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Bio-Analytical Stability Studies of Nadolol Material

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The best practices of Bio-analytical stability studies on drug samples are very crucial and essential for the drugs development process as it specify the acceptancy, purity, efficacy, prediction of strength and quality of the drugs. The main objective of this stability studies on Nadolol the proposed approach of chromatographic separation was administered in isocratic way by using asymmetric C18 column of 40:60 percent of acetonitrile and 0.1% OPA at a flow rate of 1 ml/min is a quantitative measure for drug analysis in biological matrix for more reliable, selective, reproducible and sensitive. This stability study constituents several methods 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. The results of these stability studies are accepted based on ICH guidelines represents this drug has a good stability under the present experimental conditions.

Keywords: Bio-analytical; bench-top; auto-sampler; freeze-thaw; dry-extract.

1. INTRODUCTION

Nadolol was marketed, among others, under the brand Corgard, may be medicine won't for the prevention of elevated vital sign [1,2] heart pain and atrial fibrillation [3,4]. It's also been utilized to prevent migraine [5] head-aches and complications of cirrhosis [6,7]. It is taken orally.

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Popular side effects are dizziness has symptoms [8], feeling sluggish, a slow-moving feeling pulse [9] and Reynaud syndrome [10,11]. Its high intake has serious side effects on coronary Bronchospasm and loss [12]. This is used in breastfeeding, and nursing the defiance is uncertain. Everything is, it's A beta adrenergic blocker that is non-selective [13,14] and acts by suppressing adrenergic *β*1 receptors [15] the adrenergic neurons in the heart and *β2* in blood vessels.

Nadolol is one among the well-liked β blockers within the control of patients with LQT for QT interval shortening and ventricular arrhythmia avoidance [16,17]. It's more operative than cardio-selective beta-blockers [18] such as metoprolol and propranolol within the avoidance of breakthrough Cardiac incidents [19]. Nadolol has the benefit of daily dosing and thereby increases the weakness of the patient. It is for the person whose function of the kidney decreased, and nadolol could also provide less dose often. For many neurological illnesses, such as the prevention of migraine attacks, attention/deficit/hyperactivity disorder (ADHD), it is effective, and it has been explored as a treatment for tremor and Parkinson's disease [20] Still, neither is well established [21]. Some bioanalytical studies are also supports the proposed approaches [22-24]. Its structure is shown in Fig. 1. Based on importance of Nadolol the proposed bio-analytical stability study was studied.

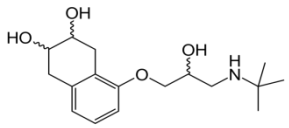

Fig. 1. Structure of nadolol

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

LCMS grade of Acetonitrile, Orthophosphoric acid and Water-HPLC graded were procured Merck Ltd. Worli, in Bombay, India. From Glenmark Pharmaceuticals, APIs of Nadolol and Bendroflumethiazide as reference standards were produced.

2.2 Instrumentation

LCMS, Make: SCIEX QTRAP 5500 Mass Spectrophotometer and Sciex software was used.

2.3 Standard and Quality Control Samples Preparation

2.3.1 Nadolol parent stock preparation

8 mg of Nadolol standard was accurately measured and dissolved in 100 ml of diluent (movable phase). Concentration of the solution is 80 µg/ml. Took 1 ml of the stock liquid saturated with 10 ml of the solvents. This is the parent stock solution of Nadolol and the concentration of the parent stock solution is 8µg/ml. In the same way internal standard parent stock was prepared.

2.3.2 Preparation standard of Nadolol solutions

The parent stock solutions of Nadolol parent stock liquid of 0.4 ml saturated into 10 ml vacuum bottles up to the mark with solvents have concentrations 320 ng/ml. In the same way internal standard stock solution was prepared.

2.3.3 Preparation of standard solution

Typical solution was prepared by taking 0.5 ml, 0.2 ml, 0.3 ml and 0.5 ml of parent liquid, 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 0.45 µ filtered then inoculated to HPLC system.

2.4 Preparation of Sample Solution

2.4.1 Sample stock preparation

One pill (contains 40 mg of Nadolol) 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 Nadolol concentration 320 ng/ml.

2.4.2 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.

2.5 Method Developed and Validation

A bioanalytical method was developed [25] and it was validated by the advanced analytical instruments like LC-MS / MS. The chromatographic conditions involve isocratic mode using Waters symmetry C_{18} (150x4.6 mm, 3.5 microns) column. A 0.1 per cent of OPA (orthophosphoric Acid) Acetonitrile and in 60:40 is employed and therefore the detection was administered during +ve mode of electron spray ionization by using LC/MS gives best results were published.

3. STABILITY STUDIES

Stability of stock solution was carried out by looking at Analyses global reaction inside the stability evaluation for the worldwide test reaction arranged through the current product structure. At the LQC and HQC concentration levels, plasma stability tests were carried out using six copies at each dose. If the shift is less than 15 percent as per US FDA guidance, Analyse was deemed stable. At room temperature, the steadiness of spiked rodent plasma experiments is placed aside; it was calculated for twenty four hrs. The safety of spiked rat plasma deposited in the auto sampler at 2-8^{o}C was measured for twenty-four hours. The durability of the auto sampler was tested by looking at the correctly infused extract plasma reports, with the samples re-injected at 2-8^{o}C for twenty four hours after storage in the auto sampler at 2-8°C twenty four hours. The reproducibility of reinjection was tested by looking at collected plasma tests that were injected promptly. With the samples that were reinvested in the wake of putting away in the auto sampler for 24 hours at 2-8^{o}C. The durability of the cold thaw was led by looking at the steadiness tests that they had been solidified at -30^{o}C except defrosted multiple occasionally, with newly spiked spikes internal samples for monitoring. Six LQC and HQC all quotes per focus the levels have been utilized for the freeze defrost soundness assessment. The concentration obtained after 24 hours were for long-term stability assessment contrasted and beginning fixation. The stability studies of some methods are discussed below.

3.1 Bench Top Stability

In this study 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 passed the Bench top stability.

3.1.1 The benchmark for approval

The Percent average exactness is in between 85-115 for eight specimens out of twelve samples. At least 80% of the matrix lot should meet the acceptance requirements. The back measured concentration accuracy percent CV of several biologic-matric have LQC and HQC is less than or equal to 15 percent.

Table 1. Nadolol stability results of Bench-Top method

Trial	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.244	40.486	80.367
	Ostensible strength range in ng/ml		
	(120.147-120.269)	(40.369-40.597)	(80.237-80.576)
	Analyte peak region		
1	$4.149x10^5$	$1.559x10^5$	$2.815x10^5$
2	$4.156x10^5$	$1.554x10^5$	$2.842x10^5$
3	$4.159x10^5$	$1.546x10^5$	$2.829x10^5$
4	$4.163x10^5$	$1.515x10^5$	$2.812x10^5$
5	$4.171x10^5$	$1.527x10^5$	$2.835x10^5$
6	$4.145x10^5$	$1.533x10^5$	$2.811x10^5$
n	6	6	6
Mean	$4.157x10^5$	$1.539x10^5$	$2.824x10^5$
SD	0.00943	0.01691	0.01315
%CV	0.23	1.10	0.47
% Mean-accuracy	98.2%	98.6%	98.9%

3.2 Auto Sampler Stability

Auto-sampler stability study was carried out on stable pooled solutions are placed in to auto-sampler inoculated to injector. The reports are placed in Table 2. It passed the Auto Sampler Stability.

3.2.1 The benchmark for approval

The repeatability results of LQC, MQC and HQC samples shows less than or equal to 15 percent and LL QC reports less than or equal to 20 percent. 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

Freeze-Thaw stability study was carried for this drug for six different strengths and the results are incorporated in Table 3 for Nadolol and it passed the freeze thaw stability.

Table 2. Auto Sampler Stability of Nadolol

Replicate No.	HQC	MQC 1	LQC
	Ostensible strength in ng/ml		
	120.334	80.497	40.432
	Ostensible strength range in ng/ml		
	(120.149-120.463)	(80.291-80.538)	(40.369-40.567)
	Area of analyte peak		
1	$4.134x10^5$	$2.852x10^5$	$1.527x10^5$
2	$4.139x10^5$	$2.864x10^5$	$1.524x10^5$
3	$4.140x10^5$	$2.828x10^5$	$1.531x10^5$
4	$4.146x10^5$	$2.836x10^5$	$1.523x10^5$
5	$4.145x10^5$	$2.854x10^5$	$1.522x10^5$
6	$4.138x10^5$	$2.826x10^5$	$1.531x10^5$
7	$4.126x10^5$	$2.844x10^5$	$1.527x10^5$
8	$4.145x10^5$	$2.861x10^5$	$1.524x10^5$
9	$4.148x10^5$	$2.872x10^5$	$1.531x10^5$
10	$4.151x10^5$	$2.851x10^5$	$1.523x10^5$
11	$4.162x10^5$	$2.861x10^5$	$1.522x10^5$
12	$4.174x10^5$	$2.836x10^5$	$1.531x10^5$
13	$4.144x10^5$	$2.827x10^5$	$1.527x10^5$
14	$4.159x10^5$	$2.852x10^5$	$1.524x10^5$
15	$4.138x10^5$	$2.843x10^5$	$1.531x10^5$
16	$4.146x10^5$	$2.839x10^5$	$1.523x10^5$
17	$4.141x10^5$	$2.868x10^5$	$1.522x10^5$
18	$4.135x10^5$	$2.877x10^5$	$1.531x10^5$
19	$4.138x10^5$	$2.872x10^5$	$1.527x10^5$
20	$4.127x10^5$	$2.861x10^5$	$1.524x10^5$
21	$4.160x10^5$	$2.853x10^5$	$1.531x10^5$
22	$4.165x10^5$	$2.848x10^5$	$1.523x10^5$
23	$4.129x10^5$	$2.867x10^5$	$1.522x10^5$
24	$4.147x10^5$	$2.843x10^5$	$1.531x10^5$
n	24	24	24
Mean	$4.145x10^5$	$2.851x10^5$	$1.526x10^5$
SD	0.01214	0.01488	0.00371
%CV	0.29	0.52	0.24
% Average Accuracy	98.3%	98.2%	98.5%

Table 3. Freeze Thaw Stability of Nadolol

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.244	40.486	80.574
	Ostensible strength range in ng/ml		
	(120.147-120.269)	(40.369-40.597)	(80.411-80.689)
	Area of analyte signal		
1	$4.105x10^5$	$1.521x10^5$	$2.828x10^5$
2	$4.108x10^5$	$1.528x10^5$	$2.826x10^5$
3	$4.116x10^5$	$1.519x10^5$	$2.821x10^5$
4	$4.119x10^5$	$1.513x10^5$	$2.827x10^5$
5	$4.125x10^5$	$1.534x10^5$	$2.839x10^5$
6	$4.108x10^5$	$1.542x10^5$	$2.808x10^5$
n	6	6	6
Average	$4.114x10^5$	$1.526x10^5$	$2.825x10^5$
SD	0.00777	0.01065	0.01015
% CV	0.19	0.70	0.36
Average percent of accuracy	98.2%	98.6%	99.2%

3.3.1 The benchmark for approval

The reported result from the approach shows that the correctness percent of the back calculated LQC, HQC strength are also in between 85-115 percent. The percent of CV is also less than or equal to 15 percent.

3.4 Wet Method of Extract

The Wet-Extract stability studies on this drug for about 12 hours and 18 hours. The results are shown in Table 4 & Table 5 and it was passed.

3.4.1 The benchmark for approval

The reported result from the approach shows that the correctness percent of the back calculated LQC, HQC strength are also in between 85-115 percent. The percent of CV is also less than or equal to 15 percent.

3.5 Dry Extract

This stability test was studied for this drug for about 12 hr and 18 hr respectively and it passed the Dry Extract stability. The results are shown in Table 6 & Table 7.

3.5.1 The benchmark for approval

The reported result from the approach shows that the correctness percent of the back calculated LQC, HQC strength are also in between 85-115 percent. The percent of CV is also less than or equal to 15 percent.

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.

3.6.1 The benchmark for approval

The reported result from the approach shows that the correctness percent of the back calculated LQC, HQC strength are also in between 85-115 percent. The percent of CV is also less than or equal to 15 percent.

3.7 Long-Term Stability

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

4. RESULTS AND DISCUSSION

Bench-Top study and Auto sampler stability study results average exactness is in between 85-115 % for eight specimens out of twelve samples. At least 80% of the matrix lot should meet the acceptance requirements. The back measured concentration accuracy percent CV of several biologic-matric have LQC and HQC is less than or equal to 15 percent shown in Table 1 & Table 2. The other stability studies Freeze

Thaw stability, Wet Extraction stability, Dry Extract stability, Short term stability and Long term stability results from Table 3 to Table 13 shows their percentages of exactness is in between 85-115 %. The repeatability results of LQC, MQC and HQC samples shows less than or equal to 15 percent and LL QC reports less than or equal to 20 percent. 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 as per ICH guidelines.

Table 4. Nadolol Stability in Wet extract at 12 Hr

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.244	40.486	80.122
	Ostensible strength range in ng/ml		
	(120.147-120.269)	(40.369-40.597)	(80.018-80.321)
	Area of analyte signal		
1	$4.156x10^5$	$1.548x10^5$	$2.859x10^5$
2	$4.151x10^5$	$1.539x10^5$	$2.866x10^5$
3	$4.162x10^5$	$1.527x10^5$	$2.861x10^5$
4	$4.174x10^5$	$1.521x10^5$	$2.870x10^5$
5	$4.169x10^5$	$1.535x10^5$	$2.852x10^5$
6	$4.178x10^5$	$1.544x10^5$	$2.863x10^5$
n	6	6	6
Average	$4.165x10^5$	$1.536x10^5$	$2.862x10^5$
SD	0.01051	0.01023	0.00618
%CV	0.25	0.67	0.22
Average percent of accuracy	98.2%	98.6%	98.9%

Table 5. Wet Extract Stability of Nadolol at 18 Hr

Trial No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.227	40.329	80.143
	Ostensible strength range in ng/ml		
	(120.117-120.245)	(40.269-40.496)	(80.059-80.295)
	Area of analyte signal		
1	$4.169x10^5$	$1.555x10^5$	$2.808x10^5$
2	$4.177x10^5$	$1.559x10^5$	$2.816x10^5$
3	$4.165x10^5$	$1.542x10^5$	$2.811x10^5$
4	$4.173x10^5$	$1.541x10^5$	$2.824x10^5$
5	$4.181x10^5$	$1.536x10^5$	$2.819x10^5$
6	$4.170x10^5$	$1.549x10^5$	$2.829x10^5$
n	6	6	6
Average	$4.173x10^5$	$1.547x10^5$	$2.818x10^5$
SD	0.00579	0.00888	0.00788
%CV	0.14	0.57	0.28
Average percent of accuracy	97.3%	98.1%	98.4%

Table 6. Nadolol Stability in Dry extract at 12 Hr

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.132	40.435	80.215
	Ostensible strength range in ng/ml		
	(120.028-120.164)	(40.129-20.426)	(80.116-80.366)
	Area of analyte signal		
1	$4.188x10^5$	$1.556x10^5$	$2.868x10^5$
2	$4.183x10^5$	$1.562x10^5$	$2.863x10^5$
3	$4.176x10^5$	$1.571x10^5$	$2.896x10^5$
4	$4.192x10^5$	$1.576x10^5$	$2.881x10^5$
5	$4.184x10^5$	$1.568x10^5$	$2.876x10^5$
6	$4.196x10^5$	$1.553x10^5$	$2.858x10^5$
n	6	6	6
Mean	$4.187x10^5$	$1.564x10^5$	$2.874x10^5$
SD	0.00709	0.00891	0.01378
% of CV	0.17	0.57	0.48
% of Mean-accuracy	98.6%	98.5%	98.4%

Table 7. Nadolol Stability in Dry extract at 18 Hr

Replicate No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.369	40.528	80.284
	Ostensible strength range in ng/ml		
	(120.187-120.425)	(40.412-40.688)	(80.127-80.356)
	Area of analyte signal		
1	$4.126x10^5$	$1.517x10^5$	$2.847x10^5$
2	$4.135x10^5$	$1.574x10^5$	$2.865x10^5$
3	$4.141x10^5$	$1.528x10^5$	$2.852x10^5$
4	$4.144x10^5$	$1.536x10^5$	$2.863x10^5$
5	$4.137x10^5$	$1.552x10^5$	$2.875x10^5$
6	$4.129x10^5$	$1.574x10^5$	$2.888x10^5$
n	6	6	6
Mean	$4.135x10^5$	$1.547x10^5$	$2.865x10^5$
SD	0.00689	0.02394	0.01501
% of CV	0.17	1.55	0.52
Mean-accuracy	97.7%	97.9%	98.1%

Table 8. Nadolol Short-Term Stability

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.254	40.127	80.168
	Ostensible strength range in ng/ml		
	(120.143-120.362)	(40.039-40.239)	(80.057-80.274)
	Area of analyte signal		
1	$4.169x10^5$	$1.526x10^5$	$2.867x10^5$
2	$4.141x10^5$	$1.535x10^5$	$2.879x10^5$
3	$4.147x10^5$	$1.542x10^5$	$2.872x10^5$
4	$4.156x10^5$	$1.544x10^5$	$2.884x10^5$
5	$4.166x10^5$	$1.558x10^5$	$2.881x10^5$
6	$4.132x10^5$	$1.574x10^5$	$2.892x10^5$
n	6	6	6

Trial no.	HQC	LQC	MQC
Average	$4.152x10^5$	$1.547x10^5$	$2.879x10^5$
SD	0.01447	0.01713	0.00884
% of CV	0.35	1.11	0.31
Average-accuracy	99.4%	99.2%	99.6%

Table 9. Nadolol Long-Term Stability at Day-1

Trial no.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.178	**40.153**	**80.146**
	Ostensible strength range in ng/ml		
	(120.143-120.234)	**(40.084-40.963)**	**(80.084-80.232)**
	Area of analyte signal		
1	$4.117x10^5$	$1.545x10^5$	$2.858x10^5$
2	$4.106x10^5$	$1.553x10^5$	$2.821x10^5$
3	$4.128x10^5$	$1.532x10^5$	$2.833x10^5$
4	$4.135x10^5$	$1.541x10^5$	$2.825x10^5$
5	$4.142x10^5$	$1.522x10^5$	$2.837x10^5$
6	$4.121x10^5$	$1.549x10^5$	$2.814x10^5$
n	6	6	6
Average	$4.125x10^5$	$1.540x10^5$	$2.831x10^5$
SD	0.01295	0.01152	0.01545
% of CV	0.31	0.75	0.55
Average-accuracy	99.2%	99.8%	99.5%

Table 10. Nadolol Long-Term Stability at Day-7

Replicate No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.351	**40.262**	**80.326**
	Ostensible strength range in ng/ml		
	(120.213-120.372)	**(40.139-40.369)**	**(80.257-40.416)**
	Area of analyte signal		
1	$3.917x10^5$	$1.424x10^5$	$2.638x10^5$
2	$3.906x10^5$	$1.423x10^5$	$2.631x10^5$
3	$3.928x10^5$	$1.422x10^5$	$2.643x10^5$
4	$3.935x10^5$	$1.421x10^5$	$2.625x10^5$
5	$3.942x10^5$	$1.422x10^5$	$2.621x10^5$
6	$3.921x10^5$	$1.429x10^5$	$2.628x10^5$
n	6	6	6
Average	$3.924x10^5$	$1.423x10^5$	$2.631x10^5$
SD	0.01295	0.00028	0.00822
%CV	0.33	0.20	0.30
Average-Accuracy	93.71%	93.18%	97.77%

Table 11. Nadolol Long-Term Stability at Day-14

Replicate No.	HQC	LQC	MQC
	Ostensible strength in ng/ml		
	120.278	**40.116**	**80.185**
	Ostensible strength range in ng/ml		
	(120.174-120.386)	**(40.047-40.223)**	**(80.077-80.242)**
	Area of analyte signal		

1	$3.691x10^5$	$1.264x10^5$	$2.528x10^5$
2	$3.692x10^5$	$1.263x10^5$	$2.511x10^5$
3	$3.691x10^5$	$1.262x10^5$	$2.523x10^5$
4	$3.690x10^5$	$1.261x10^5$	$2.525x10^5$
5	$3.691x10^5$	$1.262x10^5$	$2.521x10^5$
6	$3.691x10^5$	$1.269x10^5$	$2.528x10^5$
n	6	6	6
Average	$3.691x10^5$	$1.263x10^5$	$2.522x10^5$
SD	0.00063	0.00288	0.00635
% CV	0.02	0.23	0.25
Average-Accuracy	88.15%	82.71%	93.68%

Table 12. Nadolol Long-Term Stability at Day-21

Trial no.	**HQC**	**LQC**	**MQC**
	Ostensible strength in ng/ml		
	120.145	**40.218**	**80.365**
	Ostensible strength range in ng/ml		
	(120.093-120.202)	**(40.189-40.279)**	**(80.257-80.394)**
	Area of analyte signal		
1	$3.481x10^5$	$1.194x10^5$	$2.328x10^5$
2	$3.482x10^5$	$1.193x10^5$	$2.311x10^5$
3	$3.481x10^5$	$1.192x10^5$	$2.323x10^5$
4	$3.483x10^5$	$1.191x10^5$	$2.325x10^5$
5	$3.481x10^5$	$1.192x10^5$	$2.321x10^5$
6	$3.481x10^5$	$1.199x10^5$	$2.328x10^5$
n	6	6	6
Average	$3.481x10^5$	$1.193x10^5$	$2.3227x10^5$
SD	0.00084	0.00288	0.00635
%CV	0.02	0.24	0.27
% Average Accuracy	83.13%	89.63%	83.97%

Table 13. Nadolol Long-Term Stability at Day-28

Trial no.	**HQC**	**LQC**	**MQC**
	Ostensible strength in ng/ml		
	120.254	**40.127**	**80.168**
	Ostensible strength range in ng/ml		
	(120.143-120.362)	**(40.039-40.239)**	**(80.057-80.274)**
	Area of analyte signal		
1	$3.276x10^5$	$1.124x10^5$	$2.248x10^5$
2	$3.282x10^5$	$1.123x10^5$	$2.241x10^5$
3	$3.281x10^5$	$1.122x10^5$	$2.243x10^5$
4	$3.283x10^5$	$1.121x10^5$	$2.245x10^5$
5	$3.281x10^5$	$1.122x10^5$	$2.241x10^5$
6	$3.281x10^5$	$1.129x10^5$	$2.248x10^5$
n	6	6	6
Average	$3.2807x10^5$	$1.1235x10^5$	$2.2443x10^5$
SD	0.00242	0.00288	0.00320
%CV	0.07	0.26	0.14
% Average Accuracy	78.22%	73.54%	83.35%

5. CONCLUSIONS

The prosed method is validated and the stability of the drug Nadolol was shows good stability by studying various stability studies like Bench-Top stability, Auto sampler stability, Freeze Thaw stability, Wet Extraction stability, Dry Extract stability, Short term stability and Long term

stability results shows the method is very quick, reliable and cost effective as per ICH guidelines.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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