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Validation methods for a simultaneous determination of bisoprolol fumarate and hydrochlorothiazide multi-component products, examining system suitability, precision and robustness (Paper B)

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Abstract
A simple RP-HPLC method was developed for the determination of hydrochlorothiazide and bisoprolol fumarate in their combined pharmaceutical formulations. The separation was achieved using Cyanide column (250 × 4.6mm, 5µm particle size), both components were determined by UV detector at fixed wavelength at 228nm, for simplicity of the method an isocratic elution was selected, the optimized mobile phase was composed of methanol and buffer solution (pH=5.0) at 82:18 ratio, with flow rate of 0.9ml/min, injection volume was 10µl, and the separation was performed at 30°C. The RSD values was found to be not more than 2.0% so it is acceptable according to USP and ICH. The proposed method was found to be precise and robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions such as column temperature, flow rate and detection wavelength. RSD for the area at all different conditions for target.

Keywords: validation, bisoprolol fumarate, hydrochlorothiazide, multi-component products, suitability, precision, robustness

1. Introduction

There were many methods proposed for the analysis of bisoprolol fumarate and hydrochlorothiazide as combined drug or as separating drug or as a combined drug with other.

Different parameters affecting the method accuracy and reliability etc. were also studied eg.

A high-performance liquid chromatography method for determination of bisoprolol Fumarate and Hydrochlorothiazide was investigated by many authors eg. Patel. L. J *et al*. (2006) [10], Joshi *et al*. (2010) [6]; Ravi Varma Athota *et al*., (2016) [1]; and they used C_{18} column (250 × 4.6mm, 5µm) The limit of quantitation was 0.398 and 0.385µg/ml for bisoprolol and hydrochlorothiazide, respectively.

Bhoya *et al*. (2013) [2]; Savita's Yadav and Janhavirrao (2013) [18]; were used a high-performance thin-layer chromatographic for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide using precoated silica gel HPTLC aluminum plate 60 F254, using chloroform, ethanol and glacial acetic acid in the ratio of 5**:**1.5**:**0.2 (v/v) as mobile phase.

The use of voltammetry, chromatographic, and spectrophotometric methods for determination of bisoprolol fumarate (BIS) and hydrochlorothiazide (HCZ) was reported by Bozal *et al*. (2013) [4]; Sevinc Kurbanoglu. *et al*., (2014) [7]; validated an Ultra-performance liquid chromatographic method for the simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in their combined dosage forms and as well as in spiked human urine samples. Other authors were used HPLC-UV for determination of bisoprolol and hydrochlorothiazide such as Renuka, *et al*. (2016) [12] and Raju *et al.*, (2016) [11] and they agreed in using C_{18} column (150 x 4.6mm, 5µm) and (250 x 4.6mm). Bobade, P. S. and Ganorkar, S. B. (2017) [3]; did spectrophotometric method for the determination of bisoprolol fumarate and Hydrochlorothiazide using 0.1N Sodium hydroxide as solvent.

At our previous paper (A) a simple and sensitive RP-HPLC method was developed for the determination of hydrochlorothiazide and bisoprolol fumarate in their combined pharmaceutical formulations. The separation was achieved using Cyanide column (250 × 4.6mm, 5µm particle size), both components were determined by UV detector at fixed wavelength at 228nm, for simplicity of the method an isocratic elution was selected, the optimized mobile phase was composed of methanol and buffer solution at 82:18 ratio, with flow rate of 0.9ml/min, injection volume was 10µl, and the separation was performed at 30°C. Linearity of this method was checked using seven solutions centered with the target concentration, the concentrations range was (40–160) µg/ml for bisoprolol fumarate and (100–400) µg/ml for hydrochlorothiazide. Each solution was injected in triplicate. Plot of average area versus prepared concentrations indicates a very good linearity correlation for, (R^2 =0.999) for both components. The limit of detection for bisoprolol fumarate and hydrochlorothiazide was found to be 1.8575µg/ml and 3.57811µg/ml, respectively; whereas the limit of quantitation was found to be 6.19184 µg/ml and 11.92µg/ml; respectively.

In specificity tests, none of placebo peaks had same retention time of active ingredients peaks. This indicates that the excipients used in the formulation did not interfere in the estimation when we used this method for assay in tablets. Accuracy was evaluated for bisoprolol fumarate and hydrochlorothiazide using three concentrations in content of 50%, 100%, and 150 of target concentration. The recovery percentage for bisoprolol fumarate at the above concentrations was found to be 102.741, 100.135 and 101.845, respectively; while for hydrochlorothiazide, it was 100.543, 100.157 and 101.444 respectively. The average of recovery percentage for

bisoprolol fumarate and hydrochlorothiazide was 101.5736% and 100.7146%, respectively.

The objectives of the present work is a complementary developing of assay methods for a simultaneous determination of bisoprolol fumarate and Hydrochlorothiazide multi-component products, examining System suitability, Precision and Robustness for each developed method and comparing the obtained results with the acceptance criteria of USP and ICH guidelines, finally application of the developed method for real sample assay.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals

Bisoprolol fumarate (purity: 99.30%), Hydrochlorothiazide (purity: 99.10%) and Tetra butyl ammonium hydroxide 40% were obtained from Aurobindo, India, Unichem India and Emplura India; respectively. Methanol was of HPLC grade and all other chemicals used were of analytical grade. Purified water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis.

2.1.2 Instruments

High Performance Liquid Chromatography HPLC

Type: HPLC prominence – i
Model: LC-2030C3D
Serial No: L21455300660AE
Company: Shimadzu Corporation
Origin: Japan

Analytical Balance

Type: AY220
Serial No: O4328143000
Capacity: 220 g
Readability: 0.1 mg
Company: Shimadzu Corporation
Origin JAPAN

Ultrasonic

Model: 621.05.003
Company: ISO Lab Laborgerate -GmbH
Origin: Germany

PH-meter

Model: PHS-550
Origin: Romania.

Magnetic Stirrer

Model: LMS, 1001
Serial No: 2016017862
Company: QAIHAN LAB TECH Co-LTD
Origin: Korea

2.2. Methods

2.2.1 Optimized chromatographic conditions

Cyanide column (250 × 4.6mm, 5µm), and simple isocratic elusion, were used (one pump required) with flow-rate of 0.9ml/min, both active ingredients were detected at 228nm, injection volume was 10µl (universal loop) and analysis temperature was 30°C.

2.2.2 Buffer Solution pH 5.0

1000 ml volumetric flask was Mixed 980 ml of deionized water, 10 ml of tetra butyl ammonium hydroxide 40% was added to the flask and adjusted to pH 5.0 with glacial acetic acid, and the volume was completed to the mark with deionized water.

2.2.3 Mobile Phase

Mixture of buffer and methanol were prepared in 82:18 v/v ratio, respectively. The mixture was shaken, filtered with vacuum filtration pump through 0.45µm nylon membrane filter, and then transferred to solvent reservoir and sonicated for 5 min.

2.2.4 Standard Stock Solution

0.2500g Hydrochlorothiazide and 0.1000g bisoprolol fumarate were weighed accurately, transferred quantitatively to the same 100ml volumetric flask, 50ml Methanol was added and sonicated to 5 min, cooled, and completed to with mobile phase.

2.2.5 System Suitability

Subsequent dilutions were made from the stock solution with mobile phase to give the concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml Bisoprolol Fumarate. System suitability solution was injected six times.

2.2.6 Precision

a) Precision Standard

Subsequent dilutions were made from the stock solution with mobile phase to give the concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml bisoprolol Fumarate. System suitability solution was injected six times.

b) Preparation of working Standard Solution

5ml standard stock solution was taken in to 25ml volumetric flask, then Completed to the required volume with mobile, passed through a suitable filter 0.45µm pore size.

c) Precision sample

Five tablets were taken in to clean and dry 100 ml volumetric flask and shacked with 10 ml methanol, sonicated for 10 min, cooled, then 50 ml mobile phase was added, sonicated to 20 min, leave to reach room temperature, and then completed to required volume with mobile phase. Then 5ml was diluted with mobile phase in to 25ml volumetric flask, passed through a suitable filter 0.45µm pore size.

2.2.7 Robustness

Accuracy sample solution of target concentration was used. The sample was injected three times at each of the different conditions relative to optimum condition, five degrees more temperature, five degrees less temperature, 10% more flow rate of mobile phase, 10% less flow rate of mobile phase, 2nm above the detection wavelength and 2nm below the detection wavelength. The results were collected and subjected to statistical treatments.

3. Results and Discussion:

3.1 Bisoprolol fumarate and Hydrochlorothiazide

3.1.1 System Suitability

System suitability results for bisoprolol fumarate and hydrochlorothiazide are shown in Table (1) and Table (2)

respectively.

Table 1: System suitability results for bisoprolol fumarate

No	Area	Retention time	Tailing factor	Resolution	Theoretical plates
1	1476536	4.718	1.247	10.468	39604
2	1477324	4.71	1.241	10.438	39290
3	1475963	4.72	1.238	10.422	39390
4	1475068	4.722	1.236	10.421	39316
5	1470496	4.71	1.235	10.385	39211
6	1475461	4.719	1.234	10.358	39092
AVG	1475141.333	4.7165	1.2385	10.41533333	39317.16667
STDEV	2411.26321	0.005205766	0.00484768	0.038913579	173.3717586
RSD%	0.163459809	0.110373498	0.39141541	0.373618186	0.440956898

Table 2: System suitability results for hydrochlorothiazide

No	Area	Retention time	Tailing factor	Resolution	Theoretical plates
1	12358531	7.783	1.166	10.468	55637
2	12369606	7.775	1.161	10.438	55195
3	12365433	7.784	1.157	10.422	55143
4	12322841	7.785	1.154	10.421	55316
5	12323359	7.763	1.154	10.385	54845
6	12363664	7.779	1.151	10.358	54432
AVG	12350572.33	7.778166667	1.157166667	10.41533333	55094.66667
STDEV	21575.19108	0.008304617	0.005492419	0.038913579	413.9993559
RSD%	0.174689808	0.106768311	0.474643729	0.373618186	0.751432726

3.1.2 Precision

1) Intraday Precision

Table (3) shows results of bisoprolol fumarate and hydrochlorothiazide mixed standard for intraday precision test.

Table 3: results of bisoprolol fumarate and hydrochlorothiazide mixed standard for intraday precision test

No	Bisoprolol fumarate	Hydrochlorothiazide
1	1476536	12358531
2	1477324	12369606
3	1475963	12365433
4	1475068	12322841
5	1470496	12323359
6	1475461	12363664
AVG	1475141.333	12350572
STDEV	2411.26321	21575.19
RSD%	0.16	0.17

Table (4) and Table (5) show intraday precision for bisoprolol fumarate and hydrochlorothiazide, respectively

Table 4: Intraday results for bisoprolol fumarate

NO	1st	2nd	3rd	4th
1st trial	1509753	1494563	1505483	1506264
2nd trial	1508969	1502898	1500187	1503023
3rd trial	1509348	1506247	1505875	1500863
AVG	1509357	1501236	1503848	1503383
STDEV	392.072	6016.697	3176.86	2718.47
RSD%	0.02598	0.400783	0.21125	0.180823
Recovery	102.3195	101.76896	101.9461	101.91453
Recovery%	102.3195	101.76896	101.9461	101.91453

Table 5: Intraday results for hydrochlorothiazide

NO	1st	2nd	3rd	4th
1st trial	12405489	12311249	12376373	12316188
2nd trial	12456555	12358094	12358972	12349591
3rd trial	12460101	12363895	12312891	12350422

AVG	12440715	12344412.67	12349412	12338733.67
STDEV	30558.1	28866.67	32802.99	19529.5
RSD%	0.24563	0.233844	0.265624	0.15828
Recovery	100.72987	99.950126	99.990605	99.904145
Recovery%	100.72987	99.950126	99.990605	99.904145

Table (6) shows Summary of intraday precession for bisoprolol fumarate and hydrochlorothiazide

Table 6: Summery intraday precession for bisoprolol fumarate and hydrochlorothiazide

trial	Bisoprolol fumarate	Hydrochlorothiazide
1st	102.3195	100.7299
2nd	101.76896	99.95013
3rd	101.9461	99.99061
4th	101.9145	99.90414
AVG	101.987265	100.143695
STDEV	0.234539599	0.392396622
RSD%	0.229969496	0.391833577

2) Intraday Precision

Table (7) shows results of bisoprolol fumarate and hydrochlorothiazide mixed standard for interday precision test

Table 7: results of bisoprolol fumarate and hydrochlorothiazide mixed standard for interday precision test

No	Bisoprolol fumarate	hydrochlorothiazide
1	1476536	12358531
2	1477324	12369606
3	1475963	12365433
4	1475068	12322841
5	1470496	12323359
6	1475461	12363664
AVG	1475141.333	12350572
STDEV	2411.26321	21575.19
RSD%	0.16	0.17

Table (8) shows intraday precision for both components, respectively. Table (11) shows the summary of interday precision

Table 8: shows intraday precision for both components

Trial	Bisoprolol fumarate			Hydrochlorothiazide		
	Day1	Day2	Day3	Day1	Day2	Day3
1st trial	1508519	1505433	1505679	12447338	12353751	12332485
2nd trial	1508412	1505402	1503785	12458251	12353319	12290494
3rd trial	1505675	1504767	1504175	12461550	12352396	12334072
AVG	1507535.333	1505200.667	1504546.33	12455713	12353155.33	12319017
STDEV	1611.983974	375.8860643	1000.11266	7438.166374	692.1678505	24714.38425
RSD%	0.1069284	0.02497249	0.0664727	0.05971691	0.005603166	0.200619776
Recovery	102.195993	102.0377257	101.993368	100.851302	100.020914	99.74450307
Recovery%	102.195993	102.0377257	101.993368	100.851302	100.020914	99.74450307

Table (9) shows interday precision summary for both bisoprolol fumarate and hydrochloro-thiazide

Table 9: Interday precision summary for both bisoprolol fumarate and hydrochlorothiazide

NO	Bisoprolol fumarate	Hydrochlorothiazide
1st trial	102.195993	100.851302
2nd trial	102.0377257	100.020914
3rd trial	101.993368	99.74450307
AVG	102.0756956	100.205573
STDEV	0.106515284	0.576042693
RSD%	0.10434931	0.574860934

3.1.3 Robustness

The robustness of the method was determined as per ICH guidelines under a variety of conditions like change in Temperature,

wavelength and flow rate. The results obtained by deliberately variation in method parameters.

3.1.4.1 Robustness study of Bisoprolol fumarate

1). Optimized conditions

Table 10: Results of bisoprolol fumarate sample at optimum conditions

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1410282	4.935	41371	1.195	9.64
2	1410818	4.938	41649	1.194	9.648
3	1411423	4.937	41641	1.194	9.651
AVG	1410841	4.936667	41553.67	1.194333	9.646333
STDEV	570.8476154	0.001528	158.2445	0.000577	0.005686
RSD %	0.040461513	0.030942	0.38082	0.048341	0.058947

2). 5 ˚C more

Table 11: Results of bisoprolol fumarate after the column temperature was raised up five degrees Celsius

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1466463	4.846	43990	1.197	8.668
2	1465325	4.847	44157	1.198	8.663
3	1466023	4.847	44141	1.2	8.661
AVG	1465937	4.846667	44096	1.198333	8.664
STDEV	573.8536399	0.000577	92.14662	0.001528	0.003606
RSD %	0.03914586	0.011912	0.208968	0.127471	0.041615

3). 5 ˚C less

Table 12: Results of bisoprolol fumarate after the column temperature was decreased five-Celsius degrees

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1409363	4.942	41291	1.194	9.702
2	1411298	4.935	41269	1.196	9.631
3	1412089	4.936	41256	1.197	9.619
AVG	1410916.667	4.937667	41272	1.195667	9.650667
STDEV	1402.437283	0.003786	17.69181	0.001528	0.044859
RSD %	0.099399016	0.076675	0.042866	0.127755	0.464828

4). 5% more Flow rate

Table 13: Results of bisoprolol fumarate after the flow rate was raised up 5% of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1269184	4.454	38704	1.193	9.299
2	1267406	4.455	38698	1.191	9.297
3	1267818	4.454	38715	1.193	9.297
AVG	1268136	4.454333	38705.67	1.192333	9.297667
STDEV	930.6793218	0.000577	8.621678	0.001155	0.001155
RSD %	0.073389551	0.012962	0.022275	0.096844	0.012419

5). 5% less Flow rate

Table 14: Results of bisoprolol fumarate after the flow rate was decreased 5% of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1588774	5.549	44867	1.191	9.99
2	1588679	5.551	44957	1.192	9.997
3	1589747	5.553	45043	1.193	9.999
AVG	1589066.667	5.551	44955.67	1.192	9.995333
STDEV	591.0975667	0.002	88.00758	0.001	0.004726
RSD %	0.037197783	0.03603	0.195765	0.083893	0.04728

6). 2nm more Wavelengthd

Table 15: Results of bisoprolol fumarate after the flow rate was raised up wavelength 2nm of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1201242	4.935	41273	1.195	9.62
2	1201570	4.938	41554	1.194	9.628
3	1202095	4.937	41547	1.194	9.63
AVG	1201635.667	4.936667	41458	1.194333	9.626
STDEV	430.2747184	0.001528	160.2529	0.000577	0.005292
RSD %	0.035807419	0.030942	0.386543	0.048341	0.054971

7). 2nm less Wavelength

Table 16: Results of bisoprolol fumarate after the wavelength was decreased 2nm of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1522691	4.935	41382	1.194	9.661
2	1523097	4.938	41671	1.193	9.669
3	1523809	4.937	41652	1.193	9.671
AVG	1523199	4.936667	41568.33	1.193333	9.667
STDEV	565.9363922	0.001528	161.6488	0.000577	0.005292
RSD %	0.037154462	0.030942	0.388875	0.048381	0.054738

3.1.4.2 Robustness study of hydrochlorothiazide

1) Optimized conditions

Table 17: Results of Hydrochlorothiazide sample at optimum conditions

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11957725	7.774	56244	1.153	9.64
2	11955607	7.776	56322	1.154	9.648
3	11959046	7.774	56397	1.154	9.651
AVG	11957459.33	7.7746667	56321	1.1536667	9.6463333
STDEV	1734.824006	0.0011547	76.504902	0.0005774	0.0056862
RSD %	0.014508299	0.0148521	0.1358373	0.0500448	0.0589472

2) 5 ˚C more

Table 18: Results of Hydrochlorothiazide after the column temperature was raised up five degrees Celsius.

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11699930	7.242	56668	1.153	8.668
2	11693708	7.238	56676	1.153	8.663
3	11700910	7.238	56698	1.155	8.661
AVG	11698182.67	7.239333	56680.67	1.153667	8.664
STDEV	3906.031405	0.002309	15.53491	0.001155	0.003606
RSD %	0.03339007	0.031901	0.027408	0.10009	0.041615

3) 5 ˚C less

Table 19: Results of Hydrochlorothiazide after the column temperature was decreased five-Celsius degrees

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11963304	7.809	56350	1.153	9.702
2	11964965	7.778	55976	1.153	9.631
3	11972469	7.777	55869	1.154	9.619
AVG	11966912.7	7.788	56065	1.1533333	9.6506667
STDEV	4883.06874	0.0181934	252.54901	0.0005774	0.044859
RSD %	0.0408047	0.2336082	0.4504575	0.0500593	0.4648284

4) 5% more flow rate

Table 20: Results of Hydrochlorothiazide after the flow rate was raised up 5% of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	10800235	6.996	53330	1.156	9.299
2	10792695	6.996	53335	1.156	9.297
3	10790233	6.993	53386	1.155	9.297
AVG	10794387.67	6.995	53350.33	1.155667	9.297667
STDEV	5211.414523	0.001732	30.98925	0.000577	0.001155
RSD %	0.048278927	0.024761	0.058086	0.049958	0.012419

5) 5% less flow rate

Table 21: Results of Hydrochlorothiazide after the flow rate was decreased 5% of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	13409613	8.747	59746	1.15	9.99
2	13406495	8.748	59887	1.15	9.997
3	13413274	8.748	59982	1.15	9.999
AVG	13409794	8.747667	59871.67	1.15	9.995333
STDEV	3393.122603	0.000577	118.7448	0	0.004726
RSD %	0.025303316	0.0066	0.198332	0	0.04728

6) 2nm more wavelength

Table 22: Results of Hydrochlorothiazide after the flow rate was raised up wavelength 2nm of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	9667692	7.774	55928	1.156	9.62
2	9666205	7.775	56005	1.156	9.628
3	9669871	7.773	56085	1.157	9.63
AVG	9667922.667	7.774	56006	1.156333	9.626
STDEV	1843.853121	0.001	78.50478	0.000577	0.005292
RSD %	0.019071865	0.012863	0.140172	0.049929	0.054971

7) 2nm less wavelength

Table 23: Results of bisoprolol fumarate after the wavelength was decreased 2nm of its optimized value

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	13503899	7.775	56647	1.15	9.661
2	13500076	7.776	56720	1.151	9.669
3	13503510	7.774	56796	1.151	9.671
AVG	13502495	7.775	56721	1.150667	9.667
STDEV	2103.925141	0.001	74.50503	0.000577	0.005292
RSD %	0.015581751	0.012862	0.131354	0.050175	0.054738

4. Discussion

A simple RP-HPLC method was developed for the determination of hydrochlorothiazide and bisoprolol fumarate in their combined pharmaceutical formulations. The separation was achieved using Cyanide column (250 × 4.6mm, 5µm particle size), both components were determined by UV detector at fixed wavelength at 228nm, for simplicity of the method an isocratic elution was selected, the optimized mobile phase was composed of methanol and buffer solution at 82:18 ratio, with flow rate of 0.9ml/min, injection volume was 10µl, and the separation was performed at 30 ° C. The precision of the methods was examined by estimating the corresponding recovery percentages four times on the same day in intraday precision and three times at three different days for inter day precision. The concentrations used was 100% of target concentration as per ICH. For bisoprolol fumarate intraday precision, the RSD for the recovery percentage of assay repetitions was 0.025%, 0.40%, 0.211% and 0.18% respectively; whereas for hydrochlorothiazide RSD was 0.245%, 0.233%, 0.265% and 0.158%, respectively. For the interlay, the RSD for the recovery percentage of bisoprolol fumarate three assay repetitions was 0.106%, 0.024% and 0.066%, respectively; whereas for hydrochlorothiazide RSD was 0.059%, 0.005% and 0.20%, respectively. The RSD values was found to be not more than 2.0% so it is acceptable according to USP and ICH. The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions such as column temperature, flow rate and detection wavelength. RSD for the area at all different conditions for

target.

5. Conclusions

The proposed method is simple and reproducible and hence the method can be used in routine for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in tablet as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing good precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in multicomponent pharmaceutical preparation.

6. References

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