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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND RP- HPLC METHODS FOR THE DETERMINATION OF BRINZOLAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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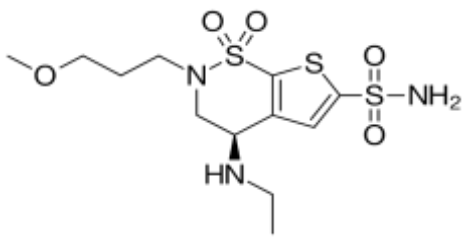
ABSTRACT

A simple, accurate, precise and specific validated stability indicating method was developed for the estimation of the Brinzolamide in ophthalmic suspension form. The chromatographic separation was achieved on STD Phenomenex C18 150 mm (4.6 x 150mm, 5µm) at ambient temperature. The separation was achieved employing a mobile phase consisting of buffer, 0.1% Formic acid – Methanol (65:35v/v) pumped through the column at a flow rate of 1 ml/min and the injection volume of 10µl. UV detector was used to detect the analyte at 254nm. The method performance was validated in compliance with the International Conference on Harmonization. Retention time of Brinzolamide found to be 3.497min. Percentage recovery obtained were 99.88 for brinzolamide respectively. Regression equation of Brinzolamide is y = 20013x-10108. The stability indicating method was performed under various stress conditions and the degradants did not interfere with Brinzolamide.The developed RP-HPLC method was highly specific, precise, sensitive and stability indicating and high resolution obtained makes this method more reliable. Hence the method developed can be adopted in regular quality control tests in industries.

INTRODUCTION:

Modern analytical chemistry generally requires precise analytical measurements at very low concentrations, with a variety of instruments. Therefore, the knowledge of instrumentation used in chemical analysis today is of paramount importance to assure future progress in various fields of scientific endeavour. This includes various disciplines of chemistry such as biochemistry, pharmaceutical chemistry, medicinal chemistry, biotechnology, and environmental sciences. The optimal usage of instrumentation with more meaningful data generation that can be interpreted reliably is possible only with the improved knowledge of the principles of the instrumentations used For measurement as well as those utilized to achieve various separations.

DRUG PROFILE



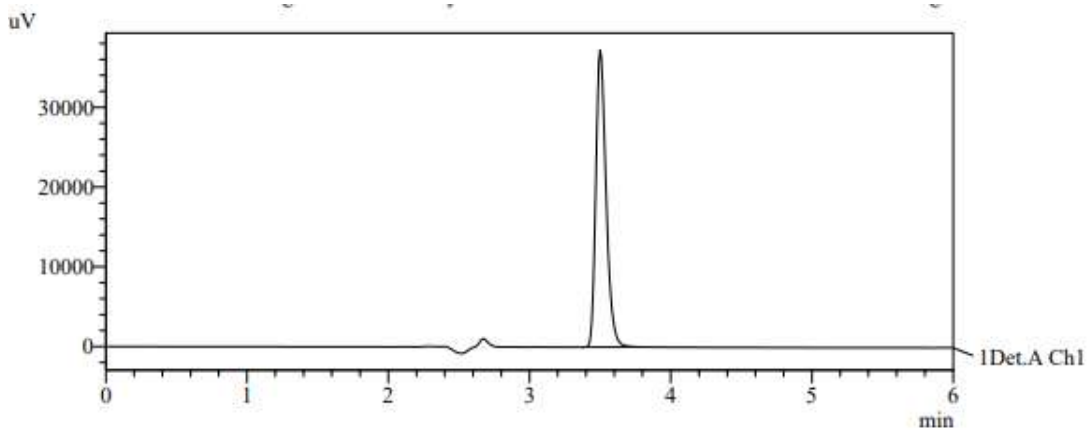
IUPAC NAME [(2)]: (4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-3,4-dihydrothienol[3,2-e]thiazine-6-sulfonamide
Molecular Formula: C12H21N3O5S3
Molecular Weight: 383.5 g/mol

Solubility: Insoluble in water; ≥15.05mg/mL in DMSO; ≥8.82mg/mL in ethanol with gentle warming and ultrasonic
pKa: 5.9 & 8.5
Therapeutic category: Carbonic anhydrase inhibitor
Melting point: 131ºC
Trail 6(Optimized method):
Chromatographic conditions:
Mobile phase : 0.1%Formic acid and Methanol (60:40%v/v)
Flow rate : 1ml/min
Column : PhenomenexC18 (4.6 x 250mm, 5µm)
Detector wave length : 254.0 nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 6min
Diluent : 0.1% formic acid
Results: In this trail, Brinzolamide was eluted with the solvent front and the plate count was within the limits with a retention time of 3.497min. The chromatogram for the optimized method.
Method:
Diluent: Based up on the solubility of the drugs, diluent was selected; 0.1% Formic acid as diluent.
Chromatographic conditions:
During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.
Preparation of 0.1% formic acid buffer: 1ml of formic acid solution was diluted to 1000ml to get 0.1% formic acid.
Preparation of Standard stock solutions: 10mg of Brinzolamide was taken into a dry 10ml volumetric flask. The volumetric flask was filled with mobile phase up to $3/4^{th}$ of the volume and the resulting solution was sonicated for 5 minutes. Then the volumetric flask was made up to the volume with mobile phase and filtered through 0.45µ membrane filter. The filtrate was then used as primary standard stock solution having concentration of1000ppm of Brinzolamide.
Preparation of Standard working solutions: Aliquots were taken from the standard stock solution and working standards were prepared in the concentration range of 2 - 10µg/ml.
Preparation of Sample stock solutions: A marketed Brinzolamide suspension 1%w/v was bought. 1ml of the suspension was taken in a clean dry 100ml volumetric flask and the volume was made up to the mark with HPLC water.
Preparation of Sample working solutions: From the above freshly prepared solution 30µl of filtered sample stock solution was transferred to 5ml volumetric flask and make up with the buffer and vertex was done, 10µl of injection was given.

SUMMARY AND CONCLUSION

Degradation data

Type of degradation	AREA	%RECOVERED	% DEGRADED
Acid	90639	87.9	12.1
Base	91711	88.9	11.1
1% Hydrogen peroxide	101758	98.7	1.3
5% Hydrogen peroxide	103012	99.9	0.1
Thermal	101758	98.7	1.3
Photo	102527	99.4	0.6



S. No	Parameters		Brinzolamide	Limit
1	Linearity Range (µg/ml)		2-10ug/ml	R< 1
2	Regression Coefficient		0.999	R< 1
3	Slope(m)		20013	R< 1
4	Intercept(c)		10108	R< 1
5	Regression Equation(y = mx+c)		y = 20013x-10108	R< 1
6	Assay (%mean assay)		99.88%	90-110%
7	Specificity		Specific	No interference of any peak
8	System Precision (%RSD)		0.4	NMT 2.0%
9	Method Precision (%RSD)		0.6	NMT 2.0%
10	Accuracy (%recovery)		99.46%	98 – 102%
11	Robustness	Flow Rate (0.9ml/min)	0.04	%RSD NMT 2.0
		Flow Rate(1.1ml/min)	0.04	%RSD NMT 2.0
		Mobile phase (60:40v/v)	0.8	%RSD NMT 2.0
		Mobile phase(70:30)	0.7	%RSD NMT 2.0
		Wave length(253nm)	0.4	%RSD NMT 2.0
		Wave length(254nm)	0.4	%RSD NMT 2.0

CONCLUSION:

A new and simple stability indicating RP-HPLC method for the estimation of Brinzolamide in ophthalmic dosage form was successfully developed and validated. The stability indicating method revealed that the Brinzolamide is stable under extreme conditions of acidic, alkaline, thermal etc. From the statistical assessment of the method It was concluded that the developed method was simple, specific, linear, accurate, precise and robust. The high resolution obtained makes this method cost effective and more acceptable. The usage of 0.1% Formic acid buffer which is a volatile in the mobile phase makes this method applicable in the LC-MS instrument.

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