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Determination of Genotoxic Alkyl Paratoluene Sulfonates in Brinzolamide Using RP-LC

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ABSTRACT

Highly sensitive method for the determination of genotoxic impurities such as Alkyl Para Toluene Sulfonates (APTSs) in brinzolamide using RP-LC has been presented in the present paper. APTSs were determined by RP-LC method using Waters Symmetry C18 (250 × 4.6 mm), 5 µ column as stationary phase. Column temperature maintained 30°C, injection volume 20 µl, flow rare was 1.5 ml/min, sample cooler temperature 5°C and run time was 30 min. pH 2.50 phosphate buffer is used. The mixture of buffer and acetonitrile in 50:50 (v/v) was used as mobile phase. The method validation has been carried as per International Conference on Harmonization guidelines. Limit of Quantitation (LOQ) was found in the range of 1.1-2.2 µg/ml for APTSs.

Keywords: Genotoxic impurities, Brinzolamide, RP-LC method, Validation and limit of quantitation

INTRODUCTION

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose [1]. These limits generally fall at low µg/ml levels. High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances [2]. Brinzolamide (BRZ), chemically (R)–4-(ethyl amino)-3,4-dihydro-2-(3-methoxy propyl)-2Hthienol[3,2-e]-1,2-thiazine-6 sulphonamide 1,1-dioxide (Figure 1), a non-competitive reversible carbonic anhydrase inhibitor is indicated for the treatment of elevated intraocular pressure in patients with glaucoma [3]. In the manufacturing process of BRZ, Methane Sulfonic Acid (MSA) and Para Toluene Sulfonic Acid (PTSA) are used as reagents and three alcohols (viz. ethanol and isopropanol) are used as solvents and hence genotoxic Methyl Paratoluene Sulfonate (MPTS) (Figure 2) and Iso Propyl Para Toluene Sulfonate (IPPTS) (Figure 3) may exist as impurities in brinzolamide drug substance. Based on maximum daily dose of BRZ (1.62 mg/day), these are to be controlled at a limit of 5 µg/ml.

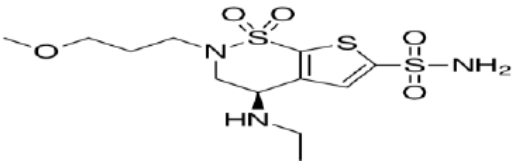

Figure 1: Chemical structure of brinzolamide

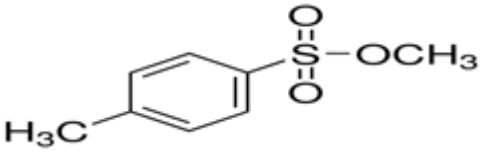

Figure 2: Chemical structure of methyl para toluene sulfonate

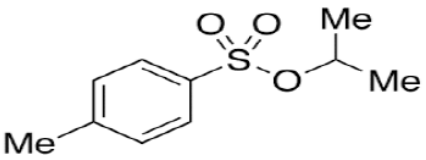

Figure 3: Chemical structure of isopropyl para toluene sulfonate

In literature, very few analytical methods such as spectrophotometric methods including derivative and simultaneous spectrophotometric methods [4-7] and RP-HPLC methods [8,9] were reported for the simultaneous determination of brinzolamide. Existing literature reported the analytical methods using hyphenated techniques for the determination of APTSs [10-12]. However, no analytical method was reported for the determination of APTSs in BRZ. Hence the author was aimed towards the development of rapid, specific and robust methods for the determination of APTSs in BRZ at trace level concentration.

EXPERIMENTAL

Chemicals and reagents

Methyl Para Toluene sulfonate (MPTS) purchased from Aarti Drugs Ltd., Mumbai, India. Potassium dihydrogen ortho phosphate and acetonitrile were procured from Merck, India. Isopropyl para toluene sulfonate and pure samples of brinzolamide were obtained from synthetic division of Century pharmaceutical Ltd. (R&D), Vadodara, and Gujarat, India.

Preparation of stock solutions

Acetonitrile was used as diluent in the present method. MPTS and IPPTS stock solutions were prepared by dissolving 20 mg each individually in 100 ml of diluent. Further diluted 10.0 ml of this into 100 ml with diluent. The mixture solution, 80 µg/ml with respect to 25 mg/ml of brinzolamide, was prepared by diluting the appropriate volumes of above stock solutions with diluent as above. A blend solution was also prepared by spiking 80 µg/m of APTSs to 25 mg/ml of brinzolamide and is used for method development.

Chromatographic conditions

RP-LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) wavelength 220 nm. Waters Symmetry C18 250 × 4.6 mm, 5 µ column was used as stationary phase. The mixture of pH 2.50 phosphate buffer and Acetonitrile in the ratio of 50:50 (v/v) was used as mobile phase. The flow rate of the mobile phase was kept at 1.5 ml/min. The injection volume was set as 20 µ. Column oven temperature and auto sampler temperature were set as 30°C and 5°C, respectively.

RESULTS AND DISCUSSION

Method development

A blend solution containing APTSs and brinzolamide was run in 1.0 mL/min flow rate. Brinzolamide eluted too extended and hence the flow rate of the mobile phase was increased from 1.0 ml/min to 1.5 ml/ min. In this condition brinzolamide eluted at an optimum retention time, but the retention times of APTSs were drastically increased. Hence, the elution order was observed from the chromatogram (Figure 4) brinzolamide solution spiked with MPTS (80 µg/ml), and IPPTS (80 µg/ml).

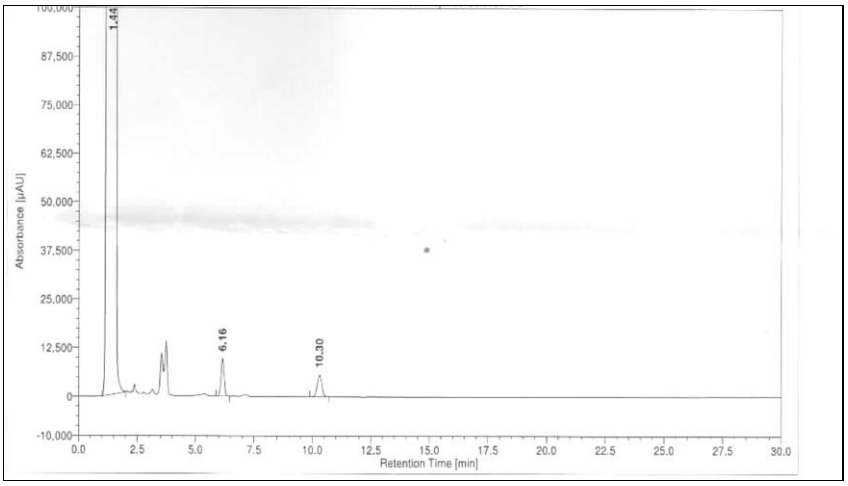

Figure 4: Spiked APTSs chromatogram of Brinzolamide

Method validation

The developed method was validated as per ICH guidelines [13] in terms of specificity, Limit of Detection (LOD), Limit of Quantitation (LOQ), precision, linearity, accuracy, robustness and system suitability and the data are presented in Table 1.

The specificity of the developed LC method was indicated by APTSs solutions (80 µg/ml each) with respect to 25 mg/ml of BRZ were injected separately and S/N ratios were recorded.

These solutions were further diluted to achieve the signal-to-noise (S/N) ratios at about 3 and 10 for determining LOD and LOQ, respectively for both the methods. The precision of the methods was checked by injecting LOQ solutions for six times. The values of RSDs for areas of each APTS were calculated.

Table 1: Validation data of Brinzolamide for the determination of MPTS and IPPTS

Parameter	MPTS	IPPTS
LOD (µg/ml)	0.4	0.7
LOQ (µg/ml)	1.2	2.1
Precision at LOQ level (RSD, %)	2.41	2.08
Precision at sixth level (RSD, %)	0.52	0.66
Intermediate precision at LOQ (RSD, %)	0.65	0.69
Linearity range (µg/ml)	1.2-131	2.1-133
Correlation coefficient	0.999	0.999
Slope	1175	986.7
Intercept	-288.6	-326.9
Accuracy at LOQ (recovery, %)		
Preparation-1	97.8	103.4
Preparation-2	96.3	100.8
Preparation-3	96.8	99.8

The intermediate precision of the method was also verified on six different days in the same laboratory using the LOQ level solutions. The low RSD values ensured the precision of the developed method. Linearity test solutions for APTSs were prepared individually at six concentration levels in the range of LOQ to 150% of the specification level 80 µg/ml. LOQ and sixth levels were injected six times and other four levels were injected thrice. The average peak areas versus concentrations were subjected to least-squares linear regression analysis. The derived correlation coefficients were above 0.9999 indicating the best fitness of the linearity curves of the developed method. Standard addition experiments were conducted in triplicate preparations to determine accuracy of the methods at LOQ level and recoveries of all the genotoxins were determined. The recoveries were found to be in the accepted range. The robustness of RP-LC method was ensure by getting the resolution between any two APTSs to be greater than 2.0, when mobile phase flow rate (1.3 ml/min and 1.7 ml/min), organic solvent ratio in mobile phase (90% and 110%) and column temperature (25°C and 35°C) were deliberately varied. The solution stability of APTSs in diluent in RP-LC method was determined by leaving APTSs mixture solution at specification level in a tightly capped volumetric flask at room temperature for 48 h and measuring the amounts of the APTSs for every 6 h. All the APTSs were found to be stable up to 48 h. The system suitability of the method was ensuring by getting the %RSD less than 10.0 for six injections of all the APTSs in RP-LC method at specification level. Brinzolamide at trace level concentration have been developed and validated as per ICH guidelines.

CONCLUSION

The proposed RP-LC method that can quantify genotoxic alkyl para toluene sulfonates in brinzolamide at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensuring by the specificity, precision, accuracy and robustness. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of brinzolamide into the market.

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