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A VALIDATED STABILITY-INDICATING HPLC ASSAY METHOD FOR EPINASTINE HCl IN BULK DRUG

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ABSTRACT

A gradient reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Epinastine hydrochloride in bulk drugs. A gradient reversed phase HPLC method was developed to separate the drug from the degradation products, using an Kromasil C18 (250 x 4.6) mm, 5 u column and the mobile phase containing 0.01 M KH_2PO_4 and buffer pH 5.2 with phosphoric acid and acetonitrile. The detection was carried out at wavelength 254 nm. The Epinastine hydrochloride was subjected to stress conditions of hydrolysis (acid, base), oxidation (30% H_2O_2) and thermal degradation. The degradation was observed for Epinastine hydrochloride in acid, base and 30% H_2O_2 and negligible degradation observed in thermal hydrolysis. The mass balance was close to 100 in all the stress conditions. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Key words: Epinastine hydrochloride, Stability indicating, RP-HPLC and Validation.

INTRODUCTION

Epinastine hydrochloride is described chemically as (RS)-3-amino-9, 13 b-dihydro-1H-dibenz (c, f) imidazo (1,5-a) azepine, is an antihistamine that is used in eye drops to treat allergic conjunctivitis. Trade named Elestat and Relestat[1,2]. Literature survey reveals that high performance liquid chromatographic methods were reported for the determination of Epinastine in bulk drugs and dosage form[3,4]. We are gratified to report a stability indicating HPLC method for the analysis and separation of drugs from the degradation products formed under ICH suggested conditions hydrolysis, oxidations, and thermal stress. In present article, reversed phase HPLC method was developed for the separation of Epinastine in bulk drug and the impurities formed from its forced degradation under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat[5,6].

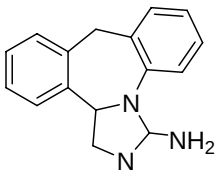

Fig. 1: Chemical structure of Epinastine HCl

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EXPERIMENTAL

Material and reagents

Epinastine hydrochloride bulk drug was made available from Merck Ltd. India (purity 99.8%). Orthophosphoric acid, hydrochloric acid was obtained from Qualigens fine chemicals, India Limited. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent used were of HPLC grade; milli-Q-water was used throughout the experiment.

Chromatographic conditions

A chromatographic system (Systronic) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and UV detector 10A-VP series with IRIS32 Plus software. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Inertsil ODS C18 stationary phase with particle size 5 micron and pore size 100 Å was used. The instrumental settings were a flow of 1 mL/min; the injection volume was 20 µL.

Mobile phase

The mobile phase contains the 0.01 M KH_2PO_4, buffer pH 5.0 and acetonitrile. The buffer was filtered through a 0.45 µm nylon filter and degassed.

Preparation of standard stock solutions

Standard stock solutions of 200 ppm of Epinastine hydrochloride in acetonitrile and water (60 : 40) were prepared in volumetric flasks. Working solutions were prepared by diluting the solutions with the same solvent.

Sample solution

Eye drop (50 mg) accurately weighed into a 100 mL calibrated dark flask contain acetonitrile and water mixture (1 : 1). The content of the flask was shaken few minutes and diluted to volume with same solvent. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure[7,8].

Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for Epinastine hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Epinastine hydrochloride bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.1 N Hydrochloric acid), alkali (0.02 N NaOH, hydrogen peroxide (30%), and heat (60^{o}C) to evaluate the ability of the proposed method to separate Epinastine hydrochloride from its degraded products. For thermal degradation, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Epinastine hydrochloride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated (Table 1).

Table 1: Summary of forced degradation results

Stress condition	Time	Assay of active substance %	Remarks
Acid hydrolysis (0.5 N HCl)	48 hrs	99.81	No degradation
Base hydrolysis (0.025 N NaOH)	2 hrs	88.36	Degradation
Oxidation (30% H_2O_2)	48 hrs	99.67	No degradation
Thermal (80°C)	7 days	99.67	No degradation

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of Epinastine hydrochloride from bulk drug and which will be also applicable for the degradable products[9]. Initially, efforts were made for the development of HPLC method quantification of standard Epinastine hydrochloride from bulk. For this purpose, Kromasil C18 (250 x 4.6) mm, 5 µ column, Water nova pack C18 (150 x 4.6) mm, 5 µ, Kromasil C18 (150 x 4.6) mm, 5 µ, Inertsil ODS 3V C18 (250 x 4.6) mm, 5 µ and Kromasil C18 (250 x 4.6) mm, 5 µ, Star ODS-II C18 (250 x 4.6) mm, 5 µ and Grace Alpha C18 (250 mm x 4.6) mm, 5 µ. Out of these used HPLC column, Kromasil 100 C18 C18 (250 x 4.6) mm, 5 µ, column found to comparatively better and gave the graph with better Gaussian shape at retention time 9.167 min. To improve the shape and width of the graph, for the above columns different solvents and buffer trials were carried with 0.2 M KH_2PO_4 and acetonitrile (60 : 40 v/v) in these trials if peak shape were not good enough, another trials were carried with water and acetonitrile (20 : 80 v/v) peak shape, were not good enough then trials with acetonitrile and water (80 : 20, v/v) with column temperature 35°C, again if peak shape were not found good, trials with K_2HPO_4, methanol and water (10 : 70 : 20, v/v/v) column temperature 35°C, trials 1.0 g KH_2PO_4 and 0.45 g 1-hexa sulphonic acid sodium salt make pH-3.5 Ortho phosphoric acid and methanol (25 : 75, v/v) peak shape obtained but retention is not good, finally trials for mobile phase-A 0.1 M KH_2PO_4 and mobile phase –B acetonitrile were carried.

Method validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table 2.

Table 2: System suitability reports

Compound (n = 3)	Retention time	% RSD	USP	Theoretical plates
Epinastine HCl	9.98	0.44	1.02	6432

Precision

The precision of the method was studied by determining the concentrations of the drug Epinastine hydrochloride in the tablet for six times[10]. The results of the precision study (Table 3) indicate the reliability of the method (RSD % < 2).

Table 3: Results of the linearity study and precision

Ingredient	Precision (% RSD)	Linearity (µg/mL)	Slopes* (n = 3)	Coefficients of correlations
Epinastine HCl	0.44	80-120	4965.4	0.99963

*Standard deviation shown in parentheses

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Epinastine hydrochloride ranged from 100.10% to 101.17% (Table 4). The average recoveries of three levels nine determinations for Epinastine hydrochloride were 100.22-100.21%.

Table 4: Results of the recovery tests for the epinastine HCl

Level of addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	50	100.10	100.22
100	100	100.52	100.40
120	150	101.17	100.21

* RSD shown in parenthesis.

^ Average recovery = the average of three levels, nine determinations

Calibration and linearity

Linearity test solutions for the method were prepared from Epinastine hydrochloride stock solutions at six concentrations levels from tested from 80% to 120% of the targeted level of the assay concentration. Standard solutions containing 80-120 µg/mL of Epinastine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear (0.999) in the mentioned concentrations.

Table 5: Results of the LOD and LOQ

Name	% LOD	% LOQ
Epinastine HCl	0.13	0.27

Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Epinastine hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL/min while the other mobile phase component was held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in Table 6.

Table 6: Results of robustness study

S. No.	Parameters	Variations	Resolutions between Epinastine HCl and base degraded product
1	Temperature	at 25 °C	8.21
		at 35 °C	7.68
2	Flow rate	0.8 mL/min	8.02
		1.2 mL/min	8.94
3	Mobile phase	40.5 mL	3.7
		49.5 mL	3.3

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.2-1.0% of the assay concentration (40 μg mL^{-1}) were prepared by dilution of the standard solutions. Each solution (20 μL) was injected five times, the areas were measured for the drug peak, and the standard deviation for the five injections was calculated for each concentration. On the basis of data obtained, the standard deviation at concentration 0 was calculated and this value was used for calculation of the LOD and LOQ. The results are shown in Table 5.

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Epinastine hydrochloride was 0.35 %. The assay values were within + 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSION

The method developed for quantitative determination of Epinastine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method was stability indicating and can be used for assessing the stability of Epinastine hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Epinastine hydrochloride in bulk drugs and pharmaceutical dosage form.

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