Hygeia:: journal for drugs and medicines
October2012-March 2013
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Pharmaceutical analysis

Simple Spectrofluorimetric determination of Buspirone hydrochloride in Bulk drug and Pharmaceutical dosage forms.

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Article history: Received: 22 June 2012, revised: 10 August 2012, accepted: 20 August 2012, Available online: 10 October 2012

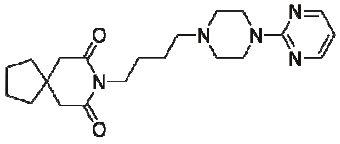
Abstract

Plan: *A spectrofluorimetric method for the estimation of Buspirone HCl in bulk drug and pharmaceutical dosage forms*
Prologue: *The method is simple, fast and accurate and can be used for routine analysis of commercial Buspirone HCl tablets.*
Methodology: *The developed method is based on the determination of fluorescence intensity of the solutions in water at 379nm after excitation at 238nm. Different experimental parameters affecting the fluorescence were carefully studied. The fluorescence intensity of the solution was stable for 5 hours. The calibration curve was linear from 50-1500ng/ml. The limit of detection (LOD) and limit of quantification (LOQ) were 20ng/ml and 45ng/ml respectively.*
Outcome: *The proposed method has been applied successfully for the determination of Buspirone HCl in pharmaceutical dosage forms. No significant interference was observed from excipients, coloring and flavoring agents commonly used in the formulation. The mean recovery of drug from tablets was 99.89%.*
Key words: *Buspirone HCl; Spectrofluorimetric method; Bulk/Pharmaceutical Dosage forms.*

1. Introduction

Buspirone is chemically 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl) butyl]-8-azaspiro[4, 5]decane-7,9-dione hydrochloride. It is a no sedating antianxiety agent. It has dopaminergic, adrenergic and serotonin modulating properties. The USP describes the assay of Buspirone HCl either in pure form or in tablets by HPLC. Several analytical methods have been reported for the determination of Buspirone in raw material, dosage forms and biological fluids. These methods include spectrophotometry,[1-4] HPLC,[5,6] polarography,[7,8] GC[9] and capillary zone electrophoresis.[10,11]

The official HPLC methods[12] and other reported methods for the determination of Buspirone require sophisticated and costly instruments and complicated procedures.



Buspirone

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Hygeia.J.D.Med. Vol.4 (2), Oct. 2012

Several N-heterocyclics, notably polyazines exhibit fluorescence[13]. The isomeric diazines ($C_4H_4N_2$) exhibit weak fluorescence. Fluorescence spectra of compounds with 2-aminopyrimidine moiety have been reported.[14] Since Buspirone is having the 2-aminopyrimidine nucleus it was thought worthwhile to develop a simple spectrofluorimetric method for determination of Buspirone HCl in pharmaceutical dosage forms which can be used as an alternative to the official method or other recommended procedures in quality control laboratories.

2. Experimental

2.1. Instrument

Fluorescence Spectrophotometer, Hitachi Ltd, Japan was used for the studies.

2.2. Chemicals and reagents

Methanol, spectroscopic grade (E. Merck India Ltd, Bombay) was used. Triple distilled water was used to prepare all solutions. Freshly prepared solutions were always used. Buspirone HCl was obtained from Alidac Genetics and Pharmaceuticals, Ahmadabad.

2.3. Determination of Fluorescence of Buspirone HCl

10 mg of Buspirone HCl was dissolved in water and made upto 100ml with water. 1ml of this solution was diluted to 100ml. The dilute aqueous solution was pre-scanned after setting the instrument parameters. The pre-scanning showed an excitation maximum at 238nm and an emission maximum at 379nm.

a) Excitation Spectrum

The emission wavelength was now fixed at 379nm and the solution was scanned for excitation spectrum from 220nm to 800nm. The excitation spectrum showed an intense sharp peak 238nm and less intense small peaks at 297nm and 378nm. (Fig. 2)

b) Emission Spectrum

The excitation wavelength was now set at 238nm and the solution was scanned for emission spectrum from 220nm to 800nm. The emission spectrum showed an intense peak at 379nm and a less intense peak at 480nm. (Fig. 3)

2.4. Determination of minimum and maximum concentration of the linearity range:

a) Minimum Concentration:

2.4.1. Preparation of Buspirone HCl Stock solution:

25 mg of Buspirone HCl reference standard was dissolved in water and made upto 250 ml with water in standard flask. This stock solution has a concentration of 100µg/ml. 2.5ml of the stock solution was diluted to 250ml with water.

Pipetted out 2, 4, 6, 8, 10, 25 and 50 ml portions of this solution to 100ml standard flasks and volume was made upto 100ml with water to get concentrations of 20, 40, 60, 80, 100, 250 and 500 ng/ml respectively. The fluorescence intensity of the solution was measured after setting the excitation wavelength at 379nm. The calibration curve was prepared by plotting fluorescence intensity (I) against drug concentration. The correlation coefficient was calculated using the data. It was found that the curve is linear at the concentration range and is sensitive even upto a very low concentration of 20ng/ml.

b) Maximum Concentration:

The stock solution was diluted with water to get a concentration of 50, 100, 250, 500, 750, 1000 and 2000ng/ml each. The fluorescence intensity of the solutions was measured at an emission wavelength of 379nm after setting the excitation wavelength at 238nm. The data obtained was plotted and correlation coefficient was calculated. It was seen that the fluorescence intensity value corresponding to the concentration 2000ng/ml was above the detectable limit of the instrument. Therefore, it was concluded that a maximum concentration of 1500ng/ml will be suitable and this limit was fixed as the upper sensitivity limit.

2.5. Preparation of Standard Curve:

The Buspirone HCl stock solution was diluted to get solutions of concentrations of 250, 500, 750, 1000, 1250 and 1500ng/ml. The fluorescence intensity was then measured at an emission wavelength of 379nm after setting the excitation wavelength at 238nm.

The fluorescence intensity was plotted against the concentration (Fig. 4) and the correlation coefficient was calculated. The curve was linear and the correlation coefficient was 0.999.

2.6. Stability Profile of the fluorescence of Buspirone HCl solution.

a) Time Scan of Buspirone HCl solution:

A solution of 1000ng/ml concentration of the drug was used. The excitation wavelength was set at 238nm and emission wavelength at 379nm. The solution was scanned for a period of 45 minutes. A straight line was obtained which showed that the fluorescence of the drug solution is stable and the method is applicable to the determination of dosage forms also.

b) Effect of Temperature on fluorescence:

A solution of 1000ng/ml was used. Eight 10ml portions of the solution were used. One portion was kept without heating and other portions were heated on a water bath at 100^{o}C for 10,20,30,40,50 and 60 minutes respectively. The solutions were cooled to room temperature. The fluorescence intensity was measured at 379nm after setting the excitation wavelength at 238nm.

The results (Table 1) revealed that the fluorescence intensity will be decreased as the heating time is increased. Since the fluorescence intensity was stable at room temperature, the study was conducted at room temperature (28^{o}C).

c) Effect of pH on the fluorescence of Buspirone HCl solution.

The effect of pH was studied by preparing the Buspirone HCl solution in various buffers and the solutions were scanned for excitation and emission peaks and fluorescence intensity was then measured.

Acid phthalate buffer solutions of pH 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 were prepared as described in USP and the pH of the buffer solutions were checked using a pH meter.

0.1ml each of the Buspirone HCl solution was pipetted out into six 10ml standard flasks. The solutions were made up with different buffer solutions.

The solutions were pre-scanned and then the excitation and emission spectra was recorded after fixing the emission and excitation wavelengths at the maxima. The excitation and emission maxima and their intensities are shown in the Table 2. The data show that the excitation and emission maxima and their intensities are different at different pH values.

d) Effect of solvent on the fluorescence of Buspirone HCl.

A 50µg/ml solution was prepared in ethanol, methanol and 0.1N sulphuric acid. The solutions were pre-scanned and the excitation and emission spectra were scanned and the intensities at the peaks were found.

The data (Table 3) show that changing the solvent from water has shifted both excitation and emission maxima and the fluorescence intensity also decreases.

2.7. Procedure for the dosage form:

Three samples of Buspirone HCl tablets were analyzed by the proposed method.

a) Extraction of Buspirone HCl tablets:

20 tablets were accurately weighed and powdered in a glass mortar. A quantity of tablet powder equivalent to 10mg of Buspirone HCl was accurately weighed and transferred to a small conical flask. 40ml of methanol was added and swirled gently for 10 minutes. This solution was filtered into a 100ml standard flask through a whatmann filter paper. The residue was extracted again with 40ml of methanol and passed through the same filter paper. The residue was washed again with 15ml of methanol and passed through the filter. The final volume was made upto 100ml with methanol. 9ml of the solution was pipetted out into a small conical flask and the solution was evaporated on a water bath. The residue was extracted with water and the solution was made upto 100ml with water.

b) Measurement of the fluorescence:

A standard curve of Buspirone HCl reference standard was prepared using 500, 1000 and 1500ng/ml concentration. The fluorescence intensity of the solutions was measured at 379nm after excitation at 238nm. Three different samples of Buspirone HCl tablets were analyzed by this method.

The results are furnished in the Table 4. Using the data the content of Buspirone HCl per tablet was determined from the standard curve.

2.8. Comparison with Official method:

The reliability and validity of the proposed method was established by parallel determination against HPLC method described in USP .

3. Results and Discussion

Analytical Performance:

Linearity and Sensitivity:

For evaluation of linearity at the selected conditions, determination of Buspirone HCl was carried out at six concentrations (n=6). The calibration curve was linear over the concentration range of 50-1500ng/ml for standard solution. The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method of analysis were 20ng/ml and 45ng/ml respectively.

Accuracy and Precision:

Accuracy, intraday and interday precisions of the method were determined. Five replicate samples in the same day, as well as on five consecutive days were assayed for intraday and interday accuracy at three different concentrations for each analyte. Accuracy was calculated as deviation of the mean from the nominal concentration. The intraday and interday precisions (expressed as the relative standard deviation (RSD%) for Buspirone HCl ranged from 0.49 to 2.48%.

Recovery:

Recovery studies were carried out, by spiking known different amounts of pure drug solutions to the pre-analyzed drug samples. The results given in the Table 5 revealed that the RSD% and percent average of recovery for preparation samples were in the range 0.97-2.13% and 99.78-100.52% (Table.5).

Recovery results suggest method to be unaffected in the presence of formulation excipients and confirm the high accuracy.

Robustness:

Robustness was examined by evaluating the influence of small variation in the experimental conditions such as working excitation and emission wavelengths ($\pm$3nm), temperature of the solution ($\pm5^{o}$C) and change in pH ($\pm$0.2). These minor changes that may take place during experimental operation did not have any significant effect on fluorescence intensity of the analysis. RSD% for the measured fluorescence intensity after the studied variations did not exceed 4.60%.

Application to Tablets:

The proposed method was successfully applied to analysis of three different commercial tablets which contain 10mg of Buspirone HCl. The mean recovery values of tablets were 99.6%, 100.4% and 101.2%. (Table 4).

Interference from excipients was observed during the estimation of Buspirone HCl tablets. The fluorescence intensity was found reduced. So to exclude the excipients from the solution, extraction was carried out using solvents like chloroform, benzene and methanol.

But when methanol was used as the extracting solvent and the residue obtained after evaporation of the methanolic extract was subjected to determination, no interference from excipients was observed and produced comparable results with HPLC method. The reproducibility of the method was investigated on three commercial samples of buspirone HCl tablets. The results obtained were reproducible. The method was compared with the official HPLC method and the results are in good conformity with it.

4. Conclusion

The overall advantages of the present spectrofluorimetric method are its simplicity, sensitivity, rapidity and no need for expensive instruments in comparison to reported techniques. This method can be used for routine determination of Buspirone HCl in bulk drug as well as in pharmaceutical preparations. This method is rapid and accurate and applicable in different dosage forms in comparison to official method and other time consuming complicated and costly techniques such as GC and HPLC. These advantages will encourage the application of the proposed method in routine quality control laboratories for determination of Buspirone in bulk drug and pharmaceutical preparations.

Table 1. Effect of Temperature on Fluorescence.

Sl.No.	*Temperature (^oC)*	*Time (Min.)*	*Fluorescence Intensity*
1	100	0	5078
2	100	10	4696
3	100	20	4285
4	100	30	3864
5	100	40	3524
6	100	50	3218
7	100	60	2965

Table 2. Effect of pH on Fluorescence:

Sl.No.	*pH*	*Excitation Maximum*		*Emission Maximum*	
		(nm)	*(Intensity)*	*(nm)*	*(Intensity)*
1	1.5	383	1653	484	972
2	2.0	237	2081	380	2149
3	3.0	313	435	407	423
4	4.0	313	608	411	595
5	5.0	312	816	405	790
6	6.0	237	3927	378	3992

Table 3.Effect of Solvent on Fluorescence:

Sl.No.	*Solvent used*	*Excitation Maximum*		*Emission Maximum*	
		(nm)	*(Intensity)*	*(nm)*	*(Intensity)*
1	Ethanol	288	1260	362	2685
2	Methanol	287	4815	362	4809
3	0.1 N H_2SO_4	307	2558	407	2437

Table 4. Data for Analysis of Buspirone HCl Tablets

Tablet sample	*Label claim*	*Mean ± SD*	*Recovery%*	*RSD%*
Tamspar	10mg	9.96 ± 0.96	99.6	0.88
Buspidac	10mg	10.04 ± 1.05	100.4	0.48
Anxipar	10mg	10.12 ± 1.3	101.2	1.50

Table 5. Data for the Recovery Study:

Sl. No.	*Amount added (ng/ml)*	*Amount found (ng/ml) (mean ± SD)*	*Recovery %*	*RSD%*
1	200	201 ± 4.1	100.52	2.13
2	400	402 ± 6.3	100.48	1.86
3	600	599 ± 7.1	99.78	0.97

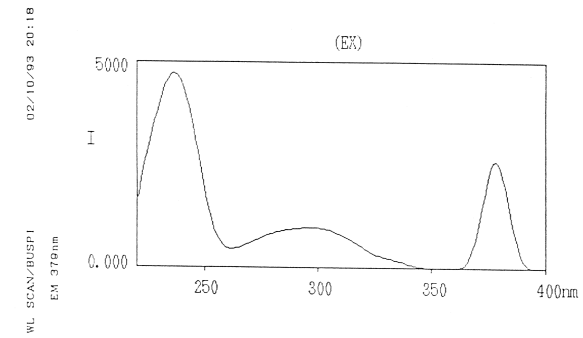

Fig.1. Excitation spectrum of Buspirone HCl in water.

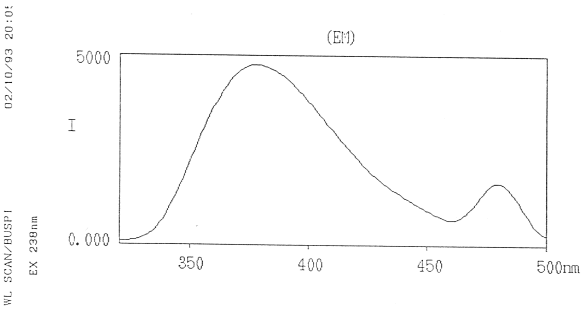

Fig.2. Emission spectrum of Buspirone HCl in water.

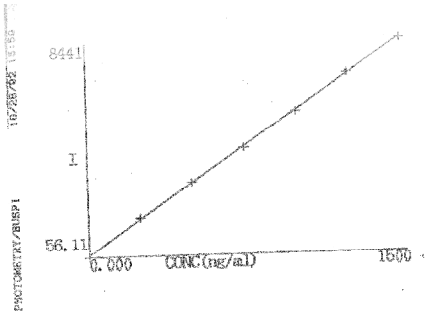

Fig.3. Standard curve for Buspirone HCl

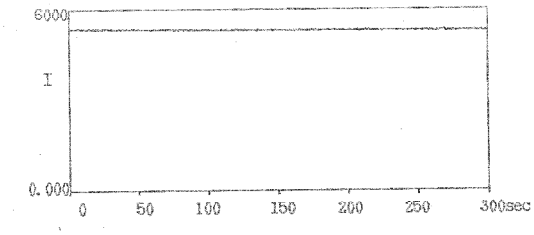

Fig.5 Time scan of Buspirone HCl in water.

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