



ORIGINAL RESEARCH ARTICLE

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Development and validation of UV spectrophotometric method for quantitative estimation of Promethazine HCl in phosphate buffer saline pH 7.4

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ABSTRACT

A new, sensitive, rapid, simple, specific and economical procedure has been developed for determination Promethazine HCl in phosphate buffer saline pH 7.4. The purpose of this analytical validation procedure is to determine a process of assessment and to validate it by laboratory experiments to prove that the method meets the minimum standard for laboratory use. This analytical method for the determination of Promethazine HCl in phosphate buffer saline pH 7.4 can be used to estimate the amount of promethazine HCl penetrated and dissolved in the blood vessels *in vitro* by penetration study. The method is based on the ultraviolet light absorbance at 251 nm which is the maximum wavelength of the concerned drug. This method can be successfully applied for determination of drug in phosphate buffer saline pH 7.4. The results of the analysis have been validated statistically and by recovery studies.

Key Words: Promethazine HCl, PBS pH 7.4, Validation, Spectrophotometer UV.

INTRODUCTION

Promethazine-hydrochloride is the generic name of (2RS)-N, N-dimethyl-1-(10H-phenothiazin-10-yl) propan-2-amine hydrochloride. It is a first generation H1 antagonist acceptor, antihistamine and antiemetic medication. Promethazine HCl is the first choice in the case of morning sickness in the UK (Anonymous, 2007). Another effect of the drug is its powerful sedativeness, although it is rarely used specifically for this purpose. Having a pKa of 9.1, Promethazine HCl is a weak base (Alam *et al.*, 2007). The drug is available as a white-yellowish crystalline, odorless powder form. Oxidation resulted in color change to blue that occurs slowly when it is exposed to air. Promethazine HCl salt is very easily soluble in water and soluble in alcohol (Saif and Anwar, 2005). Oral route is the most widely used route of administration for this drug. Recently, another alternative route has been explored for its potential beneficiary effect over the oral route. Promethazine HCl transport test was conducted to determine its ability to penetrate skin. Analysis of the amount of promethazine HCl penetrated into the blood can be compared with determination of the amount of Promethazine HCl dissolved in phosphate buffer saline pH 7.4. Assay of Promethazine HCl in phosphate buffer saline pH 7.4 can be useful to measure the amount of promethazine HCl included in the penetration study from the system.

The purpose of development and validation of analytical methods is to ensure that a suitable method for analysis of a particular analyte is specific, accurate and precise. Main purpose of this study is to validate the experimental conditions and parameters to be followed in the determination of Promethazine HCl in phosphate buffer saline pH 7.4.

MATERIALS AND METHODS

Chemicals and reagents

Promethazine HCl working standard was a generous gift from Dong Gang City Hong Da Pharmaceutical Co. LTD (Batch No. 110802; Exp. date- 4th August 2015) from PT Berlico Mulia Farma, Sleman, Indonesia. Sodium dihydrogen phosphate, sodium chloride, potassium dihydrogen phosphate and all other chemicals were of analytical grade.

Instrument

UV-Vis spectrophotometer (Genesys 10S) and (Hitachi U 1800), Analytical Scales (AdvenC 2140), pH meter (Hanna HI 8314).

Preparation of phosphate buffer saline pH 7.4

Phosphate buffer saline pH 7.4 solution 1000 ml was prepared by mixing 2.86g NaH₂PO₄ and 0.2g KH₂PO₄ in distilled water. 8 grams NaCl was added to the mixture. Solution was stirred with a stirrer and the pH of the solution was adjusted to 7.4 with the addition of a suitable buffer component (Nugroho, 2004).

Determination of the maximum wavelength of promethazine HCl in phosphate buffer saline pH 7.4

Stock solution of Promethazine HCl in phosphate buffer saline pH 7.4 was made by carefully measuring approximately 100.0 mg of promethazine HCl and dissolving it in phosphate buffer saline pH 7.4 in a 200.0ml volumetric flask. 10.0 ml of the stock solution was filled into spectrophotometer cuvette and scanned over wavelength range of 200-550 nm. The maximum wavelength of promethazine HCl in phosphate buffer saline pH 7.4 were found is 251 nm.

Validation of the assay method of promethazine HCl

Linearity is the ability of the analysis procedure for obtaining the results of the experiment are directly proportional to the concentration of analyte in the sample (ICH, 1996). This parameter is a measure of how well the calibration curve that connects between the response (y) and concentration (x). Linearity is measured by a single

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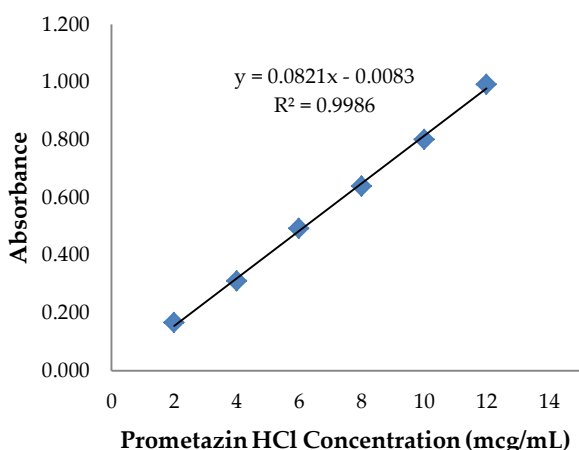


Figure 1: Graphical representation of linearity.

measurement at different concentrations and then determined the value of the slope and intercept and also correlation coefficient. Linearity test performed at a concentration of 0.125 to 12 mg/ml. Results from absorbance measurements at various concentrations were obtained value of $r = 0.9996$, proving the value of good linearity as approaching a value of 1.

Linear regression equation of the standard curve shown in the equation is $\hat{Y} = 0.08 X + 0.002$. Accuracy is the closeness of test results between the results obtained with the true value (true value) or the value of the reference. It describes the systematic error of the measurement results. Accuracy of the method was determined by measuring the concentration of promethazine HCl with 6.0 µg/ml of dilution of the stock solution in phosphate buffer saline pH 7.4 in 9 samples solution. The obtained values of recovery studies were in the range of 98.7-103.6% which indicates the accuracy of the proposed method. ICH demands that 'a good accuracy value' should be in the range of 95-105% of the true value.

Intermediate precision values were determined by testing the samples in different days, different analysts, and using different equipments. According to the International conference of Harmonisation (ICH) (1996), a good value any precision measurement of a sample has a value of RSD not more than 2%. Precision was determined by measuring the levels of promethazine HCl with concentration 6.0 mg/ml of dilution of the parent solution in phosphate buffer saline pH 7.4 were analyzed 6 times. Precision was indicated in the coefficient of variation. The resulting intermediate precision values are as follows 1.51% and 1.54%

RESULTS AND DISCUSSION

The analytical method development and validation for the Promethazine HCl in phosphate buffer saline pH 7.4 was carried out. The linearity calibration curve (Figure 1) shows linear response over the range of concentration used. The precision data shows that the reproducibility of the assay procedure was satisfactory. The accuracy of the method was determined by recovery studies that was carried out by percentage recovery calculation. The robustness of the method was determined by analyzing the same sample under different laboratory, by different analyst and using operational and environmental conditions; the degree of reproducibility shows results are within their limit (Table 1-4).

Table 1: Data for method precision test.

Sample Conc. (µg/mL)	Number of Measurement	Absorbance	Relative Std Deviation
6.0	1	0.48	1.50 %
	2	0.492	
	3	0.482	
	4	0.485	
	5	0.492	
	6	0.474	

Table 2: Data for linearity test.

Sl No.	Promethazine HCl Conc. (µg/mL)	Absorbance	Correlation Coefficient
1	2	0.165	0.999
2	4	0.311	
3	6	0.492	
4	8	0.638	
5	10	0.801	

Table 3: Data for accuracy test.

Sample No.	Claim Concentration (µg/mL)	Absorbance	Observation Concentration (µg/mL)	C_{obs}/C_{claim}	% Recovery
1	6.0	0.510	6.031	1.005	100.5%
2	6.0	0.501	5.923	0.987	98.7%
3	6.0	0.518	6.136	1.022	102.2%
4	6.0	0.510	6.037	1.006	100.6%
5	6.0	0.519	6.143	1.024	102.4%
6	6.0	0.502	5.949	0.989	98.9%
7	6.0	0.525	6.225	1.036	103.6%
8	6.0	0.509	6.026	1.004	100.4%
9	6.0	0.510	6.030	1.006	100.6%

Table 4: Data for intermediate precision.

Variable Parameters	Coefficient of variation (%)
Analyst -1	1.59
Analyst -2	1.68
Equipment -1 (UV-Vis Spectrophotometer Model Genyensis 10)	1.64
Equipment -2 (UV-Vis Spectrophotometer Model Hitachi U 1800)	1.82
Day -1	1.51
Day -2	1.54

CONCLUSION

From the above data it can be concluded that all validation parameters (like method precision, accuracy, linearity) met the predetermined acceptance criteria.

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