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A reversed-phase high performance liquid chromatographic method for determination of Epinephrine in pharmaceutical formulation

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Abstract

A simple, specific, sensitive, precise, and accurate high performance liquid chromatography method was developed for the determination of Epinephrine in pharmaceutical injection forms. Epinephrine is a catechol ($C_6H_6O_2$) group containing secondary amine substance[1]. The method was carried out on reverse phase C-18 column (Luna Phenomenex) (250×4mm, 5µm particle size) using a mixture of water: methanol: acetic acid in the ratio of 85:10:5 (pH adjusted to 3.1 with ammonium acetate) as mobile phase. 3,4 Dihydroxy benzylamine was used as internal standard. The detection was carried out by UV-detector at 280 nm. The calibration curve was found to be linear in the range of 10ng/ml to 100ng/ml. The intra-day and inter day RSD for 60 ng/ml was found to be 0.123 and 0.685 respectively.

Key words: HPLC, Epinephrine, Pharmaceutical Formulation.

INTRODUCTION

Epinephrine chemically known as 4[1R]-1-Hydroxy -2 –(methylamine) thyl]-1,2-benzenediol is monoamines linked to a benzene ring with two vicinal hydroxyl groups (Catechol group)[2]. They are endogenously synthesized from amino acid precursors by metabolic pathways in mammalian cells that usually involve decarboxylation of the parent amino acid[3]. Similarly, they can be generated exogenously also in the intestinal tract by bacteria-induced decarboxylation of amino acids released by the enzymatic hydrolysis of dietary proteins. Also several methods are involved to synthesize epinephrine and like catecholamines [4].

Epinephrine (adrenaline) stimulate the metabolism in the cells, lead to a higher rate of fat and glycogen conversion which altogether leads to more efficiency of the body for physical work [5]. The effect of Epinephrine is mainly an increased blood pressure [6]. The plasma levels of catecholamines and their metabolites are required for the evaluation of neuroendocrine disorders and the role of the autonomic nervous system in several physiological and pathological situations [7].

The concentration of the major catecholamines Norepinephrine (NE), Epinephrine (E) and dopamine (DA) in biological tissues is modified by physiological factors and influenced by pharmacological agents [8]. It is therefore essential to be able to elucidate its variation in quantitative chemical tests and in turnover studies. Thus, CA profile measurement is of clinical importance as a diagnostic tool in clinical test laboratories [9].Epinephrine generally available in injection form is used to treat emergency treatment of severe allergic reactions (including anaphylaxis) to insect bites or stings, medicines, foods, or other substances. So, its Injection form must meet the purity aspects.

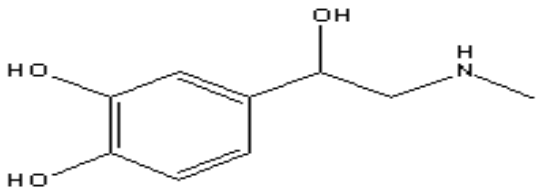

Fig.1. Structure of Epinephrine (Adrenaline)

HPLC methods are most reliable and most accurate methods as compared to other analytical techniques in order to measure the Epinephrine. Literature survey reveals the Existing Methods for Measurement of Catecholamine Concentration are LC/LC-MS methods including HPLC-Electrochemical detector and HPLC-Photodioade array detector, HPLC-Florescence detector from biological samples (as plasma, urine, milk) and pharmaceutical dosage forms[10-13]. Other methodology generally adopted are RIA (Radio Immune Assay) and ELISA (Enzyme linked immuosorbent assay) [14]. RIA method is accurate but it is expensive and chance of radio isotope contamination in concern of health hazards. Also Radio safety level regarding Govt. regulation is required to follow. ELISA method is commonly employed for urinary metanephrine [15]. The objective of the present work was to develop a simple, efficient and reproducible method for quantitative determination of epinephrine in pharmaceutical preparations.

MATERIALS AND METHODS

Reagents and chemicals

All solvents were of HPLC grade and reagents were analytical grade. Acetic acid and acid washed alumina were obtained from CDH, methanol and Ammonium acetate were obtained from Qualigens fine chemicals. Standard epinephrine and 3, 4 Dihydroxy Benzyl amine were obtained from Sigma–Aldrich (St Louis, MO, USA). Injection formulation of Epinephrine was obtained from Rathi Labs Hindustan (P) Ltd. Patna. All the solvents and solution were filtered through Cellulose nitrate membrane filter (size 0.45µm) and sonicated before use.

Instrumentation

Quantitative estimation was performed on isocratic high performance liquid chromatography system (HPLC Shimazdu,) with C18 column reverse phase (Column-C18 (2) (Phenomenex Luna Column, particle size 250X4.6MM) and UV detector. A guard column (Phenomenex Co.USA) and Rheodyne injection valve with 20 µl loop were used. The HPLC system was equipped with Data acquisition and analysis unit-DS-200 (USA).

HPLC conditions

The eluting mobile phase was mixture water: methanol: acetic acid in the ratio of 85:10:5 (pH adjusted to 3.1 with ammonium acetate) as mobile phase. Flux was maintained 1.0 ml/min. The UV detection was made at 280 nm and all analyses were done on isocratic conditions HPLC system.

Standard solution preparation

A stock solution of the drug (Epinephrine Injection) and internal standard were prepared by dissolving 1ml of 1 mg/ml epinephrine and 1mg of 3,4 DHBA in 100 ml volumetric flask and final volume was maintained using mobile phase. Standard solutions of E and DHBA were prepared by suitable dilution of stock solution with mobile phase.

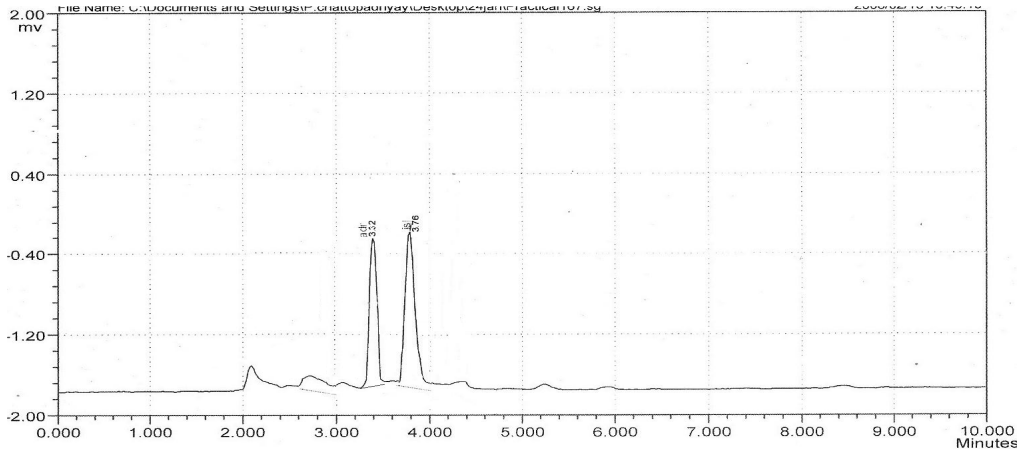

Fig.2. Chromatogram representing peak of Epinephrine and DHBA(Internal standard)

Sample preparation

Samples (1mg equivalent) of different commercial samples of were pipette out accurately, dissolved in and made up to 100 ml with a solution in water. The resultant solution was diluted 10-fold with mobile phase prior to analysis. From that peak area, the drug content in the injection was quantified.

Calibration curves

Ten sets of standard solution were prepared in mobile phase containing Epinephrine at a concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ml along with a fixed concentration of DHBA as internal standard. Each of these drug solutions (20 µl) was injected by Hamilton syringe in triplicate into the chromatographic system. The peak area and retention time was recorded, mean value of peak areas were plotted against different concentrations.

RESULTS AND DISCUSSION

The present method was developed to quantify and access the purity of Epinephrine in pharmaceutical dosage form. In order to develop an efficient method for analysis of drug in pharmaceutical formulation, several trials were made for the Parameters such as wavelength, mobile phase composition, optimum pH and concentration of the standard solutions. The results of study showed that the proposed RP-HPLC method is simple, precise and accurate. It will be useful for the determination of Epinephrine in its pharmaceutical dosage forms.

The drug content was analyzed and found to be 98.76 % of amount claimed (average). The proposed method is simple and less time consuming for sample preparation in comparison to methods as HPLC-Amperometric and HPLC-Florescence detector which requires complicated sample pretreatment procedure. The result of assay of Epinephrine injection are in table 1.

Table 1: Assay of Epinephrine injection by the proposed HPLC method

Labeled Claim (mg/ml)	Observed* (mg/ml)	% Purity
1	0.988±0.0012	98.8

* *mean of three values*

The retention time (RT) of E and Internal Standard were found 3.32 and 3.76 min respectively. The calibration curve showed linearity over a concentration range from 10 to 100 ng/ml. It was found linear with a correlation coefficient (r^2) of 0.9977, the representative linear regression equation being Y= 342.6X + 253.33 (fig. 2).

Best resolution of peak was found in water: methanol: acetic acid in the ratio of 85:10:5 (pH adjusted to 3.1 with ammonium acetate) as mobile phase and flow rate 1.0 ml/min, after several trials with different composition of mobile phase and different Ph range.

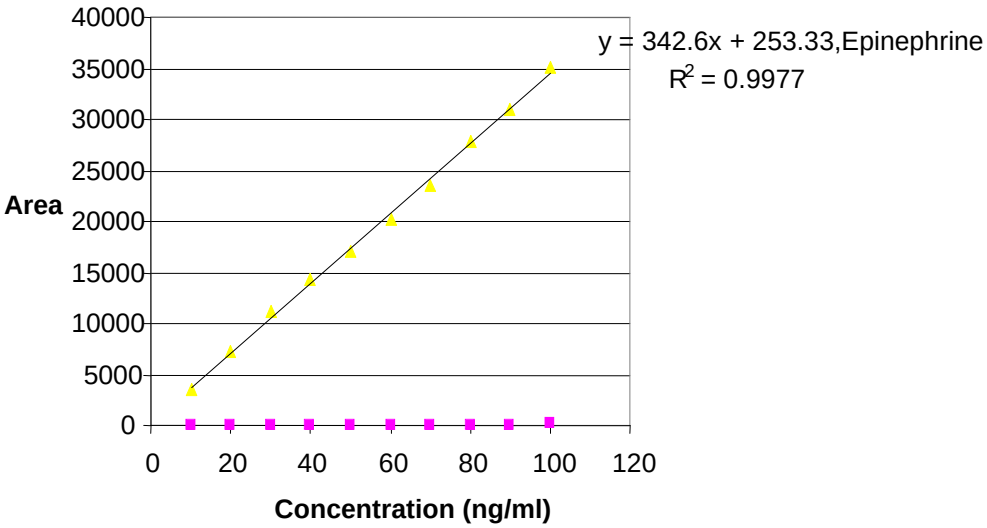

Fig.3 Calibration Curve of Epinephrine (Standard)

The accuracy and precision study were also performed which indicated statistically acceptable result. Recovery test was performed in triplicate and average recovery was found 99.08 %, indicating that the proposed method for the estimation of Epinephrine is highly accurate.

Method Validation

The developed method was further validated as per ICH guidelines under the points as Accuracy, Method precision, Intermediate precision, Limit of detection (LOD) and limit of quantitation (LOQ), ruggedness [16]. The study for ruggedness was done as performed by two different analysts. The results of estimation by proposed method are very much similar under variety of conditions. The assay results of Epinephrine in injection dosage forms were comparable with the value of labeled claim. The obtained results are shown in [Table 1]

Intra and inter-day RSD was found to be 0.123 and 0.685 respectively for assay of Epinephrine in pharmaceutical dosage form by developed method (table-3).

Table 2: Inter and intra-day Relative standard deviation for Epinephrine assay in pharmaceutical dosage forms by the developed RP-HPLC method.

Conc. of E in (ng/ml)	Observed conc. of E* (%Relative standard deviation)	
	Intra-day	Inter-day
60	0.123	0.685

** mean of three values*

The intra- and inter-day precisions were performed with concentrations 60 ng/ml for the Epinephrine estimation. The values of %RSD revealed that developed method is correct.

For the accuracy study, standard addition method was employed. The known amount of reference standard of Epinephrine was incorporated to pre analyzed sample of epinephrine. The result obtained indicated accuracy of method. During the process of study, the CO_2 ice pack was used to preserve the sample till analysis to avoid degradation of samples. Each analysis was done in triplicate. The result of accuracy study is presented in table 2.

For precision study of the instrument, fixed concentration of E (50ng/ml) was injected (n=5) by repeating and relative standard deviation was found to be 0.1712. The LOD and LOQ were found to be 0.1097and 0.6077 ng/ml for E.

CONCLUSION

As the quality control aspects are concerned, the developed method may be employed to evaluate purity and to quantify the epinephrine in Pharmaceutical injection form. The validation study reveals that method is accurate, precise, and quick. Thus this method may be used in routine analysis of Epinephrine injection formulation.

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