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Preparation and evaluation of molecularly imprinted polymer liquid chromatography column for the determination of enantiomeric impurity of l-Ephedrine sulfate-validated methods according to the ICH guidelines

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ABSTRACT

A sensitive, simple, specific, precise, accurate and rugged method for determination of enantiomeric impurity of l-Ephedrine Sulfate (l-EPS) a drugs substance has been developed. The separation of enantiomer is by molecularly imprinted polymer (MIP) column. In this study molecular imprinting technology was employed to prepare a specific affinity sorbent for the resolution of Ephedrine Sulfate (commonly used as a decongestant). The separation of isomer is by molecularly imprinted polymer (MIP) column 250mm × 4.6mm with 10µ particle size. Column was maintained at 10°C. The UV/Vis detector was operated at 254nm. Flow rate of the mobile phase was 0.8ml/min. The method offers excellent separation of two enentiomers with resolution (R) is not less than 2 and tailing factor not more than 3.5. The method was validated for the quantification of d-Ephedrine enantiomer impurity (d-EPS) in the bulk drug l-Ephedrine Sulfate (l-EPS), was performed according to the International Conference on Harmonization (ICH) guidelines[1-4]. Calibration curves showed excellent linearity over the concentration range 0.025mg/ml to 0.1mg/ml (50% to 200 %) for d-EPS 0.05mg/ml (0.5% with respect to test concentration 10mg/ml). Precision of the method the relative standard deviation was 0.99% limit of detection (LOD) and limit of quantification (LOQ) of the method for d-EPS were 0.2% and 0.5% respectively. Average recovery of the d-EPS was between 87.3% to 107.8%. This method was employed in determining enantiomeric impurity of bulk drug l-EPS.

KEYWORDS

Enantiomer;
Enantiomeric impurity;
Molecularly imprinted polymers (MIP);
l-Ephedrine Sulfate (l-EPS);
d-Ephedrine Sulfate (d-EPS).

INTRODUCTION

Ephedrine is a sympathomimetic amine commonly used as a stimulant, appetite suppressant, concentration aid, decongestant, and to treat hypotension associated with anaesthesia. Ephedrine is similar in structure to the synthetic derivatives amphetamine and methamphetamine. Chemically, it is an alkaloid derived from various plants in the genus *Ephedra* (family Ephedraceae). It is most usually marketed in the l-Ephedrine hydrochloride and sulfate forms, Active pharmaceutical ingredient (API)[5,6].

A stimulant used in the treatment of asthma and respiratory ailments. *Ephedra sinica*, a herb containing

ephedrine, is included in some herbal supplements used by athletes and others to accelerate fat loss and enhance feelings of physical well-being. These supplements are sometimes promoted as 'fat burners'. There is no conclusive scientific evidence that ephedra-containing supplements improves athletic performance.

CHEMISTRY

Ephedrine exhibits optical isomerism and has two chiral centres. By convention the enantiomers with opposite stereochemistry around the chiral centres are designated ephedrine, while pseudoephedrine has same stereochemistry around the chiral carbons. That is, (1R, 2R)- and (1S, 2S)-enantiomers are designated pseudoephedrine; while (1R, 2S)- and (1S, 2R)-enantiomers are designated ephedrine. The isomer which is marketed is (-)-(1R, 2S)-ephedrine. As with other phenylethylamines, it is also somewhat chemically similar to methamphetamine, although the amphetamines are more potent and have additional biological effects[7,18,19].

Ephedrine may also be referred to as: (αR)-α-[(1S)-1-(methylamino) ethyl] benzenemethanol, α-[1-(methylamino)ethyl]benzyl alcohol, or L-erythro-2-(methylamino)-1-phenylpropan-1-ol[15]. Ephedrine sulfate has a melting point of 248°C-252°C, $(C_{10}H_{15}N_O)_2$. H_2SO_4 (2:1 salt) CAS No. [134-72-5], Molecular weight 428.54[8,10,12].

Manufacture

Although Ephedrine occurs naturally as an alkaloid in certain plant species (for example, as a constituent of extracts from the ephedra species, also known as Ma Huang, in which it occurs together with other isomers of ephedrine)[17,18,19], the majority of Ephedrine produced for commercial use is derived from yeast fermentation of dextrose in the presence of benzaldehyde. In this process, specialized strains of yeast (typically a variety of Candida utilis or Saccharomyces cerevisiae) are added to large vats containing water, dextrose and the enzyme pyruvate decarboxylase (such as found in beets and other plants, inter alia). After the yeast has begun fermenting the dextrose, the benzaldehyde is added to the vats and in this environment the yeast convert the precursor ingredients to l-Phenylacetylcarbinol (l-PAC). l-PAC is then chemically converted to ephedrine via reductive amination. The bulk of Ephedrine is produced by commercial pharmaceutical manufacturers in India and China, where economic and industrial conditions favor the mass production of Ephedrine for export.

This article describes developments and validations of a new analytical method, which can detect quantify trace levels of d-EPS in l-EPS.

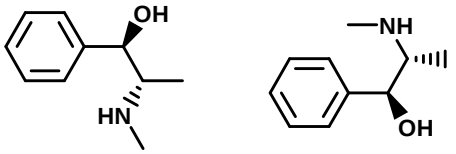
EXPERIMENTAL

Materials

Methacrylic acid (99%), ethylene glycol dimethacrylate (EGDMA, 98%) and 2,2'-Azobisisobutyronitrile (AIBN) were obtained from Merck (Germany). Acetone, Methanol, Chloroform and Acetic acid chromatography grade were from Merck India. l-Ephedrine Sulfate and d-Ephedrine Sulfate were synthesized by Medicinal Chemistry group. Both the compounds were characterized[5] for their identity and purity, their enantiomeric purity was monitored by specific optical rotation using polarimeter and were purified till equal opposite values for specific optical rotation were obtained.

Synthesis of molecular imprinting stationary phase

The MIP stationary phase was prepared by bulk polymerization at a low temperature[16]. Briefly, l-Ephedrine Sulfate 100mg, methacrylic acid (0.25ml), EGDMA (2.5ml), and AIBN (25mg) were dissolved in 5 ml of chloroform in a glass vial. After degassing and nitrogen purging for 5 minutes the vial was sealed and allowed to polymerize at 5°C for 6 h under UV (365nm, 100 W lamp) irradiation. For each preparation of MIP, either (+)-Ephedrine sulfate or (-)-Ephedrine sulfate was used as the template. Methacrylic acid was used here as the functional monomer, EGDMA as the crosslinking monomer and AIBN as the free radical initiator. After polymerization, the chloroform was removed. The product in the form of a white solid was dried in a vacuum oven for 7 hours at room temperature. The resultant bulk polymer was finally ground and sieved. The fraction of particles having size of 10μ was collected for packing in chromatographic columns.



(1R, 2S)-2-(Methylamino) -1-phenylpropan-1-ol (1S, 2R)-2-(Methylamino) -1-phenylpropan-1-ol

Figure 1 : Chemical structures of (-)-Ephedrine and (+)- Ephedrine

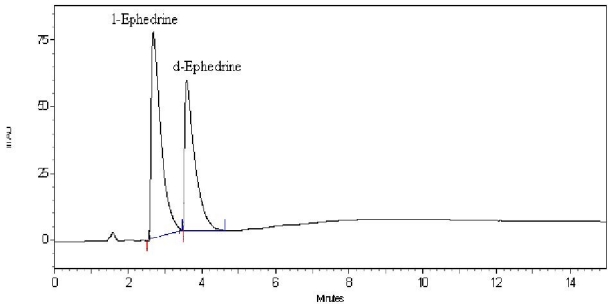


Figure 2 : HPLC Chromatogram of l-Ephedrine (l-EPS) and d-Ephedrine (d-EPS). Chromatograms with peak of l-EPS, Retention time 2.8 minutes and d-EPS, Retention time of 3.8 minutes

Liquid chromatography

HPLC system used was Shimadzu 2010 AHT and Agilent 1200 series. Shimadzu system comprised of degasser, quaternary pump, auto injector, column oven and UV/Vis detector. The signal was acquired and processed using LC-solution software and Agilent-1200 series system comprised of degasser, quaternary pump, auto injector, and column compartment and variable wavelength detector. The system was controlled through EZ Chrome Elite version 3.2.1.

MIP particles were suspended in methanol by sonication and then slurry packed into 25cm×0.46cm ID. stainless steel columns using an air-driven fluid pump with acetone as the solvent. The back- pressure for packing was 350 bar. Template molecules were removed from the columns by continuously washing with methanol-acetic acid (8:2, v/v) until a stable baseline was reached. For the HPLC analysis, a 20µL sample solution was injected and eluted isocratically at a flow-rate of 0.8ml/min, using a mixture of water, acetic acid and methanol (2:7:91v/v) as the mobile phase. The temperature was kept at 10°C. The effluent solution was constantly monitored by measuring the absorbance at 254nm.

Preparation of solutions and chromatographic conditions

Preparation of solutions

System suitability solution (SS)

Accurately weigh about 100mg each of l-Ephedrine Sulfate and d-Ephedrine Sulfate into a 10ml volumetric flask, dissolve and dilute to volume with mobile phase.

Reference solution

Stock impurity solution

Accurately weigh 50mg of d-Ephedrine Sulfate in to a 100ml volumetric flask, dissolve and dilute to volume with mobile phase.

Reference solution R

Accurately weigh about 100mg of l-EPS in a 10ml volumetric flask, add 1ml of the Stock impurity solution dissolve and dilute to volume with mobile phase and shake well.

Test solution

Accurately weigh about 100mg of the test (l-EPS) in a 10ml volumetric flask, dissolve and dilute to volume with mobile phase and inject.

System suitability

Performance of the method was determined by injecting System suitability solution (SS) resolution mixture (10mg/ml of l-EPS and 10mg/ml of l-PSE). Method performance criteria were resolution (R) between two entiomer peaks should be not less than 2.0, tailing factor (TF) not more than 3.5.[9,11,13,15].

Linearity

Linearity of response for d-EPS was determined in the range of 0.025 to 0.1mg/ml (50% to 200% of the limit concentration (0.05mg/ml), 0.5% with respect to test concentration 10mg/ml. The % RSD for linearity solution is not more than 15.0% (L1 – L5).There should be no non-linear trend at the ends of the plotted fitted line. Correlation coefficient (R^2) is NLT 0.99[1-3,15].

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ of d-EPS was determined by Signal-to-Noise method. Solutions of enantiomer was prepared in the range of 0.2% and 0.5% with respect to test respectively and injected in six times. The % RSD

for LOQ solution must not be higher than 15.0%. The signal-to-noise ratio (S/N) in LOQ solution should be about 10:1 for d-EPS. The signal-to-noise ratio (S/N) in LOD solution should be about 3:1 for d-EPS[1-3,15].

Precision and accuracy and ruggedness

Precision of the method was determined by injecting six different test preparations and determining % RSD of impurity (d-EPS) values. Accuracy of the method was determined by recovery studies. d-EPS was spiked in pre-analyzed test of l-EPS and its percent recovery was determined. Ruggedness of the method was determined by performing quantification of d-EPS on two different HPLC systems (HPLC system used was Shimadzu 2010 AHT and Agilent 1200 series) and columns by two analysts[1-3,15].

RESULTS AND DISCUSSION

The objective of this work was to develop a precise and accurate method to determine enantiomeric purity of l-EPS. Various options were attempted to develop such method. Resolution of 2.7 was obtained by using Mobile phase consist of a mixture of water, acetic acid and methanol (2:7:91v/v) as the mobile phase with molecularly imprinted polymer (MIP) column 250mm × 4.6mm with 10µ particle size[6]. Column was maintained at 10°C. The UV/Vis detector was operated at 254nm. Flow rate of the mobile phase was 0.8ml/min. This method was further validated for estimation of d-EPS (enantiomeric impurity) in l-EPS bulk drug, gives acceptable resolution required to quantify presence of d-EPS in l-EPS.

A representative chromatogram showing resolution of enantiomers is shown in figure 2. An excellent resolution (R = 2.5) between the two peaks along with tailing factor (TF) for l-EPS and d-EPS was 2.7 and 3.3 respectively.

The described method was found to be linear for d-EPS in the range of 0.025 to 0.1mg/ml (50% to 200% of the limit concentration i.e. 0.05mg/ml (0.5%). The % RSD for linearity solution is between 10% to 13.5% respectively. There is no non-linear trend at the ends of the plotted fitted line, Correlation coefficients (R2) was 0.99. LOD and LOQ for d-EPS was determined by Signal-to-Noise method. LOD and LOQ for d-EPS was found to be 0.2 % (S/N = 3:1) with respect to l-EPS drug matrix and 0.5 % (S/N = 11:1) with respect to l-EPS drug matrix respectively. The % RSD for LOQ solution is 13.6%. The method found to be Precise for the impurity d-EPS detected %RSD = 10.45%, accurate for the amount spiked and amount found of d-EPS are shown in TABLE 1 and rugged as content of d-EPS was did not deviate significantly on two systems with overall relative standard deviation 8.5%.

TABLE 1 : Recovery of d-EPS

Amount added (%)	Amount found (%)	Recovery (%)	Mean	SD	RSD (%)
0.204	0.178	87.3	90.4	6.69	7.4
0.408	0.44	107.8	97.2	9.9	10.19
0.51	0.45	88.2	92.8	6.87	7.40
0.816	0.73	89.5	91.9	6.5	7.1
1.02	1.1	107.8	94.8	11.3	11.9

SD: Standard deviation, RSD: Relative standard deviation

CONCLUSION

The demands of the pharmaceutical industry require to develop a suitable chiral separation because most of the active pharmaceutical ingredients (API) are chiral in nature. Enantiomerically pure drug compounds will be one of the criteria for APIs to be accepted by the US Food and Drug Administration (FDA).

The increasing demand for optically pure drugs has resulted in an interest in developing tools for efficient chiral separation. In the present study we used a simple molecule, ephedrine sulfate as the template for the preparation of MIP chromatographic column packed with MIP particles were effective for the resolution of ephedrine enantiomers. The recognition and binding of template molecules was based on interactions between amino and hydroxyl groups of the template and the carboxyl group of methacrylic acid, a host molecule in the MIP. An excellent resolution obtained in the present work suggest that molecular imprinting is a promising method for the separation and quantification of chiral compounds, and support the application of pharmaceutical analysis.

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