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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION PAMABROM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Development and validation of simple, accurate, precise and economical UV spectrophotometric method for pamabrom in bulk and tablet dosage form. Pamabrom shows the maximum absorbance (λmax) at 279 nm in distilled water and was utilized for its determination. Drug was found to follow the Beer-Lambert's law in the concentration range of 2-20µg/ml. The suggested method is validated by using ICH validation parameters like accuracy, precision, linearity, LOD and LOQ respectively. Standard deviation and RSD for intra-day and inter-day precision studies was found to be less than ± 2. The limit of detection and limit of quantification was found to be 0.239µg/ml & 0.725µg/ml. The proposed method was successfully applied to the determination of pamabrom in pharmaceutical formulations without any interference from excipients. Results of the analysis were validated statistically and found to be satisfactory. The aim of present work is to develop the simple, economical, accurate, UV method for the estimation of pamabrom in bulk drug and tablet dosage form. This method was validated as per ICH guidelines.

INTRODUCTION

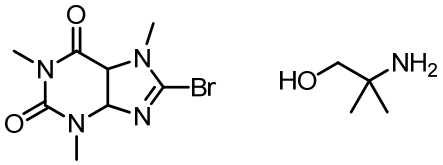

Figure 1:-Chemical Structure of Pamabrom

Pamabrom is chemically, mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate (1:1)[1]. It is official in US pharmacopoeia[2]. It is assayed by liquid chromatography as per USP[3]. Pamabrom, a xanthine derivative, is a safe and effective diuretic in relieving the water-accumulation symptoms of water-weight gain, bloating, swelling, and full feeling associated with the premenstrual and menstrual periods. As an over-the-counter diuretic, pamabrom is typically recommended to women to alleviate symptoms associated with menstrual cycles. Physicians also prescribe the use of such a water pill for other conditions involving water-weight gain. The medication works, as all diuretics, by pulling excessive water from throughout the body and increasing how frequently patients need to urinate. By flushing excess water from the system through increased urination, patients gain relief from the uncomfortable bloating and swelling associated with water-weight gain[4].

Literature survey reveals plasma HPLC method for estimation of Pamabrom in pharmaceutical dosage form[5-6]. The suggested method is validated by using ICH validation parameters like accuracy, precision, linearity, LOD and LOQ respectively[7 - 13]. However, there is no analytical method reported for the estimation of pamabrom. Aim of the present work was to develop the simple, economical, accurate, UV method for the estimation of Pamabrom in bulk drug and tablet dosage form. This method was validated as per ICH guidelines.

MATERIAL AND METHODS

Chemical and reagents

Active pharmaceutical ingredient of pamabrom were received as gift samples from Pan drug Ltd, Ahmedabad, India. Distilled water was used for the preparation of solutions. All the apparatus used for the experiment were graded.

Instrumentation

A Shimadzu UV 1100 series Spectrophotometer was used with 1 cm matched quartz cells. UV Spectra of standard and sample solutions were recorded in 1cm quartz cells at the wavelength range of 200- 400 nm.

Analytical method development

Preparation of standard stock solution

Standard stock solutions (100µg/ml) Pamabrom were prepared by dissolving accurately 10 mg of each drug separately in water in 100 ml volumetric flask. The working standard solutions of these drugs were further diluted with water to get required concentration of Pamabrom.

Preparation of sample solution for tablet dosage form

Twenty tablets were weighed and crushed to fine powder. A quantity of powder equivalent to 50 mg of Pamabrom was added in a 100 ml volumetric flask. 70 ml of distilled water was added in a volumetric flask, sonicatad for 20 minutes and final volume was made-up to 100 ml with distilled water. This solution was filtered through the Whatmann filter paper No. 41.

Preparation of calibration curve

Aliquots of standard stock solution were diluted with water to get a series of concentration in the range of 2-30µg/mL and scanned in the spectrum mode from the wavelength range 400-200 nm. Calibration curve was plotted as absorbance Vs concentration at λmax 279 as shown in fig.2. The correlation coefficient was found to be 0.9998. The optimized spectrophotometric method was applied to the direct determination of pamabrom in tablet dosage form.

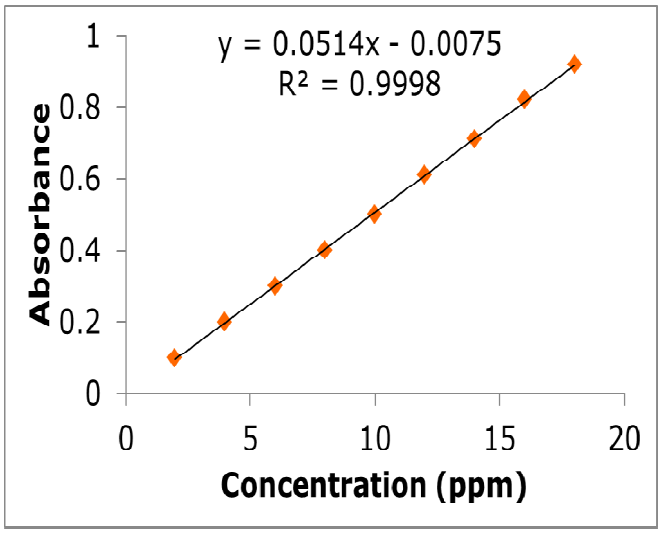

Fig. 2- Calibration Curve of Pamabrom

Validation of the proposed method

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantification.

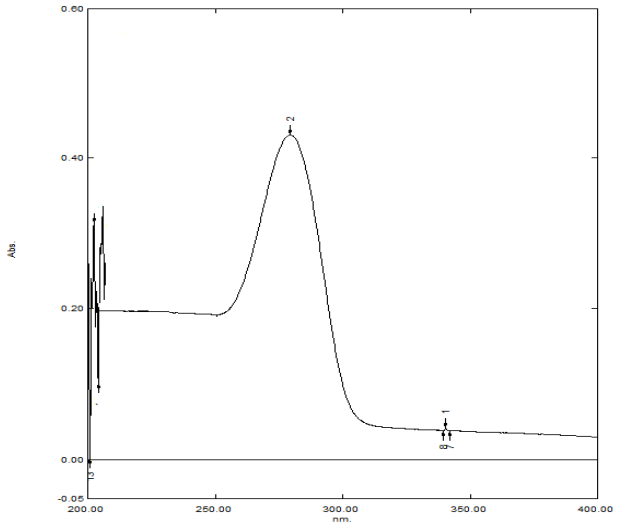

Fig. 3. UV Spectra of pamabrom at λ max 279nm.

Table 1: Optical Characteristics

Parameters	Result
Beer's law limit(µg/ml)	2-20µg/ml
Correlation coefficient	0.9998
Regression equation(Y*)	0.051x - 0.0075
Slope(a)	0.051x
Intercept(b)	0.0075

Limit of Detection (LOD)

LOD was calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S}$$

Where,

σ = the standard deviation of the response for the lowest conc. in the range

S = the slope of the calibration curve.

The limit of detection (LOD) was calculated by the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves . The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value result shown in Table 4.

Limit of Quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated for the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves. result shown in Table 4.

Linearity

The linearity of Pamabrom by analyzed by preparing different concentration of the standard drug solution. The graph of concentration verses absorbance was found to be linear in the concentration range of 2-20 µg/ml. The regression equation was calculated and linearity coefficient was found to be 0.9998 at λmax 279 nm.

Precision

Estimation of Precision of Pamabrom by proposed method was ascertained by replicate analysis of homogenous samples of tablet powder at different time intervals on the same day (Intra-day precision) and on second day (Inter-day precision).

Table 2: Precision

Drug	**Conc. Of drug(µg/ml)**	**Intraday precision Mean abs ± % RSD***	**Interday precision Mean abs ± % RSD***
Pamabrom	2.5	0.116 ± 0.131	0.114 ± 0.132
	5	0.250 ± 0.040	0.250 ± 0.041
	7.5	0.353 ± 0.016	0.354 ± 0.015

*Average of six determinations ,R.S.D.: Relative standard deviation

Accuracy

The accuracy of the method was determined by calculating percent recovery of pamabrom by standard addition method. The recovery experiments were carried out in triplicate (80, 100 and 120 %) by spiking previously analyzed samples of the tablet dosage form with three different concentrations of standards.

Table 3: Accuracy study

Drug	Level	Amount of sample (µg/ml)	Amount of standard spiked (µg/ml)	Total Amount recovered (µg/ml)	% Recovery ± RSD*
Pamabrom	80%	1.0	0.8	1.79	98.87 ± 0.155
	100%	1.0	1.0	1.95	99.90 ± 0.102
	120%	1.0	1.2	2.18	101.12 ± 0.108

*Average of six determinations, R.S.D.: Relative standard deviation

Robustness

Robustness of the method was determined by carrying out the analysis under different temperature condition. The respective absorbance of 18µg/ml was noted and the result was indicated as % RSD

Table 4: Summary of validation

Parameter	Result
Linearity indicated by correlation coefficient	0.9998
Limit of Detection	0.23µg/ml
Limit of Quantification	0.72µg/ml
Range	2-20µg/ml
Linear regression equation	0.051x - 0.007
Robustness indicated by % RSD	0.93%

RESULTS AND DISCUSSION

The proposed methods for estimation of Pamabrom were found to be simple, rapid, accurate and economic. Since not a single method reported for analysis of the drug earlier by UV spectrophotometric method, the developed methods can be used for routine analysis of drug in dosage forms. absorbance are measured at 279 nm and linearity was observed in the concentration range of 2-20 µg/ml in distilled water. The linearity coefficient was found 0.9998 at 279nm. Marketed brand of tablet was analysed and amount of drug determined by proposed method ranges from 98.87to101.12% and the drug were obtained at each added concentration, indicating that the method was accurate as shown in Table No 3. The developed method was found to be precise as the % RSD values for intraday and inter-day were found to be less than 2%. The LOD and LOQ were found to be 0.23µg/ml and 0.720µg/ml respectively and in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2. The results of the methods lie within the prescribed limit, showing that method is free from interference from excipients.

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