Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 6, Issue 10, 2014

Original Article

ANALYTICAL QUALITY BY DESIGN APPROACH FOR DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHODS IN THE ESTIMATION OF TROSPIUM CHLORIDE FROM CAPSULE DOSAGE FORM

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Received: 03 Aug 2014 Revised and Accepted: 05 Sep 2014

ABSTRACT

Objective: The aim of present work is to develop and validate two spectrophotometric methods for trospium chloride estimation in capsule dosage form using Analytical Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines.

Methods: Variable parameters like type of sample preparation, solvent, wavelength, instrumental parameters such as slit width, scan speed and sampling interval etc. were designed into Ishikawa diagram and critical parameters were determined by observation as well as by using principal component analysis.

Results: In simple spectrophotometric method, trospium chloride was estimated at 258 nm using 0.1 N sodium hydroxide. Beer's law was obeyed in the concentration range 200-1000 µg/ml (r^2 = 0.999). In first derivative spectrophotometric method, beer's law was obeyed in concentration range 20-100 µg/ml (r^2 = 0.999) at 226.6 nm using 0.1 N sodium hydroxide. The proposed methods were validated according to ICH Q2 (R1) analytical method validation guidelines.

Conclusion: The proposed methods were found to be accurate, precise, and economical and can be applicable for routine quality control analysis of trospium chloride on pharmaceutical dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.

Keywords: Quality by Design (QbD), Trospium chloride, UV Spectrophotometry, ICH Q8 (R2), Principal component analysis.

INTRODUCTION

Analytical quality by design is the new emerging field for the analysis of drugs in the industries. The impact of quality by design in pharmaceutical analysis is an increased understanding of method variability as more thorough approaches are applied for defining method performance [1]. Implementation of quality by design for analytical methods during development allows for enhanced understanding of the analytical method focusing on robustness and ruggedness, thereby facilitating method transfer and providing opportunities for continual improvement. Analytical quality by design finally results in significant reductions of working capital requirements, resource costs and non-value added time [2].

Trospium Chloride is new upcoming molecule official in British Pharmacopoeia 2012 [3]. Trospium Chloride (Fig 1) is an antispasmodic, antimuscarinic agent. It is used in the treatment of overactive bladder with urge incontinence. Literature survey revealed that pharmacological activity was studied along with its bioavailability in various dosage forms [4, 5]. In analytical study, trospium chloride has been estimated using sophisticated techniques like high-performance Liquid Chromatography-Tandem Mass Spectrometry, LC-MS/MS, RP-HPLC and others [6-10]. Also, fluorimetric determinations and Stability Indicating RP-UFLC method have been reported [11-13]. These methods are very advanced and highly sophisticated techniques have been utilized making it more economical and time consuming. Henceforth it was planned to develop spectrophotometric methods including simple spectrophotometric method (method 1) and first derivative spectrophotometric method (method 2) based on new concept called Quality by Design as per ICH Q8 (R2) guidelines [14]. Also, the methods includes statistical tool which help to make the data systematic. The methods have been validated according to ICH Q2 (R1) guidelines [15].

QbD Approach

ICH Q8 (R2) guideline introduces a concept of Quality by Design which is defined as - "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". According to this guideline, the fishbone diagram which is also called as Ishikawa diagram was designed (Fig. 2) and variable parameters were carried out as discussed below.

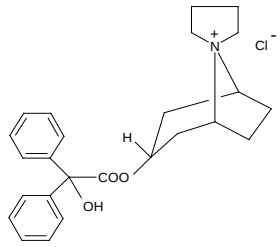

Fig. 1: Structure of trospium chloride

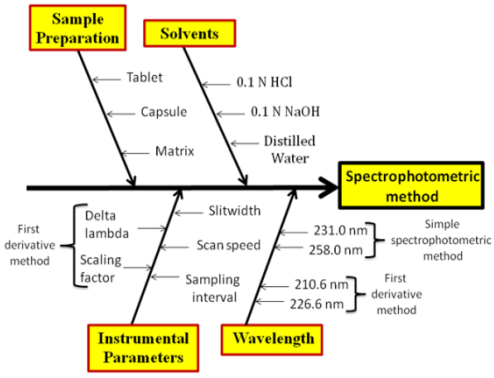

Fig. 2: Fishbone diagram

Sample preparation

Three samples were prepared for the estimation of trospium chloride. First one was a matrix containing lactose monohydrate, microcrystalline cellulose and starch for which the average weight was taken and analysed. In the second sample, analysis was performed on tablets which were prepared at the laboratory scale. Similarly for the capsules, the granules were tolerated. The average weight was taken and analysed further. Percentage recovery study was carried out for all these samples. Among the three sample preparations, the precise results were obtained in the capsule preparation (Table 1).

Table 1: Recovery studies in three sample preparations

Recovery	Matrix	Tablet	Capsule
80 %	87.6	88.1	98.4
100 %	84.5	91.3	99.2
120 %	81.1	93.7	98.6
Average (%)	84.4	91.03	98.7

Solvents

The ideal property of a solvent is that the drug should be completely soluble in the solvent used and should give constant results. To serve this purpose, three solvents were tried that are 0.1 N hydrochloric acid, 0.1 N sodium hydroxide and distilled water. The spectrum was sharp in 0.1 N sodium hydroxide.

Instrumental parameters

The instrumental parameters were varied in the particular range. Scan speed was varied as fast, medium, slow, very slow over the range 400 – 200 nm, while slitwidth and sampling interval were varied in particular range of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 nm and 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 nm respectively. Similarly for method 2, delta lambda was varied in the range 2, 4, 6, 8 and scaling factor was varied as 1, 2, 3, 4, and 5. For all these variable parameters, the absorbances were recorded over the concentration range according to the respective method**.**

Wavelength

The spectrum was showing two maximum wavelengths that are 258.0 nm and 231.0 nm (Fig. 3). The absorbances at 231.0 nm were not reproducible, while the absorbances at 258.0 nm were reproducible as well as precise for method 1. Similarly, after conversion of spectrum in the first derivative mode (Fig. 4), two maximum wavelengths that are 210.6 nm and 226.6 nm were observed and absorbances at 226.6 nm were reproducible for method 2.

By comparing the spectral shape, sharpness and absorbances of linearity, the critical parameters were selected in case of sample preparation, solvent and wavelength. For instrumental parameters, critical parameters were extracted by principal component analysis using SPSS software.

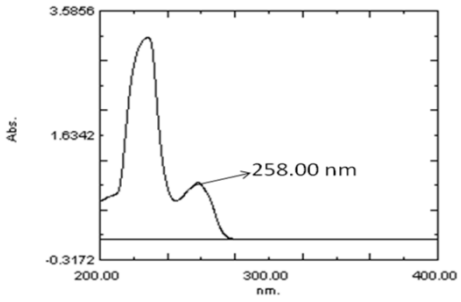

Fig. 3: Spectrum of Trospium chloride in 0.1 N sodium hydroxide

In principal component analysis, all these parameters were entered in variable entry window of SPSS software. Simultaneously, all the values of variable parameters were arranged in a datasheet. This datasheet was then substituted in the data entry window of SPSS software. Then the program was run to get principal components (critical parameters).

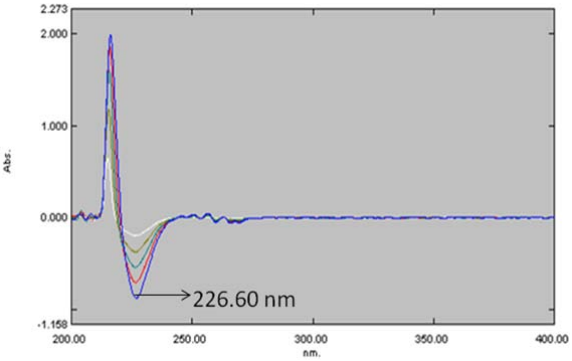

Fig. 4: First derivative Spectrum of Trospium chloride in 0.1 N sodium hydroxide

MATERIALS AND METHODS

Chemicals and Instrumentation

Trospium chloride was procured from Sidmak laboratories, Valsad, Daman. All chemicals and reagents used were of analytical grade and purchased from Merck chemicals, India. Rospium XR extended release capsules manufactured by Cipla containing the Trospium chloride equivalent to 60 mg were used as a dosage form. UV – 2450 double beam spectrophotometer for making Shimadzu having software UV Probe and version UV Probe was used for both methods.

Preparation of stock standard and working solution

A stock solution was prepared by weighing 10 mg of trospium chloride in 10 ml volumetric flask and dissolved in 0.1 N sodium hydroxide to obtain a concentration 1000 µg/ml. Working solution (100 µg/ml) was prepared by diluting 1 ml of stock solution to 10 ml and it was used for initial spectral scan in spectrophotometric method and further dilutions for linearity were prepared from the stock solution by alligation method.

Spectrophotometric conditions

According to extracted parameters, 0.1 N sodium hydroxide was used as solvent and Rospium XR capsules were used as a dosage form for both methods. In case of wavelength, 258.0 nm was selected for method 1 and 226.6 nm was selected for method 2. For both methods, instrumental parameters selected were slitwidth 1.0, scan speed medium, sampling interval 0.2 while for method 2, delta lambda 2 and scaling factor 5 was used.

Linearity Studies

Trospium chloride obeyed beer's law in the concentration range of 200–1000 µg/ml at 258 nm using 0.1 N sodium hydroxide for method 1. While in the Method 2, the absorbances were linear in the concentration range of 20–100 µg/ml at 226.60 nm in 0.1 N sodium hydroxide. The absorbances were plotted against the corresponding concentrations to obtain the calibration graphs.

Estimation of trospium chloride in standard mixture and capsule dosage form

Trospium chloride was estimated by preparing standard mixture of label claim 60 mg and it was diluted to make 600 µg/ml and 60 µg/ml for method 1 and 2 respectively. For the estimation in formulation, twenty capsules were weighed and average weight was noted. Then the granules in capsule were triturated and capsule powder equivalent to average weight was weighed and transferred to 10 ml volumetric flask and diluted with 0.1 N sodium hydroxide. It was shaken for 5 minutes followed by immediate filtration and volume was adjusted up to 10 ml. From this solution, 1.0 ml was withdrawn and further diluted up to 10 ml using 0.1 N sodium

hydroxide. This concentration 600 µg/ml was used for the estimation in method 1 while concentration 60 µg/ml was prepared by diluting 0.1 ml of stock up to 10 ml and used in method 2. Finally percentage amount was calculated (Table 2).

Table 2: Estimation of trospium chloride

Method		Amount taken (n=3)	Amount found (%)	% RSD
1	Standard mixture	600 µg/ml	99.8	0.26
	Capsule	600 µg/ml	98.7	0.42
2	Standard mixture	60 µg/ml	100.2	0.30
	Capsule	60 µg/ml	99.2	0.32

Validation of spectrophotometric methods

Precision

Precision studies were performed by using standard solutions containing three concentrations that are 400, 600, 800 µg/ml for method 1 and 40, 60, 80 µg/ml for method 2.

Repeatability

The precision of the methods in terms of repeatability was determined by analyzing three concentrations per three replicates of trospium chloride standard solutions. Depending on absorbances obtained for each concentration, standard deviation and percentage relative standard deviation was calculated.

Intermediate precision

Intermediate precision was assessed by analyzing trospium chloride standard solutions on three consecutive days over a period of one week. Same parameters were calculated for intermediate precision. The results of the repeatability and intermediate precision are as shown in Table 3.

Table 3: Precision data

Method	Concentration	Repeatability (% RSD) (n = 3)	Intermediate precision (% RSD) (n = 3)
1	400	0.31	1.41
	600	0.26	1.67
	800	0.21	1.80
	mean	0.26	1.63
2	40	0.42	1.54
	60	0.33	1.29
	80	0.37	1.92
	mean	0.38	1.58

Accuracy

Accuracy was determined by using the standard addition method at three different levels. Sample stock solution of capsule dosage form having concentration 600 µg/ml was used for method 1 and 60 µg/ml was utilized for method 2. To the above solutions, 80%, 100% and 120% of standard drug solution was spiked and accordingly percentage recovery was calculated as stated in table 4.

Table 4: Accuracy data

Method	Level	Concentration (µg/ml)		% Recovery	Mean
		Tablet	Standard		
1	80 %	600	480	98.4	98.8
	100 %	600	600	99.3	
	120 %	600	720	98.7	
2	80 %	60	48	99.5	99.0
	100 %	60	60	98.7	
	120 %	60	72	99.2	

Specificity

For determination of interference of other excipients, 200 µg/ml of lactose monohydrate, microcrystalline cellulose and starch were added to 200 µg/ml standard drug solution for method 1. Similarly for method 2, 20 µg/ml of each excipient was added to 200 µg/ml standard drug solution. Interference of excipients was determined by finding percentage concentration of the standard drug in each dilution.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations 1 and 2.

$LOD = 3.3 \times \sigma / S$ ..... (1)

$LOQ = 10 \times \sigma / S$ ..... (2)

Where σ is the standard deviation of intercept, S is the slope of the calibration curve.

Robustness

Robustness was carried out by changing the concentration of the solvent. For both methods, the absorbances were taken by using 0.05 N and 0.2 N sodium hydroxide as a solvent.

RESULTS

Two spectrophotometric methods were proposed according to QbD approach. The statistical tool known as principal component analysis was utilized for extraction of critical parameters (Table 5).

Table 5: Extracted critical parameters

Method 1				Method 2			
By observation		By Principal component analysis		By observation		By Principal component analysis	
Parameter	Extracted result	Parameter	Extracted result	Parameter	Extracted result	Parameter	Extracted result
Solvent	0.1 N NaOH	Scan speed	Medium	Solvent	0.1 N NaOH	Scan speed	Medium
Wavelength	258 nm	Slitwidth	1.0	Wavelength	226.6 nm	Slitwidth	1.0
Sample preparation	Capsule	Sampling interval	0.2	Sample preparation	Capsule	Sampling interval	0.2
						Delta lambda	2
						Scaling factor	5

In method 1, trospium chloride followed linearity in the concentration range 200 – 1000 µg/ml. The proposed method was applied to capsule dosage form and amount of drug estimated was 98.7% showing good agreement with the label claim. Precision data showed % RSD value less than 2 for repeatability as well as intermediate precision. Accuracy studies showed mean percentage recovery 98.8%. Specificity studies resulted in null interference of excipients with the drug. Limit of detection and limit of quantitation was found to be 0.71 and 2.17 respectively. Changing in concentration of solvent does not affected the absorbances. The statistical data of validation is summarized in Table 6.

In method 2, linearity was in the concentration range 20 – 100 µg/ml. The proposed method was applied to capsule dosage form and amount of drug estimated was 99.2% showing good agreement with the label claim. % RSD value was less than 2 for repeatability as well as intermediate precision. Accuracy studies showed mean percentage recovery 99.0%. Specificity studies did not show any interference of excipients with the drug. Limit of detection and limit of quantitation was found to be 2.57 and 7.81 respectively. Changing in concentration of solvent does not affected the absorbances. The statistical data of validation is summarized in Table 6.

Table 6: Statistical data of Validation

Method	Method 1	Method 2
λ max (nm)	258.00	226.60
Linearity range (µg/ml)	200-1000	20-100
Regression equation	Y = 0.00094 X + 0.019	Y = 0.0084 X + 0.030
Correlation coefficient	0.9999	0.9999
Percentage recovery	98.8	99.2
LOD	0.71	2.57
LOQ	2.17	7.81
Precision (% RSD)		
Repeatability	0.26	0.38
Intermediate precision	1.63	1.58
Standard error	0.15×10^{-4}	0.78×10^{-4}
Molar absorptivity (lit. .mole-1. .cm-1)	41682.60	37278.80
Sandell's sensitivity (µg/cm²/0.001)	0.0102	0.0154
Specificity (% R. .S. .D.)	0.73	0.58
Robustness (% R. .S. .D.)	0.34	0.21

CONCLUSION

The proposed spectrophotometric methods can be concluded as accurate, precise, robust, specific and economic. In comparison, first order spectrophotometric method was found to be more sensitive than simple spectrophotometric method. Implementation of QbD approach resulted in more robust methods with less time consumption, consistency, reliability and quality data. The proposed spectrophotometric methods along with QbD approach can be used as an alternative tool in the drug quality control laboratories for quantitative determination of trospium chloride.

ACKNOWLEDGEMENT

The authors are thankful to the management, Principal and the staff of M. .G. .V's Pharmacy College, Panchavati, Nashik for their kind help and support. They also thanks to Dr. S. J. Surana, Principal of R. .C. .Patel Institute of pharmaceutical Education and Research, Shirpur, for providing required facilities.

CONFLICT OF INTEREST

There is no any competing interest among the authors. Authors declare no conflict of interest.

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