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Research Article

METHOD DEVELOPMENT AND VALIDATION OF CIS-BROMOBENZOATE BY RP-HPLC IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of the study was to develop and validate RP-HPLC method for the analysis of cisbromobenzoate in marketed tablets and pure form.The methods was validated in terms of system sutiability,linearity, accuracy (%Recovery), precision (system precision& intermediate precision), speficity,ruggedness ,and robustness. The method found to be linear (R2 =0.999) and accurate. The methods was also found precise (% RSD< 2%) and robust. All validation parameters were within the acceptable range as per International Conference on Harmonization (ICH). Parameters of validation prove the precision of the method and its applicability for the determination of cisbromobenzoate in pharmaceutical tablet formulations.

Keywords: Cisbromobenzoate, RP-HPLC, Method validation.

INTRODUCTION

Cisbromobenzoate is designated chemically Cis-2-(Bromo methyl)-2-(2,4-dichlorophenyl)-1,3-dioxalan-4ylmethyl benzoate. Literature survey reveals that the drug can be estimated by spectrophotometric methods, ionic spectrophotometric methods and Chromatographic methods[1-6]. In the present investigation RP-HPLC method is a carried out with simple solvent system Water: Acetonitrile (75:25). Cisbromobenzoate is an Anti-Fungal[7-11]. Cisbromobenzoate is Anti-Fungal because it interferes with the fungal synthesis of ergo sterol, a constituent of fungal cell membranes, as well as certain enzymes[12].

MATERIALS AND INSTRUMENTS

MATERIALS AND METHOD

Instruments used

- Electronic balance (SARTORIOUS)
- UV-Visible spectrophotometer-UV2600-Shimadzu
- Heating mantle-BTI
- HPLC – WATERS Model NO.486 series Compact System Consisting of Kromofil-C18 ODS column
- Digital pH meter(POLOMAN)
- Filter paper 0.45microns
- Sonicator(FAST CLEAN)

CHEMICALS

Reference standard of cisbromobenzoate was supplied as gift sample from Sun Pharmaceutical Laboratories Limited, Hyderabad with purity of 99.987%

- Purified water HPLC Grade
- Acetonitrile

PROCEDURE

Preparation of standard stock solutions and Wavelength selection

The standard stock solution of drug was prepared by dissolving 50mg of the drug in 50 ml standard flask using Mobile phase(Acetonitrile(75): water(25)) as a solvent to give a concentration of 1000 µg/ml. This stock solution on further dilutions is used for establishing following parameters.

They were subjected to scanning from 200-400nm.

The dilutions were made using Mobile phase and scanned

From the different absorbance values obtained Maximum absorbance at 227nm was selected for the present work.

Method Development and validation

The RP HPLC procedure was optimized with a view to develop an effective method for the estimation of cisbromobenzoate in tablet dosage

forms. A Kromofil-C18 ODS, (250X4.6)mm, 5µ column(temp 35°C) was used as a stationary phase and the separation was achieved by using mobile phase consisting of Acetonitrile and water were mixed in the ratio of 75:25v/v in isocratic mode at the flow rate of 1 ml/min with PDA detection at 2240nm. Chromatogram of standard solution containing cisbromobenzoate

Standard preparation

Weigh accurately about 100mg of standard as Cis-bromobenzoate and place in 100ml volumetric flask and dissolves the powder n small quantity of mobile phase, then make up the volume with mobile phase up to the mark, then pipette out 1ml of this solution and place n 10ml volumetric flask and make up to the mark with mobile phase(in 10ml volumetric flask containing 100ppm/ml)

Sample preparation

Weigh and finely powder 20 tablets and Weigh a quantity of powder equivalent to about 100mg of standard Cis-bromobenzoate and place in 100ml volumetric flask and dissolves the powder in small quantity of mobile phase, then make up the volume with mobile phase up to the mark, then pipette out 1ml of this solution and place n 10ml volumetric flask and make up to the mark with mobile phase (in 10ml volumetric flask containing 100ppm/ml).

Validation parameters

System suitability test

The purpose of System suitability test is to ensure that the complete testing including instrument, method and analyst is suitable for the intended application .In this method the six replicates of standard solutions of Cis-bromobenzoate were injected and studied the suitability parameters like plate number (N), resolution (R), retention time (Rt), tailing factor and %RSD were studied with the help of standard chromatograms. The values obtained demonstrated the suitability of the system for the analysis of this drug, system suitability parameters may fall within ± 3 % standard deviation range during routine analysis.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the found value. Accuracy was calculated with respect to above prepared solution at the levels of 50%, 100% and 150% of the normal or target concentration. The accuracy of the method was demonstrated through recovery experiment on 3 samples at concentration 50%, 100%, 150% of the actual concentration employed in the usual procedure and the peak area of peaks is calculated.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. In this method Six individual preparations of drug substance were Cis-bromobenzoate were prepared and peak areas are calculated

According to the ICH, precision should be performed at three different levels:

- System precision (Repeatability)
- Method precision (Reproducibility)
- Intermediate precision (Ruggedness)

Specificity

It is the ability to assess unequivocally the analyte in the presence of components (Blank, placebo, impurities interference) which may be expected to be present.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness

In this method the relative standard deviation for the 6-replicated assay preparations is not more than 2.0%

1. System to system variability
2. Column to column variability
3. Analyst to analyst variability
4. Bench top stability of standard and sample solution
5. Refrigerator stability of standard and sample solution
6. Bench top stability of M.P

LOD (limit of detection)

Minimum conc.of the std compound in which the peak of the std get merged with noise called the LOD

$$\text{LOD} = \frac{3}{\frac{s}{n}\,value\ of\ std.} \text{Xconc. of std.}$$

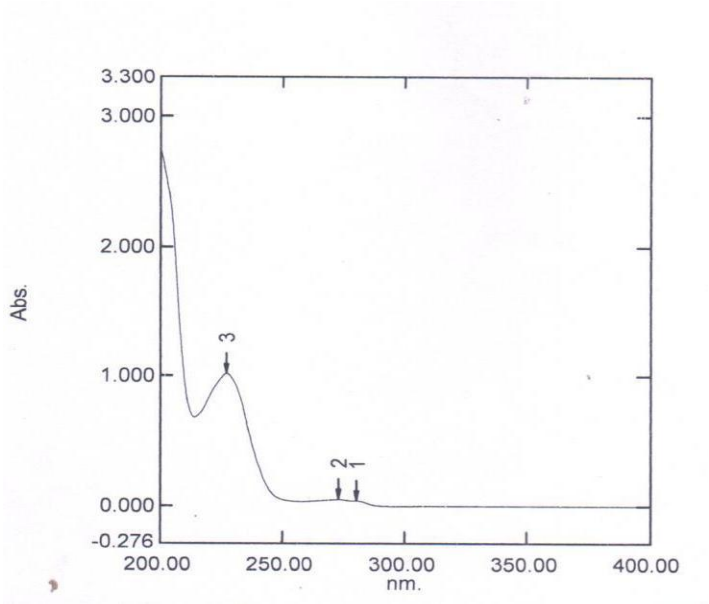
LOQ (limit of quantitation)

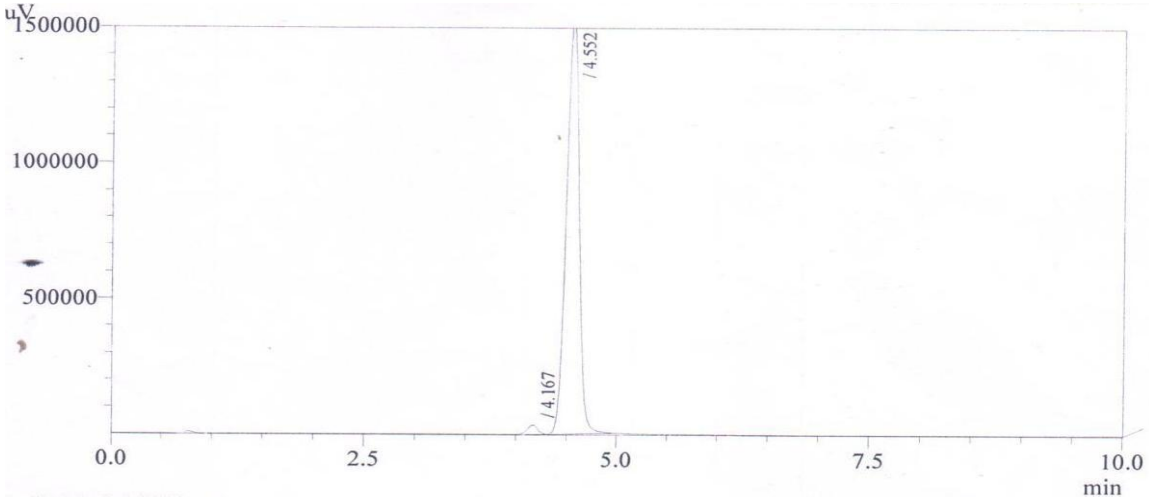
Minimum conc.of the std compound in which the peak of the std get detected and quantified is called the LOQ

$$\textbf{LOD} = \frac{10}{\frac{s}{n}\,value\ of\ std.} \text{Xconc. of std.}$$

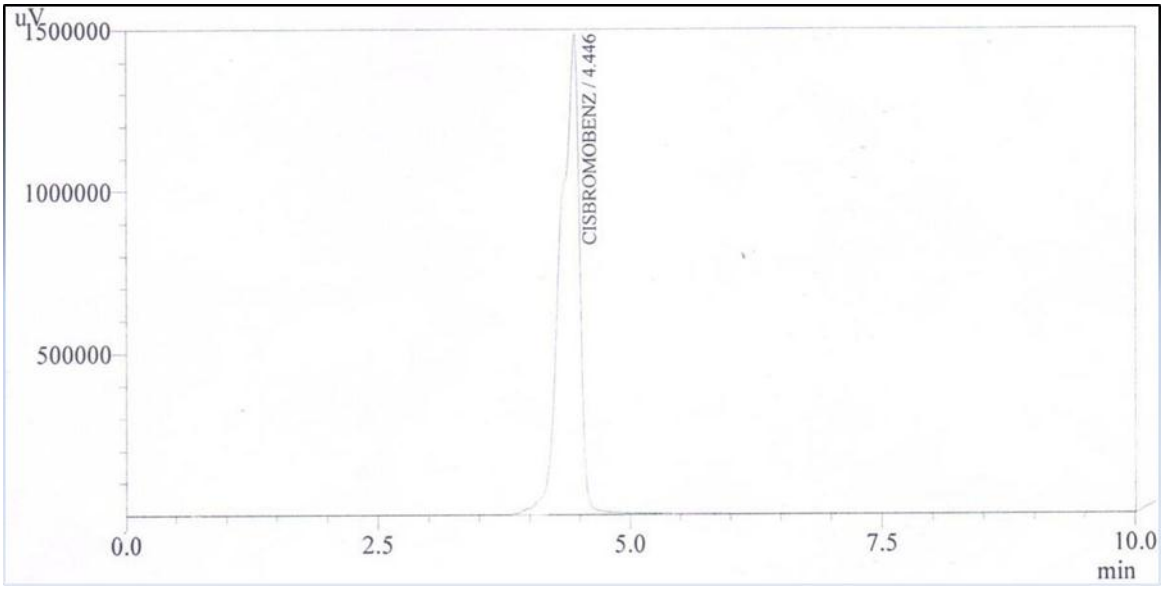
RESULTS AND DISCUSSION

Selection of wavelength maximum





Chromatogram of Standard Cis Bromobenzoate



Chromatogram of Sample Cis Bromobenzoate

Table 1: Linearity results

S.no	Concentration (µg/ mL)	Area
1	L-25%	5202059
2	L-50%	9951905
3	L-75%	14935964
4	L-100%	19672453
5	L-125%	23692683
6	L-150%	27810850

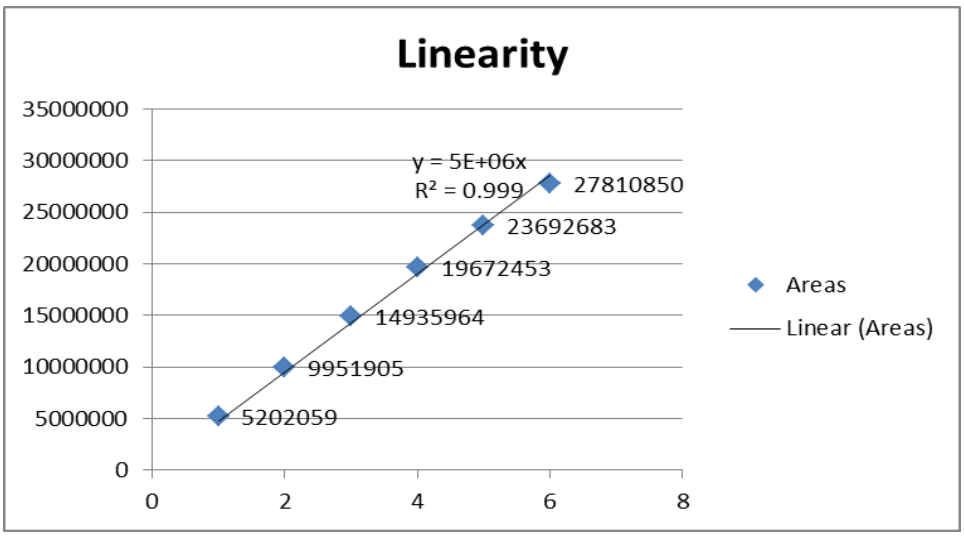


Table 2: System suitability results

S.no	Name	R_t	Area	Theoretical plates	Tailng factor
1	Blank	-	-	-	-
2	Stanard-1	4.446	19614533	2483.281	0.723
3	Stanard-2	4.446	19398795	2492.119	0.724
4	Stanard-3	4.446	19590403	2484.209	0.723
5	Stanard-4	4.446	19562027	2484.906	0.723
6	Stanard-5	4.446	195136991	2486.532	0.723
7	Sample	4.446	19549854	2485.370	0.723

Table 3: Accuracy results

S. No	% Spike Level	Amount added (mg)	% Recovery	Mean % Recovery	% RSD
1.	50%	100.00	100.4	100.35	0.1
2.		100.00	100.38		
3.		100.00	100.29		
1.	100%	200.00	99.7	100.03	0.2
2.		200.00	100.5		
3.		200.00	99.9		
1.	150%	150.00	94.30	100.66	0.2
2.		150.00	94.20		
3.		150.00	96.50		

Table 4: System Precision results

S.no	Name (no.of injections)	Area	R_t	Theoretical plates	Tailing factor
1	Blank	-	-	-	-
2	Standard	19614533	4.446	2483.281	0.723
3	Sample 8µg/ml	19456644	4.446	2488.624	0.722
4	Sample 8µg/ml	19427498	4.446	2490.487	0.724
5	Sample 8µg/ml	19424832	4.446	2490.254	0.723
6	Sample 8µg/ml	19398795	4.446	2492.119	0.724
7	Sample 8µg/ml	19586657	4.446	2484.209	0.723

Intermediate Precision

Intermediate Precision Day-1

Table 5: Intermediate Precision Day-1 results

S.no	Name	R_t	Area	Theoretical plates	Tailing factor
1	Blank	-	-	-	-
2	Stanard-1	4.446	19445074	2489.323	0.723
3	Stanard-2	4.446	19426066	2490.254	0.723
4	Stanard-3	4.446	19358276	2493.518	0.723
5	Stanard-4	4.446	19483226	2487.694	0.723
6	Stanard-5	4.446	19460951	2488.857	0.723
7	Sample	4.446	1946890	2488.392	0.723

Table 6: Intermediate Precision Day-2 results

S.no	Name	R_t	Area	Theoretical plates	Tailing factor
1	Blank	-	-	-	-
2	Stanard-1	4.446	19368574	2492.818	0.723
3	Stanard-2	4.446	19479770	2487.927	0.723
4	Stanard-3	4.446	19419333	2490.254	0.723
5	Stanard-4	4.446	19513691	2486.532	0.723
6	Stanard-5	4.446	19440132	2489.788	0.723
7	Sample	4.446	1941604	2490.720	0.723

Table 7: Cumulative Precision results

Type of precision	% of assay
1.System Precision	99.8
2.Intermediate precision	
i.Day-1	99.976
ii.Day-2	99.655

Table 8: Ruggedness Analyst-1

S.no	Name	Area
1	Blank	-
2	Stanard-1	19449090
3	Stanard-2	19408237
4	Stanard-3	19494709
5	Stanard-4	19456644
6	Stanard-5	19005391
7	Sample	1945644

Table 9: Ruggedness Analyst-2

S.no	Name	Area
1	Blank	-
2	Stanard-1	19455850
3	Stanard-2	19470531
4	Stanard-3	19469069
5	Stanard-4	19495975
6	Stanard-5	19514068
7	Sample	19470531

Table 10: Ruggedness results

Ruggedness	Percentage
Analyst-1	100.28%
Analyst-2	99.77%

Table 11: Robustness

changes in chromatogram conditions	Normal	Variation	Rt	Peak area
Mobilephase(water: Acetonitrille, %v/v)	75:25	5.582	27140	11230
Flow rate (mL/min)	1.0	1.5	4.925	12182
Change in wavelength(nm)	227	243	5.200	28823

The analytical RP-HPLC method was developed by studying different parameters. Initially maximum absorbance was found to be at 227nm and the peak purity was excellent.In this method to optimize the mobile phase, various combinations of water, and acetonitrile were studied on an Kromofil- C18 ODS column .Initially, the ratio of water and acetonitrile 75:25 v/v resulted in peaks with good shape. A flow rate of 1.0mL/min was found to be optimum in the range of 0.8-1.2 mL/min resulting in short retention time, baseline stability and minimum noise and the retention times of cis-bromobenzoate was found to be 4.446 min.

The developed method is validated in accordance with the ICH guidelines with all of the results within the limits. Quantitative linearity was obeyed in the concentration range of 25-150 µg/mL of cis-bromobenzoate the mean percentage recoveries for cis-bromobenzoate was found to be 100.34%. The high percentage recovery indicates that the proposed method was highly accurate.

Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The % RSD values of Bisoprolol were found to be 0.2%. The low % RSD value (below 2) indicates that the method was Precise.

Robustness of the method was performed by varying the flow rate of mobile phase. From that data it was found the asymmetric factor was less than 2.0 and theoretical plates were more than 2000 for peak, which illustrates the good robustness of the developed method

Finally the developed HPLC method was applied for estimation of Bisoprolol Pharmaceutical dosage forms. No interfering peaks were found in the chromatogram indicating that excipients used in capsule formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

CONCLUSION

The proposed method of analysis is novel, simple, cost-effective, environment friendly, safe, accurate and Reproducible. This method

can be routinely employed in the analysis of cis-bromobenzoate in tablet formulations.

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