Original Research Article

Bio-analytical approach for stability studies of Bendroflumethiazide materials

Abstract

In this paper a comprehensive study of stability related,and evidence based best practices of Bio-analytical stability on Bendroflumethiazide drug samples are studied. The proposed approach is very significantand essential for the drugs development processaddress the specify the acceptancy, purity, efficacy, prediction of strength and quality of the drugs. The stability study constituents several methods like Bench-Top, Auto-sampler, Freeze-Thaw, Dry-extract, Wet-extract, Short-term, long-Term stability studies at relative intervals results the complete stability information about the drug under the proposed and validated method. Thereported out comes of this methos shows this drug have good stability according to ICH guidelines.

Key words: Bio-analytical/ Bendroflumethiazide/ Auto-sampler/ Short term/ Wet-extract/ ICH guidelines.

1. INTRODUCTION

Bendroflumethiazide formerly, bendrofluazide, brand name Aprinox is a thiamine diuretic used for the treat of hypertension. Bendroflumethiazide may be a thiamine diuretic [1-2] that acts at the start of the distal convoluted tubule (DCT) by inhibiting sodium reabsorption. As a consequence of more sodium hitting the supply ducts, water is lost. Bendroflumethiazide mark has a roleto play in the treatment of minor coronary wreck,but the diuretic loop could be safer for overloadreduction. The Best use of bendroflumethiazide in hypertension [3] at present. Its structure shown in Figure 1 and it was patented in 1958 & it was used for medical usage [4-7] in 1960. The mechanism of action of this drug as diuretic [8] nature it inhibit reabsorption of active chlorine increases the excretion of NaCl and H_2O. Which results exchange mechanism of Sodium-Potassium. In the hypertensive mechanism with carbonic anhydrase leads to forms very smooth muscles due to conductance between activated calcium-potassium. In the present study the stability study of the proposed validated method for quantification and validation of Bendroflumethiazide to asses the stability of the drug.

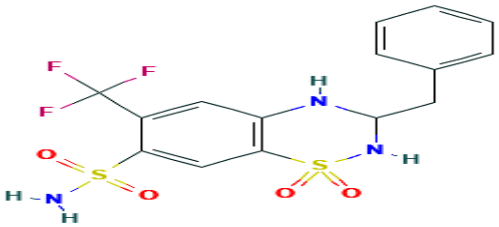

Figure 1: Structure of Bendroflumethiazide's

2. MATERIALS AND METHODS

2.1 Chemical and reagents:

Chromatographic graded Acetonitrile, Orthophosphoric acid were procured Merck Ltd. Worli, in Bombay, India and aqueous Water with marked HPLC graded was used. From Glenmark Pharmaceuticals, APIs of Bendroflumethiazide as reference standards were produced.

2.2 Instrumentation:

Sciex software enabled Liquid Chromatographic Mass Spectrometry (LC-MS) SCIEX QTRAP 5500 was used for chromatographic analysis.

2.3 Standard and quality control samples preparation

2.3 Preparation of Bendroflumethiazide parent stock

To prepare the parent stock solution of Bendroflumethiazide in concentration is 0.1 µg/ml, first step prepare solution of Bendroflumethiazide in concentration 100 µg/ml by weight 10 mg of Bendroflumethiazide standard and dissolved it in 100 ml of diluent. Second step take 1 ml of first solution prepared (100 µg/ml) and dissolved it with 10 ml of solvent to finally prepare the parent stock solution in concentration is 0.1 µg/ml.

2.4 Preparation standard of Bendroflumethiazide solutions

The parent stock solutions of Bendroflumethiazide parent stock liquid of 0.4 ml saturated into 10 ml vacuumbottlesup to the mark with solvents have concentrations 320 ng/ml&40 ng/ml respectively. In the same way internal standard stock solution was prepared.

2.5 Preparation of standard solution

Typical solution was prepared by taking 0.5 ml, 0.2 ml, 0.3 ml and 0.5 ml of parentliquid, internal standard stock solution, plasma, acetonitrile and diluent in a centrifuged tube and centrifuged for about 15 min to mixing the contents at 5000 rpm excessive managed solution was isolated and filtered by micro filter with pore size 0.45 µ then inoculated to HPLC system.

2.6 Sample stock preparation

One pill (5 mg of Bendroflumethiazide) was weighed, note the average weight of the tablet. The pill was taken into a mortar and crushed into fine powder. 13.4 mg of tablet powder was weighed accurately and dissolved in 100 ml of diluent. From this take 0.8 ml and diluted to 100 ml with diluents. This is the sample stock with Bendroflumethiazide concentration 40 µg/ml.

2.7 Sample solution preparation

For sample preparation take 0.2 ml of plasma, 0.5 ml of sample stock, 0.3 ml of acetonitrile and 0.5 ml of IS, 0.5 ml of diluent were taken into a centrifuge tube and centrifuged about 15 min to precipitate all the proteins with 5000 rpm and collect the excessive solution into a vial and inject it into HPLC system.

2.8 Method Developed and Validation

A method was developed and validated [9] by LC-MS with isocratic approach have waters symmetry C_{18} column with dimensions 150x4.6 mm with pore size 3.5 microns for chromatographic analysis. The author also studied few drugs, and their bioanalytical quantification and validation studies gave good results [10-12]. The solvent Orthophosphoric

acid with 0.1 % strength and acetonitrile are taken in 60:40 proportions are administered into chromatogram for positive electron spry ionization method.

2.9 Stability Studies of the Proposed method

In-order to check the feasibility and stability of the proposed validated method by studying various Bio-analytical stability related studies like Bench-Top, Auto-sampler, Freeze-Thaw, Dry-extract, Wet-extract, Short-term, long-Term stability studies at various intervals gave the complete stability information about these drugs. As per the US FDA guidelines theLQC and HQC strengths & their plasma stability studies on six different copies are injected for each dose. In auto sampler stability the spiked rat plasma was placed at temperature 2-8^{o} C for about twenty-four hours.

3. RESULTS AND DISCUSSIONS

3.1 Bench Top Stability: In Bench-Topmethod the sample solutions are placed on bench-top during the experiment for about six to twenty-four hours of the procedure of extraction after remove from the fridge took six replications have low and high strengths then inoculate to chromatogram the results are shown in Table 1 and it allows the Bench top stability.

Table: 1Bendroflumethiazidestability results of Bench-Topmethod

Replicate No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.755	5.257	10.125
	Ostensible strength range in ng/ml		
	(15.751-15.759)	(5.252-5.259)	(10.017-10.232)
	Area of analyte-peak		
1	$1.041x10^5$	$0.361x10^5$	$0.788x10^5$
2	$1.022x10^5$	$0.378x10^5$	$0.775x10^5$
3	$1.036x10^5$	$0.385x10^5$	$0.779x10^5$
4	$1.018x10^5$	$0.381x10^5$	$0.785x10^5$
5	$1.027x10^5$	$0.393x10^5$	$0.782x10^5$
6	$1.034x10^5$	$0.367x10^5$	$0.794x10^5$
n	6	6	6
Mean	$1.030x10^5$	$0.378x10^5$	$0.784x10^5$
SD	0.00882	0.01176	0.00674
%CV	0.86	3.12	0.86
% Mean	98.8%	98.5%	99.7%

Accuracy			

Acceptance criteria: The Percent average exactness is in between 85-115 for eight specimens out of twelve samples. The minimum requirement of 80% of the matrix lot should meet the acceptance criteria. The reverse calculated strengths of LQC and HQC is less than or equal to 15 %.

3.2 Auto Sampler Stability: In Auto-sampler stability method the pooled solutions are placed in to auto-sampler inoculated to injector. The reports are placed in Table 2 and it acceptancy was passed the Auto Sampler Stability.

Table: 2Auto Sampler Stability of Bendroflumethiazide

Replicate No.	HQC	MQC	LQC
	Ostensible strength in ng/ml		
	15.756	10.526	5.257
	Ostensible strength range in ng/ml		
	(15.751-15.759)	(10.522-10.528)	(5.253-5.259)
	Analyte peak region		
1	$1.061x10^5$	$0.748x10^5$	$0.342x10^5$
2	$1.066x10^5$	$0.756x10^5$	$0.333x10^5$
3	$1.064x10^5$	$0.749x10^5$	$0.330x10^5$
4	$1.069x10^5$	$0.732x10^5$	$0.364x10^5$
5	$1.073x10^5$	$0.726x10^5$	$0.351x10^5$
6	$1.075x10^5$	$0.738x10^5$	$0.335x10^5$
7	$1.061x10^5$	$0.349x10^5$	$0.349x10^5$
8	$1.074x10^5$	$0.755x10^5$	$0.351x10^5$
9	$1.082x10^5$	$0.764x10^5$	$0.355x10^5$
10	$1.056x10^5$	$0.761x10^5$	$0.342x10^5$
11	$1.047x10^5$	$0.774x10^5$	$0.363x10^5$
12	$1.055x10^5$	$0.753x10^5$	$0.347x10^5$
13	$1.061x10^5$	$0.742x10^5$	$0.338x10^5$
14	$1.062x10^5$	$0.749x10^5$	$0.326x10^5$
15	$1.078x10^5$	$0.736x10^5$	$0.339x10^5$
16	$1.069x10^5$	$0.738x10^5$	$0.341x10^5$
17	$1.057x10^5$	$0.769x10^5$	$0.374x10^5$
18	$1.042x10^5$	$0.772x10^5$	$0.371x10^5$
19	$1.066x10^5$	$0.774x10^5$	$0.369x10^5$
20	$1.053x10^5$	$0.758x10^5$	$0.364x10^5$
21	$1.072x10^5$	$0.743x10^5$	$0.350x10^5$
22	$1.081x10^5$	$0.750x10^5$	$0.355x10^5$
23	$1.049x10^5$	$0.749x10^5$	$0.362x10^5$
24	$1.063x10^5$	$0.764x10^5$	$0.361x10^5$
n	24	24	24

Average	$1.064x10^5$	$0.757x10^5$	$0.351x10^5$
SD	0.01059	0.01277	0.01355
%CV	0.99	1.69	3.87
% Average Accuracy	98.8%	98.6%	98.4%

Acceptance criteria: The reports of LQC, MQC and HQC samples shows less than or equal to 15 % and LL QC reports less than or equal to 20 %. The Percent average exactness is in between 80-115 for sixteen specimens out of twenty-four samples. At least 80% of the matrix lot should meet the acceptance requirements. The back measured concentration accuracy percent LQC, MQC & HQC is in the above boundaries and LL QC is in between 80-120 percent.

3.3 Freeze-Thaw stability: For six different concentrations of this drug samples the Freeze-Thaw stability study was carried and the results are placed in Table 3forBendroflumethiazide. It passed the freeze thaw stability.

Table: 3 BendroflumethiazideFreeze Thaw Stability

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.755	5.257	10.425
	Ostensible strength range in ng/ml		
	(15.751-15.759)	(5.252-5.259)	(10.321-10.581)
	Area of analyte signal		
1	$1.026x10^5$	$0.314x10^5$	$0.727x10^5$
2	$1.021x10^5$	$0.308x10^5$	$0.741x10^5$
3	$1.032x10^5$	$0.314x10^5$	$0.732x10^5$
4	$1.037x10^5$	$0.301x10^5$	$0.749x10^5$
5	$1.041x10^5$	$0.322x10^5$	$0.725x10^5$
6	$1.045x10^5$	$0.335x10^5$	$0.718x10^5$
n	6	6	6
Average	$1.034x10^5$	$0.316x10^5$	$0.732x10^5$
SD	0.00911	0.01178	0.01131
%CV	0.88	3.73	1.55
Average percent of accuracy	98.8%	98.5%	99.5%

Acceptance criteria: Thecriteria to accept this method have strength in between 85-115 % of LQC, HQC and the % CV is less than or equal to 15 %.

3.4 Wet method of Extract: At different time intervals of 12 hours, 18 hours Wet-Extract stabilitywas studied on these drugs reported the results are shown in Table 4 & Table 5. It was passed.

Table: 4 Bendroflumethiazide stability in Wet extract at 12 Hr

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.755	5.257	10.111
	Ostensible strength range in ng/ml		
	(15.751-15.759)	(5.252-5.259)	(10.079-10.222)
	Area of analyte signal		
1	$1.074x10^5$	$0.332x10^5$	$0.779x10^5$
2	$1.073x10^5$	$0.338x10^5$	$0.785x10^5$
3	$1.068x10^5$	$0.342x10^5$	$0.782x10^5$
4	$1.085x10^5$	$0.347x10^5$	$0.774x10^5$
5	$1.079x10^5$	$0.355x10^5$	$0.762x10^5$
6	$1.081x10^5$	$0.363x10^5$	$0.775x10^5$
n	6	6	6
Mean	$1.077x10^5$	$0.346x10^5$	$0.776x10^5$
SD	0.00615	0.01137	0.00808
%CV	0.57	3.29	1.04
% Mean Accuracy	99.8%	98.5%	98.1%

Table: 5 Bendroflumethiazide stability in Wet extract at 18 Hr

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.623	5.236	10.235
	Ostensible strength range in ng/ml		
	(15.521-15.759)	(5.212-5.259)	(10.104-10.368)
	Area of analyte signal		
1	$1.041x10^5$	$0.387x10^5$	$0.789x10^5$
2	$1.045x10^5$	$0.376x10^5$	$0.781x10^5$
3	$1.052x10^5$	$0.383x10^5$	$0.774x10^5$
4	$1.038x10^5$	$0.377x10^5$	$0.777x10^5$
5	$1.044x10^5$	$0.359x10^5$	$0.750x10^5$
6	$1.059x10^5$	$0.368x10^5$	$0.763x10^5$
n	6	6	6
Average	$1.047x10^5$	$0.375x10^5$	$0.772x10^5$
SD	0.00771	0.01018	0.01388

%CV	0.74	2.71	1.80
%Average Accuracy	99.1%	98.2%	98.8%

Acceptance criteria: The criteria to accept this method have strength in between 85-115 % of LQC, HQC and the % CV is less than or equal to 15 %.

3.5 Dry Extract: Dry Extract stability was performed at two different time intervals of 12 hours and 18 hours for this drug shows the reported results are accepted. The results are shown in Table 6& Table 7.

Table: 6 Bendroflumethiazide stability in Dry extract at 12 Hr

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.418	5.528	10.329
	Ostensible strength range in ng/ml		
	(15.328-15.629)	(5.310-5.759)	(10.215-10.426)
	Area of analyte signal		
1	$1.023x10^5$	$0.359x10^5$	$0.736x10^5$
2	$1.027x10^5$	$0.362x10^5$	$0.724x10^5$
3	$1.032x10^5$	$0.366x10^5$	$0.728x10^5$
4	$1.038x10^5$	$0.374x10^5$	$0.739x10^5$
5	$1.029x10^5$	$0.373x10^5$	$0.741x10^5$
6	$1.044x10^5$	$0.358x10^5$	$0.753x10^5$
n	6	6	6
Average	$1.032x10^5$	$0.365x10^5$	$0.737x10^5$
SD	0.00768	0.00692	0.01026
%CV	0.74	1.89	1.39
%Average Accuracy	99.5%	98.3%	98.7%

Table: 7 Bendroflumethiazide stability in Dry extract at 18 Hr

Replicate No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.529	5.341	10.255
	Ostensible strength range in ng/ml		
	(15.478-15.759)	(5.242-5.413)	(10.174-10.316)
	Area of analyte signal		
1	$1.027x10^5$	$0.341x10^5$	$0.741x10^5$
2	$1.022x10^5$	$0.358x10^5$	$0.732x10^5$
3	$1.036x10^5$	$0.363x10^5$	$0.747x10^5$

4	$1.029x10^5$	$0.372x10^5$	$0.758x10^5$
5	$1.034x10^5$	$0.355x10^5$	$0.712x10^5$
6	$1.040x10^5$	$0.348x10^5$	$0.726x10^5$
n	6	6	6
Average	$1.031x10^5$	$0.356x10^5$	$0.736x10^5$
SD	0.00656	0.01094	0.01626
%CV	0.64	3.07	2.21
Mean-accuracy	99.2%	98.4%	98.5%

Acceptance criteria: The criteria to accept this method have strength in between 85-115 % of LQC, HQC and the % CV is less than or equal to 15 %.

3.6 Short-Term Stability: The Short-Term study on these drugs for different strengths were studied and It was allowed. The results are shown in Table 8.

Table: 8 Bendroflumethiazide Short-Term Stability

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.315	5.758	10.621
	Ostensible strength range in ng/ml		
	(15.428-15.751)	(5.689-5.896)	(10.524-10.783)
	Area of analyte signal		
1	$1.036x10^5$	$0.357x10^5$	$0.787x10^5$
2	$1.047x10^5$	$0.341x10^5$	$0.782x10^5$
3	$1.028x10^5$	$0.358x10^5$	$0.786x10^5$
4	$1.041x10^5$	$0.366x10^5$	$0.796x10^5$
5	$1.055x10^5$	$0.350x10^5$	$0.790x10^5$
6	$1.063x10^5$	$0.374x10^5$	$0.791x10^5$
n	6	6	6
Mean	$1.045x10^5$	$0.358x10^5$	$0.789x10^5$
SD	0.01276	0.01160	0.00480
%CV	1.22	3.24	0.61
% Mean Accuracy	99.8%	98.7%	98.4%

Acceptance criteria: The criteria to accept this method have strength in between 85-115 % of LQC, HQC and the % CV is less than or equal to 15 %.

3.7 Long-Term Stability: In long term stability study, reveals how these drugs are stable can be studied for about 1, 7, 14, 21 and 28 days shows the %CV and average accuracy for

Bendroflumethiazide is found to be within the acceptable limit and it passed the Long-Term stability. The results are shown in Table 9 – Table 13.

Table: 9 Bendroflumethiazide Long-Term Stability at Day-1

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.358	5.758	10.621
	Ostensible strength range in ng/ml		
	(15.2758-15.512)	(5.258-5.896)	(10.524-10.778)
	Area of analyte signal		
1	$1.042x10^5$	$0.347x10^5$	$0.734x10^5$
2	$1.057x10^5$	$0.326x10^5$	$0.725x10^5$
3	$1.063x10^5$	$0.378x10^5$	$0.701x10^5$
4	$1.041x10^5$	$0.374x10^5$	$0.726x10^5$
5	$1.032x10^5$	$0.381x10^5$	$0.718x10^5$
6	$1.058x10^5$	$0.386x10^5$	$0.722x10^5$
n	6	6	6
Mean	$1.049x10^5$	$0.365x10^5$	$0.721x10^5$
SD	0.01219	0.02363	0.01114
%CV	1.16	6.47	1.54
Mean-Accurcay	98.8%	98.5%	98.2%

Table: 10 BendroflumethiazideLong-Term Stability at Day-7

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.125	5.268	10.241
	Ostensible strength range in ng/ml		
	(15.104-15.187)	(5.122-5.342)	(10.127-10.263)
	Area of analyte signal		
1	$0.942x10^5$	$0.325x10^5$	$0.704x10^5$
2	$0.957x10^5$	$0.326x10^5$	$0.705x10^5$
3	$0.963x10^5$	$0.324x10^5$	$0.701x10^5$
4	$0.941x10^5$	$0.325x10^5$	$0.706x10^5$
5	$0.932x10^5$	$0.328x10^5$	$0.708x10^5$
6	$0.958x10^5$	$0.327x10^5$	$0.702x10^5$
n	6	6	6
Mean	$0.948x10^5$	$0.325x10^5$	$0.704x10^5$
SD	0.01219	0.00147	0.00258
%CV	1.28	0.45	0.37
% Mean-Accuracy	90.71%	89.77%	98.59%

Table: 11 Bendroflumethiazide Long-Term Stability at Day-14

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.321	5.711	10.521
	Ostensible strength range in ng/ml		
	(15.212-15.341)	(5.676-5.831)	(10.502-10.683)
	Area of analyte signal		
1	$0.912x10^5$	$0.275x10^5$	$0.634x10^5$
2	$0.913x10^5$	$0.276x10^5$	$0.635x10^5$
3	$0.903x10^5$	$0.274x10^5$	$0.631x10^5$
4	$0.901x10^5$	$0.275x10^5$	$0.636x10^5$
5	$0.912x10^5$	$0.278x10^5$	$0.638x10^5$
6	$0.914x10^5$	$0.277x10^5$	$0.632x10^5$
n	6	6	6
Mean	$0.9092x10^5$	$0.275x10^5$	$0.634x10^5$
SD	0.00564	0.00147	0.00258
%CV	0.62	0.53	0.41
% Mean Accuracy	100.05%	75.96%	88.79%

Table: 12 Bendroflumethiazide Long-Term Stability at Day-21

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.324	5.158	10.121
	Ostensible strength range in ng/ml		
	(15.227-15.451)	(5.089-5.196)	(10.074-10.153)
	Area of analyte signal		
1	$0.852x10^5$	$0.255x10^5$	$0.594x10^5$
2	$0.853x10^5$	$0.256x10^5$	$0.595x10^5$
3	$0.853x10^5$	$0.254x10^5$	$0.591x10^5$
4	$0.851x10^5$	$0.255x10^5$	$0.596x10^5$
5	$0.852x10^5$	$0.258x10^5$	$0.598x10^5$
6	$0.854x10^5$	$0.257x10^5$	$0.592x10^5$
n	6	6	6
Average	$0.852x10^5$	$0.255x10^5$	$0.5943x10^5$
SD	0.00105	0.00147	0.00258
%CV	0.12	0.58	0.43
%Average Accuracy	81.53%	70.44%	83.19%

Table: 13 Bendroflumethiazide Long-Term Stability at Day-28

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	15.315	5.758	10.621
	Ostensible strength range in ng/ml		
	(15.428-15.751)	(5.689-5.896)	(10.524-10.783)
	Area of analyte signal		
1	$0.802x10^5$	$0.241x10^5$	$0.562x10^5$
2	$0.803x10^5$	$0.241x10^5$	$0.565x10^5$
3	$0.803x10^5$	$0.242x10^5$	$0.561x10^5$
4	$0.801x10^5$	$0.243x10^5$	$0.596x10^5$
5	$0.802x10^5$	$0.242x10^5$	$0.568x10^5$
6	$0.804x10^5$	$0.241x10^5$	$0.562x10^5$
n	6	6	6
Mean	$0.8025x10^5$	$0.2417x10^5$	$0.569x10^5$
SD	0.00105	0.00082	0.01348
%CV	0.13	0.34	2.37
% Mean Accuracy	76.79%	66.76%	79.69%

4. CONCLUSIONS

The proposed bio-analytical stability studies on Bendroflumethiazide constitutes several studies like Bench-Top stability, Auto sampler stability, Freeze Thaw stability, Wet Extraction stability, Dry Extract stability, Short term stability and Long term stabilityresults supports the method is validated and the drug Bendroflumethiazideshows good stability under the various experimental conditions repots their percentages of exactness is in between 85-115 %. The LQC, MQC and HQC samples shows less than or equal to 15 % and LL QC reports less than or equal to 20 %. The proposed methods meets the minimum criteria of 80% of the matrix lot. The reverse calculatedand measured strengths of accuracy percent LQC, MQC & HQC is in the above boundaries and LL QC is in between 80-120 %.

5. COMPETING INTERESTS DISCLAIMER:

The authors are declared there is no competing of interest to publish this work. There is no financial support to do this research work from any funding resources.

6. REFERENSES

1. Dvorak M M, Joussineau C D, Carter D H, Pisitkun T, Knepper M A, Gamba G, Kemp P J, Riccardi D., Thiazide Diuretics Directly Induce Osteoblast Differentiation and

Mineralized Nodule Formation by Interacting with a Sodium Chloride Co-Transporter in Bone. Journal of the American Society of Nephrology, 2007, Vol. 18(9), pp. 2509–2516.

2. Moser M., Fifty Years of Thiazide Diuretic Therapy for Hypertension. Archives of Internal Medicine, 2009, Vol. 169(20), pp. 1851–1851.
3. Sato A, Terata K, Miura H, Toyama K, Lober4iza F R, Hatoum O A, Saito T, Sakuma I, Gutterman D D., Mechanism of vasodilation to adenosine in coronary arterioles from patients with heart disease. American Journal of Physiology-Heart and Circulatory Physiology,2005, Vol. 288(4), pp.H1633-H1640.
4. Monroy A, Plata C, Hebert SC, Gamba G: Characterization of the thiazide-sensitive Na(+)-Cl(-) cotransporter: a new model for ions and diuretics interaction. Am J Physiol Renal Physiol. 2000 Jul;279(1):F161-9.
5. Naveen V. M. K, Veeraswami B andSrinivasa Rao G. High Response Bio-Analytical Validation approach of Nadolol and Bendroflumethiazide by LC-MS/MS on Rat plasma. International Journal of Research in Pharmaceutical Sciences. 2020; 11(SPL4), 2272-2279.
6. Naveen VMK, Veeraswami B and Srinivasa Rao G. Bio analytical validation for Nadolol and Bendroflumethiazide material. Mat. Tod. Proce 2021; 46(1): 503-505.
7. Veeraswami B and Naveen VMK, Development and validation of RP-HPLC method for the estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. Asian J. Pharm. Clin. Res 2019; 12(2): 267-271.
8. Naveen V. M. K. and Veeraswami B. Highly accurate and New approach for quantification of Gramicidin in medication by RP-HPLC. International Journal of Research in Pharmaceutical Sciences 2020;11(SPL4), 3053-3058.
9. Naveen V. M. K, Veeraswami B andSrinivasa Rao G. High Response Bio-Analytical Validation approach of Nadolol and Bendroflumethiazide by LC-MS/MS on Rat plasma. International Journal of Research in Pharmaceutical Sciences. 2020; 11(SPL4), 2272-2279.
10. Naveen VMK, Veeraswami B and Srinivasa Rao G. Bio analytical validation for Nadolol and Bendroflumethiazide material. Mat. Tod. Proce 2021; 46(1): 503-505.
11. Veeraswami B and Naveen VMK, Development and validation of RP-HPLC method for the estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. Asian J. Pharm. Clin. Res 2019; 12(2): 267-271.
12. Naveen V. M. K. and Veeraswami B. Highly accurate and New approach for quantification of Gramicidin in medication by RP-HPLC. International Journal of Research in Pharmaceutical Sciences 2020;11(SPL4), 3053-3058.