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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 succesfully 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 abillity 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.

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MATERIALS AND METHODS

Chemicals and reagents
Promethazine HCl working standard was a generous gift from Dong Gang City Hong Da Pharamceutical 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 NaH_2PO_4 and 0.2g KH_2PO_4 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