.36	199.94	197.14
4	198.01	199.36	198.01
5	199.94	200.23	198.18
6	198.96	199.35	198.81
Mean	199.38	199.97	198.61
SD	0.83	0.49	1.08
%RSD	0.40	0.20	0.50

TABLE 5: INTER DAY PRECISION RESULTS OF MONO ISOPROPYLAMINE (200µg/g) SPIKED IN ISOPROTERENOL HYDROCHLORIDE DRUG SUBSTANCE.

n	Mono isopropylamine content(µg/g)		
	Day 1	Day 2	Day 3
1	199.06	200.99	199.18
2	199.73	198.83	198.83
3	199.00	200.71	199.91
4	199.53	197.80	200.43
5	199.41	197.80	200.12
6	199.76	197.85	196.89
Mean	199.35	199.22	199.69
SD	0.32	1.49	1.29
%RSD	0.20	0.70	0.60

Linearity:

Standard solutions of derivatized mono isopropyl amine at different concentrations level 5 to 60µg/ml were prepared. Calibration curve was constructed by plotting the concentration level of mono isopropylamine versus corresponding absorbance at 420 nm. The results show an excellent correlation between absorbance and concentration level of mono isopropyl amine within the concentration range (5.0-60 µg/ml) are given in (**Table 6**)**.** The correlation coefficients were greater than 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 5.0 - 60 µg/ml

TABLE 6: CALIBRATION DATA FOR MONO ISOPROPYLAMINE STANDARD.

n	Mono isopropylamine (µg/ml)	Absorbance
1	5	0.072
2	10	0.112
3	20	0.224
4	30	0.327
5	40	0.445
6	50	0.550
7	60	0.686
Regression equation		y = 0.11x + 0.003
Correlation coefficient (r^2)		0.998

Accuracy:

Accuracy of the method was performed by recovery experiments using standard addition method technique. The recoveries of I, II and III were determined by spiking mono isopropyl amine at three different levels ranging from 100 to 300µg/g into carisoprodol and Isoproterenol hydrochloride drug substances. These samples were prepared as per the procedure and analysed in triplicate and the percentage recoveries were calculated (**Table 7** and **Table 8**). The recovery value of mono isopropylamine ranged from 95.7 to 98.23 and the average recovery of three levels (nine determinations). The accepted limits of recovery are 95% - 99% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

TABLE 7: RECOVERY RESULTS FROM SPIKING OF CARISOPRODOL DRUG SUBSTANCE WITH MONO ISOPROPYLAMINE.

Accuracy (Average of triplicates)	Level – I (LOQ)	Level – II (100%)	Level – III (150%)
Added(µg/g)	99.99	199.64	299.04
Found(µg/g)	96.48	195.55	286.22
Recovery(%)	96.50	97.97	95.70
RSD(%)	0	0.70	0.20

TABLE 8: RECOVERY RESULTS FROM SPIKING OF ISOPROTERENOL HYDROCHLORIDE DRUG SUBSTANCE WITH MONO ISOPROPYLAMINE.

Accuracy (Average of triplicates)	Level – I (LOQ)	Level – II (100%)	Level – III (150%)
Added(µg/g)	100.19	199.18	300.05
Found(µg/g)	98.43	194.81	290.39
Recovery(%)	98.23	97.80	96.80
RSD(%)	0.9	1.3	0.6

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the mono isopropylamine content despite deliberate variations done concerning compositions of colored reagent and solvents.

Sensitivity:

The sensitivity of measurement of mono isopropylamine content by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD). The

limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 3.3µg/ml and 10.0 µg/ml respectively. Optical characteristics results are summarized in (**Table 9**)

TABLE 9: OPTICAL CHARACTERISTICS OF MONO ISOPROPYLAMINE CONTENT IN DRUG SUBSTANCE.

Parameters	Results
Detection wavelength(nm)	420
Beer's law limits(µg/ml)	5 - 60
Regression equation(y=mx+c)	y = 0.011x + 0.003
Correlation coefficient(r2)	0.998
LOQ(µg/ml)	10
LOD(µg/ml)	3.3

CONCLUSION: A sensitive cost effective UV spectrophotometric method was developed, optimized and validated for the quantitative determination of mono isopropylamine in Carisoprodol and Isoproterenol hydrochloride drug substances as per ICH guidelines.

ACKNOWLEDGMENTS: The authors express their sincere gratitude to APL Research Centre-II(A division of Aurobindo pharma Ltd.) located at Hyderabad, India, for providing the analytical support to pursue this work, and are also grateful to colleagues who helped us in this work and also thankful to the authorities of Andhra university.

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How to cite this article:
Nagaraju C, Gorla R, Ray UK, Kanteti N, Kumar SH and Vidavalur S: A Simple and Sensitive Spectrophotometric Method for the Determination of Trace Level Mono Isopropylamine in Pharmaceutical Drug Substances. Int J Pharm Sci Res 2016; 7(2): 834-39.doi: 10.13040/IJPSR.0975-8232.7(2).834-39.