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Analytical Method Development And ICH-Guided Validation of A RP-HPLC Procedure for Buspirone Estimation in Bulk and Tablets

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Abstract

Buspirone is a widely prescribed anxiolytic drug which requires reliable analytical techniques to ensure the safety, quality, efficiency and consistency of its pharmaceutical dosage forms. Although various chromatographic methods have been reported, many lack optimal sensitivity, short run time performance or comprehensive validation in accordance with regulatory expectations. So, there's need for an efficient RP-HPLC method that is rapid, cost-effective, and suitable for routine quality-control applications in both bulk and tablet formulations. The present study aims to develop and validate a robust RP-HPLC method for the quantitative estimation of Buspirone in bulk and pharmaceutical formulations. Buspirone was analyzed under isocratic conditions using Shimadzu C18 column using 0.1 M acetate buffer (pH 5) and acetonitrile (45:55) at 1 ml/min and 25 °C with chromatographic detection at 245 nm. The developed method has achieved a sharp and well resolved Buspirone peak with a retention time of 3.4 minutes achieving rapid analysis. The calibration curve gave excellent linearity from 1 to 5 µg/ml range, with a correlation coefficient of 0.9998 showing its reliability. Sensitivity parameters showed LOD and LOQ values of 6.36 µg/ml and 19.28 µg/ml, respectively. Precision for both intra-day and inter-day analysis showed %RSD values less than 2% and repeatability measurements confirmed consistent peak areas. Validation parameters such as linearity, accuracy, precision, robustness, and sensitivity complied with ICH guidelines confirming the consistency, reliability, efficiency and suitability of method for routine applications such as drug development, routine QC analysis and broader pharmaceutical research. This study offers a practical and economical RP-HPLC method for Buspirone estimation.

Keywords

Buspirone Quantification, RP-HPLC Method Development, Pharmaceutical Quality Control, Analytical Method Validation, ICH-Compliant Validation

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INTRODUCTION:

Buspirone is chemically known as 8-{4-[4-(pyrimidin-2-yl) piperazin-1-yl] butyl}-8-azaspiro [4.5] decane-7,9-dione. The chemical structure of Buspirone is given in Fig. 1.

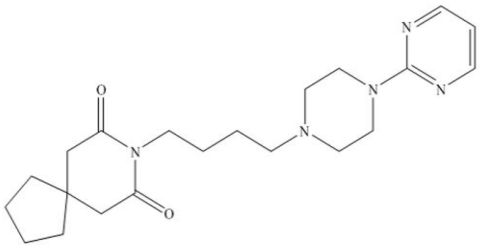

Fig. 1: Chemical Structure of Buspirone

It is a non-benzodiazepine anxiolytic drug that offers a favorable side effect profile compared to other anxiety medicines. It is primarily prescribed for generalized anxiety disorder (GAD) and in some cases as a secondary option for treating depression.[1-3] The drug was developed in 1968 and patented in 1975. It is most commonly sold under the brand name Buspar® and received FDA approval in 1986.[4-6] Typically, it is given as a daily dose of 15 mg. Clinical studies have demonstrated that Buspirone effectively relieves GAD symptoms by interacting with two specific subtypes of the 5-HT1A serotonin receptors.[7-12]

Analytical methods using RP-HPLC offers varying sensitivity and linearity but they have challenges too. The limitations of previous studies are shown in cause-and-effect diagram ("Fig. 2") which was used to categorize and review the effect of all the possible factors.

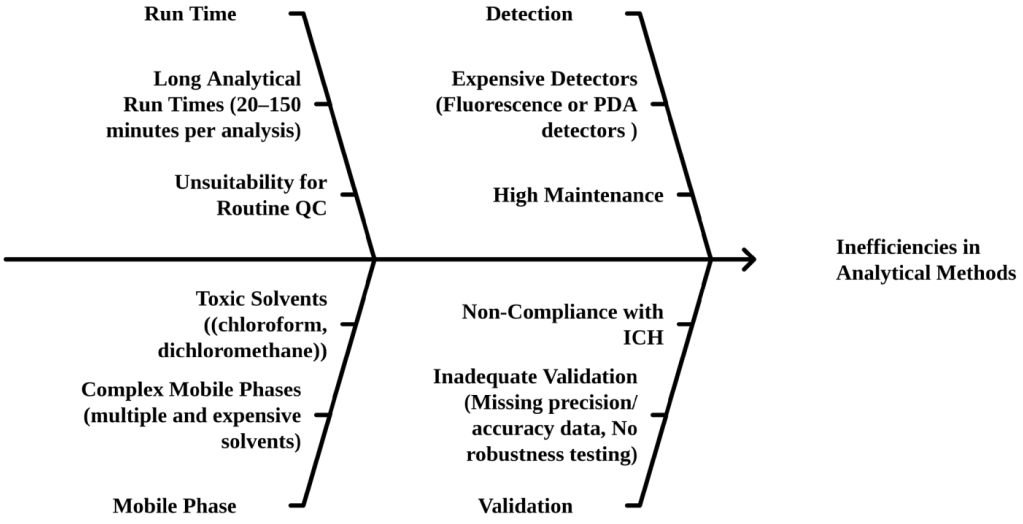

Fig. 2: Cause and effect diagram to identify limitations of previous methods

This study focuses on developing and validating a novel RP-HPLC method for Buspirone in bulk and tablet dosage forms to give a better alternative by reducing toxic solvent usage and by using green chemistry approaches which makes it eco-friendly. The objective is to optimize chromatographic conditions for rapid analysis with reduced retention time and to ensure ICH-compliant validation (precision, accuracy, linearity, LOD/LOQ) and analyzing its applicability in tablet formulations.

METHODOLOGY

Materials and methods

Drug

The API and tablet of Buspirone was received as a gift sample from Strides Alathur Pvt. Ltd., Alathur, Chengalpattu, Tamil Nadu, India.

Chemicals and reagents

HPLC Grade Acetonitrile was bought from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India.

HPLC Grade water, Sodium Acetate and Acetic acid were procured from Isochem laboratories, Angamaly, Kochi.

Table 1: Instruments Used

Instrument/ Component	Details
HPLC Device	Shimadzu LC-20AD
UV Detector	Shimadzu SPD-M20A
Software	LC lab solution
Column	Shim-pack Solar C18
Column Dimensions	250 mm × 4.6 mm
Particle Size	5 µm
Injector	Rheodyne injector 20µl
pH meter	Alpha-01
Sonicator	Labman sonicator, Model No: LMUC-3
Analytical balance	Wensar

Selection of wavelength

UV spectroscopy was used to determine the wavelength of drug and 245 nm was chosen as its λmax because of its good absorbance. Therefore, based on the spectrum it was determined that the detection wavelength under study is 245 nm.

Table 2: Fixed chromatographic conditions

Parameter	Conditions
Stationary Phase	C18 Column (250 mm × 4.6 mm, 5 µm)
Mobile Phase	0.1 M acetate buffer (pH 5) : acetonitrile
Mobile Phase ratio	45:55 v/v
Detection Wavelength	245 nm
Flow Rate	1 mL/min
Injection Volume	20 µL
Run Time	10 minutes

Preparation of Stock solutions

Stock solution-1

10 mg of Buspirone API was weighed and transferred into 10ml volumetric flasks and the volume was made up to 10 ml using acetonitrile as a solvent. The mixture was then sonicated for 5 minutes until thoroughly dissolved. The final concentration of Buspirone was 1000 µg/ml.

Stock solution-2

1 ml aliquot of Buspirone from stock solution-1 was transferred to 10 ml volumetric flasks and the volume was made up to 10 ml with water. The solution was sonicated for 5 minutes, and the final concentration of Buspirone obtained was 100 µg/ml.

Preparation of acetate buffer

5.772 g of sodium acetate and 1.778 g of acetic acid were weighed and dissolved using 800 ml of HPLC grade water in a 1000ml volumetric flask. The solution was adjusted to desired a pH (5.0) using 10 N HCL and the volume was made up to 1L using HPLC grade water. The solution was then filtered through a membrane of 0.45µm pore size and used for the study.

Preparation of mobile phase

A mixture of 0.1M acetate buffer (pH 5) and acetonitrile in 45:55 ratio was prepared and degassed in an ultra sonicator for 5 minutes.

Preparation of working standard solution

Working standard solutions were prepared by pipetting out 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml from stock solution- 2 and the volume was made up to 10 ml with mobile phase. Then the mixture was sonicated for 5 mins to dissolve it completely. Calibration curve was prepared from the concentration range of 1-5 µg/ml for Buspirone.

Analysis of formulation

20 tablets of Buspirone were weighed and powdered. An accurately weighed quantity of finely powdered tablets equivalent to 3mg of Buspirone was trasferred into a 100ml volumetric flask and mobile phase was added. It was then sonicated for 5mins, then filtered with Whatman filter paper (No.41) and diluted to required concentration (3 µg/ml) with mobile phase. The chromatogram was obtained at 245nm and the amount of drug was calculated.

RESULTS

Method development and optimization of chromatographic conditions

To achieve good resolution different proportions of solvents like acetonitrile and 0.1M acetate buffer of different pH conditions were tested. However, 0.1M acetate buffer of pH 5 and acetonitrile in the ratio of 45:55 achieved satisfactory results at flow rate 1 ml/ min measured at a detection wavelength of 245nm. The chromatogram of optimized standard is shown in "Fig. 3". The system suitability parameters such as retention time, tailing factor and theoretical plates for optimized standard chromatogram were tabulated.

Method Validation

Linearity

Linearity was studied by analyzing five standard solutions in the concentration range of 1 – 5 µg/ml for Buspirone by RP-HPLC. Calibration curve with concentration on x-axis Vs peak area on y-axis was plotted. Linearity was measured by using calibration data, regression analysis as well as coefficient of correlation. The regression statistics was displayed in "Table 3". The correlation coefficient (r^2) of Buspirone was found to be 0.9998. The linearity graph is shown in "Fig. 4". This shows better detector response in different concentrations for the method.

Table 3: Regression data

Parameters	RP-HPLC
Range	1-5 µg/ml
R^2	0.9998
Slope	1368087
Y-intercept	287135

Table 4: Intra-day precision data

Parameters	RP-HPLC
Concentration (µg/ ml)	3
Standard deviation	45372.940
%RSD	1.043 **%**

Table 5: Inter-day precision data

Parameters	RP-HPLC
Concentration (µg/ ml)	3
Standard deviation	46399.0404
%RSD	1.066 **%**

Table 6: Repeatability data

Parameters	RP-HPLC
Concentration (µg/ ml)	3
Standard deviation	45183.174
%RSD	1.041 **%**

Table 7: Accuracy data

Drug Concentration	Recovery level %	Amount spiked (µg)	Amount found (µg)	% Recovery
Buspirone (3 µg/ml)	80 **%**	8	10.96	99.64 %
	100 **%**	10	13.15	101.16 %
	120 **%**	12	14.93	99.59%

Table 8: LOD and LOQ data

Drug	RP-HPLC	
	LOD	LOQ
Buspirone	6.36 µg/ml	19.28 µg/ml

Table 9: Robustness

Parameter	Changes done	Peak area	%RSD
Detection wavelength	244nm	4399759	1.19 **%**
	245nm	4349269	
	246nm	4295959	
Flow rate	0.9 ml/min	4394645	1.068 **%**
	1.0 ml/min	4349269	
	1.1 ml/min	4301759	

Table no 10: Ruggedness

Concentration	Parameters	Peak area
3 µg/ ml	Analyst 1	4349269
	Analyst 2	4301899
	Analyst 3	4379674
%RSD		0.902 %

Table 11: System suitability parameters of RP-HPLC

Parameters	Buspirone
Retention time	3.4 mins
Peak area	3738634
Theoretical plate	5456
Tailing factor	1.12

Table no 12: RP-HPLC analytical method validation parameters

PARAMETERS	RP-HPLC
λ_{max}	245nm
Regression equation (y= mx+c)	y =1,368,087x + 287,135
Regression coefficient (r^2)	0.9998
Limit of detection (LOD)	6.36 µg/ml
Limit of quantitation (LOQ)	19.28 µg/ml
% RSD for Buspirone 3 µg/ml (Repeatability)	1.04 %
% RSD for Buspirone 3 µg/ml (Inter-day precision)	1.06 %
% RSD for Buspirone 3 µg/ml (Intra-day precision)	1.043 %
Robustness indicated by % RSD (λ_{max} ±1nm)	1.13 %
Robustness indicated by % RSD (flow rate ±0.1ml/min)	1.068 %
Ruggedness indicated by % RSD (different analysts)	0.902 %

Table No.13: Analysis of formulation

Drug	Label claim (µg)	Amount found (µg)	% Label claim
Buspirone	10 µg	9.72 µg	97.20%

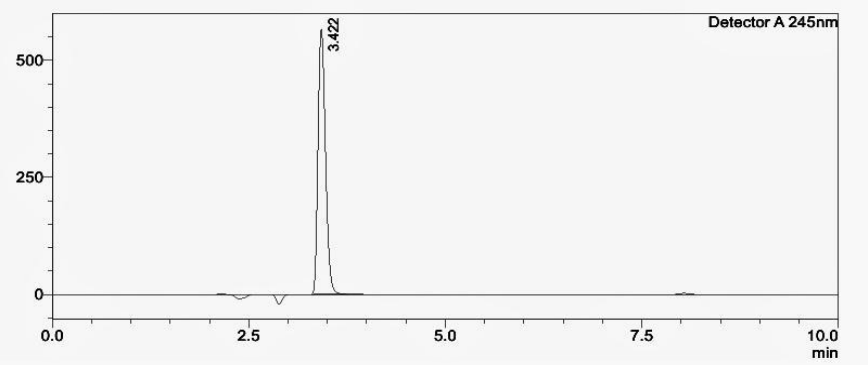

Fig. 3 Representative chromatogram of Buspirone at 245nm for RP-HPLC

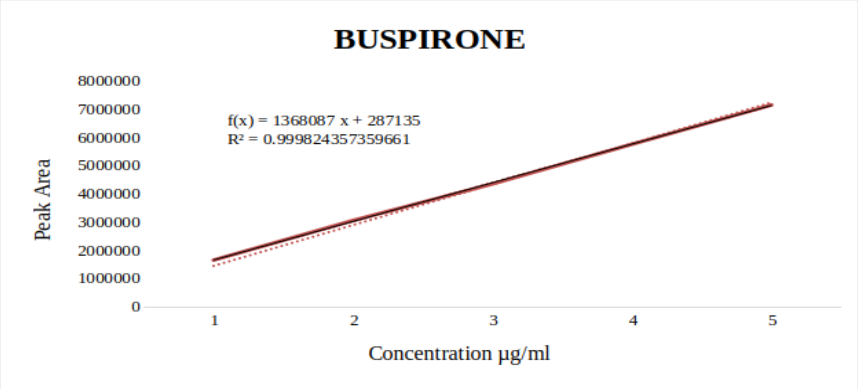

Fig. 4 Linearity graph of Buspirone at 245nm for RP-HPLC

Precision

Precision of the analytical method was demonstrated by intra-day precision, inter-day precision and repeatability. % RSD was calculated and results were represented in "Table 4, 5 & 6". The coefficient of variation (% RSD) was less than 2% which shows the method was precise.

Accuracy

Accuracy of the optimized method was determined by % recovery. It was found out by replicate analysis of samples containing known amount of the analyte. Standard addition was used for accuracy determinations at the three levels (80%, 100%, and 120%). The solution was prepared at three levels and was injected three times. Based on the area of the peak, the % recovery was calculated and results were responded in "Table 7". The recovery range for Buspirone was found to be 99.64 %, 101.16 % and 99.59 % by RP-HPLC method at 80%, 100%, and 120% levels respectively.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection and quantitation of the drug were calculated with standard deviation and slope. The LOD and LOQ of each method was calculated and represented in "Table 8". LOD and LOQ of were calculated by using the following formula,

LOD = 3.3*σ / S

LOQ = 10*σ / S

where, σ = standard deviation and S = slope

Robustness

Robustness of the HPLC method was determined by the analysis of appropriate concentrations of Buspirone at different wavelengths (245 ± 1nm) and different flow rates (± 0.1ml/min). Values of % RSD were calculated and are shown in "Table 9".

Ruggedness

Ruggedness is the ability to replicate results from different analysts under normal and expected operational conditions. Appropriate concentrations of Buspirone were analyzed by different analyst. % RSD was calculated and values are shown in "Table 10".

System suitability

The determination of the system suitability of RP-HPLC method was accomplished by using the concentration of 3 µg/ml. The system suitability was assessed based on the retention time, peak area, theoretical plates, and asymmetry factor. The optimized method is acceptable as system suitability parameters are valid as they passed the criteria of acceptability, as shown in "Table 11".

DISCUSSION

The working conditions for the RP-HPLC method were established for Buspirone in bulk and tablet dosage form. The correlation coefficient (r^2) for Buspirone was found to be 0.9998. The proposed method was validated as per ICH Q_2R_1 guidelines. LOD and LOQ indicates the sensitivity of the method. The coefficient of variation (% RSD) was less than 2% which shows the method was precise. According to the cited research article, the retention time of Buspirone was in the range of 4.27 to 23.60 min. The common detection wavelengths for Buspirone are around 210 - 245 nm, depending on the method used.[13-24] But as per the newly developed analytical method, retention time was found to be 3.422min for Buspirone by using flow rate of 1 ml/ min and the elution was completed within 5 min. Hence, simple, sensitive and accurate analytical method has been developed and validated as per guidelines for the estimation of Buspirone in bulk and tablet dosage form by RP-HPLC.

CONCLUSION

RP-HPLC method was developed for the estimation of Buspirone and validated as per ICH guidelines. With Shim pack Solar C18 Column, the drug got eluted with good peak and the pressure was within

the limit. This method gives reliable results with short analysis time using mobile phase of 0.1 M acetate buffer (pH 5) and acetonitrile in the ratio of 45:55. Retention time was found to be 3.4 min. System suitability parameters were in the desired limit. This method has been developed and optimized as per ICH Q2 (R1) guidelines. Validation parameters, linearity and correlation coefficient were found to be 1-5 µg/ml and 0.9998 respectively. Precision was found to be < 2%. LOD and LOQ were found to be 6.36 µg/ml,19.28 µg/ml. System suitability, tailing factor was 1.12 and theoretical plate were 5456. % RSD for all the validation parameters were within the limit (NMT 2.0). Thus, the developed analytical method was simple, economical, less time consuming and all the validation parameters were within the limits.

ABBREVIATIONS

RP-HPLC- Reverse Phase High Performance Liquid Chromatography, UV- Ultra Violet, ICH- International Council for Harmonization, SD- Standard deviation, RSD- Relative Standard Deviation, LOD- Limit of detection, LOQ- Limit of quantitation, NMT- Not more than, µg/ml- Micro gram per milli litre.

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