Development and Validation of an Analytical Procedure to Measure Residual Solvents in Betaxolol Hydrochloride

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ABSTRACT

Any pharmaceutical industry's primary goal is to consistently manufacture goods with the required qualities and attributes at a reasonable cost. The discovery, development, and assessment of medications in pharmaceutical formulations depend on the creation of a methodology. This review article's primary goal was to examine the evolution and validation of the process used for the drug from the beginning of formulation to the full commercial batch of product. The results must be trustworthy when an analytical method is used to generate results for the quality of samples related to medicine. The pharmaceutical sector has published validation policies that outline how to conduct validation, methods of validation and how the validation policy conforms to the requirements of GMP legislation. Validation is crucial to the efficient operation of pharmaceutical companies. Every step of the process, from the raw material to the final product, was validated for stability. The validation parameters are described in terms of accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), and system appropriateness testing using specific substances as an example. The method was appropriately established. The routine and stability analysis make use of every validation parameter.

KEYWORDS

Validation, Limit of quantitation, Limit of detection, system suitability and specificity, Linearity,

INTRODUCTION

From a clinical and toxicological perspective, determining the enantiomeric purity of medications requires careful consideration. Before a novel racemic medication is approved, the enantiomers must be clearly and analytically separated, and each enantiomer's pharmacological effects and metabolic routes must be investigated independently [1]. This suggests that the need for relevant enantioselective technology and pure enantiomeric substances will only grow. In every phase of the drug development process, enantiomeric resolutions have gained importance. Thus, it is more than required to develop novel techniques for effective chiral separations and quantification [2].

Betaxolol hydrochloride, a selective βblocker with a sustained effect that blocks the majority of β1-adrenergic receptors, is one of the medications in this class. exhibits a hypotensive action and guards against hypertension brought on by mental stress, physical exertion, and other variables [3].

As a result, the solvent may occasionally be an essential component of the synthetic process and may not be eliminated during the production process. To comply with safety-based restrictions,

ingredient and product specifications, good manufacturing practices, or other quality-based requirements, residual solvents should be eliminated to the greatest extent possible because they have no therapeutic benefit. Determining permissible residual solvent levels in pharmaceutical medication and dietary supplement items for patient safety is the aim of this[4-5].

The same medicinal drugs are made by many producers using various organic solvents. As a result, in pharmaceutical analysis and control, residual solvent analysis becomes a difficult analytical task[6]. Regular quality control testing often reveals unknown residual solvents. When using the current official techniques for their determination, a mistake could happen. Therefore, we must create a quick, sensitive technique that can detect and measure all leftover medications [7].

[In this research paper, the solvents of active pharmaceutical ingredients (APIs) were examined for residual solvents using a validated gas chromatography (GC) method. Acetone, ethyl acetate, T-butyl alcohol, benzene, tetrahydrofuran, Dimethyl amide and toluene were the solvents that were examined. Specificity, precision, linearity, limit of detection (LOD), and limit of quantitation (LOQ). Is the part of the method validation process, which was carried out in compliance with ICH principles. System suitability tests verified that all parameters were within acceptable bounds and that the procedure performed as intended.

Material and methods

Chemicals and Reagents: Samples of Betaxolol Hydrochloride were obtained from the Pharmaceutical Company. Analytical-grade chemicals were used. Analytical and HPLC Grade Chemicals were used without any purification. Acetone, Pet Ether and N-Methyl-2-pyrrolidone from Merck Company. Tetrahydrofuran, Tertiary butanol, Acetic Acid and Isopropyl Alcohol from LOBA company. Cyclohexane, Benzene and Toluene from SD-Fine chemicals, Dimethyl acetamide from Baker and Dichloromethane from Thermo chemicals.

Instrument: GCHS, RDS/AL/GC-1, Instrument made by Perkin–Elmer and Analytical Balance by Contech.

Column: DB-5 (30m x 0.53 mm x 5.0 µm), Agilent with no AMD/CAP/050

Objective: Validated the GCHS method for the determination of Residual solvents in Betaxolol HCI and to demonstrate that the method is appropriate for its intended use.

Scope*:* The report is applicable only for the determination of Residual solvents in Betaxolol HCI as per the method of analysis.

Background: Acetone, Dichloromethane, Tertiary butanol, Petroleum ether, Cyclohexane, tetrahydrofuran, and Dimethyl acetamide were used as the solvents in the synthesis process of key starting materials of Betaxolol HCI and API. So, the content of Residual solvents in Betaxolol HCI has to be determined. The GCHS method has been developed In-house for the determination of

Residual solvents in Betaxolol HCl. The method was validated as per the current ICH guidelines in order to give evidence of its reliability and suitability at Supriya Life Science Ltd.

RESULTS AND DISCUSSION:

***System suitability*:** System suitability validation is a quick pre-analysis check that the analytical system is operating properly and is suitable for the planned run [8]. It is carried out before each batch of samples to ensure that parameters like theoretical plates, peak resolution, retention time repeatability, and signal-to-noise satisfy predetermined acceptance criteria. System suitability was determined by performing six replicate injections of the standard solution as per the method of analysis and analyzed.

Table-l: Summary of System Suitability

Inj No.	Acetone		Tertiary butanol		Dichloromethane	
	RT (Mins)	Area	RT (Mins)	Area	RT (Mins)	Area
Inj-1	8.959	2860932	10.280	1248889	10.877	72411
Inj-2	8.964	2817225	10.284	1227954	10.885	71521
Inj-3	8.943	2858896	10.266	1223429	10.865	70128
Inj-4	9.945	2764292	10.268	1190630	10.869	68167
Inj-5	8.943	2779092	10.265	1198505	10.863	70146
Inj-6	8.945	2812275	10.270	1224918	10.865	75742
Average	**8.950**	**2815452**	**10.272**	**1219054**	**10.871**	**71353**
SD	**0.009**	**39775.5**	**0.01**	**21221.57**	**0.01**	**2589.17**
% RSD	**0.10**	**1.42**	**0.08**	**1.74**	**0.08**	**3.63**

Table-2: Summary of System Suitability

Inj No.	Pet Ether-1		Pet Ether-2		Pet Ether-3		Sum of all functions
	RT (Mins)	Area	RT (Mins)	Area	RT (Mins)	Area	
Inj-1	12.432	219193	13.225	175457	14.098	430710	825360
Inj-2	12.435	214005	13.243	170727	14.107	429808	814540
Inj-3	12.415	221025	13.212	176907	14.077	433816	831748
Inj-4	12.416	266898	13.224	167769	14.087	417802	792469
Inj-5	12.415	217468	13.215	174983	14.088	429835	822286
Inj-6	12.417	218629	13.223	171079	14.088	429492	819200
Average	**12.422**	**-**	**13.224**	**-**	**14.090**	**-**	**817601**
SD	**0.009**	**-**	**0.01**	**-**	**0.01**	**-**	**13608**
% RSD	**0.07**	**-**	**0.08**	**-**	**0.07**	**-**	**1.66**

Table-3: Summary of System Suitability

Inj No.	Tetrahydrofuran		Cyclohexane		Toluene		Dimethyl acetamide	
	RT (Mins)	Area	RT (Mins)	Area	RT (Mins)	Area	RT (Mins)	Area
Inj-1	16.090	528306	18.252	2066408	29.984	304704	28.605	15486
Inj-2	16.020	521730	18.264	2028486	24.997	302577	28.609	15700
Inj-3	15.995	527844	18.236	2064124	24.975	302211	28.584	15656
Inj-4	15.999	512528	18.237	2019054	24.979	297097	28.595	15117
Inj-5	16.001	517449	18.241	2030147	24.975	293219	28.591	14918
Inj-6	16.001	520423	18.244	2070126	24.984	302438	28.593	15433
Average	**16.004**	**521380**	**18.245**	**2046391**	**24.982**	**300374**	**28.596**	**15385**
SD	**0.009**	**0.01**	**0.01**	**22848.45**	**0.01**	**4316.70**	**0.01**	**308.25**
% RSD	**0.06**	**1.17**	**0.06**	**1.12**	**0.03**	**1.44**	**0.03**	**2.00**

Acceptance criteria: % RSD of the area of six replicate injections of each residual solvent from the standard solution should be NMT 10.0.

Specificity: ICH Q2(R2)[9] requires proof that no interfering signal compromises identification or quantification. Specificity is the analytical method's capacity to detect the intended analyte unambiguously in the presence of anticipated components (impurities, degradants, excipients, and matrix). Each solvent and a solvent mixture solution was made separately, and each solution was examined using the appropriate analysis technique. Table 4 provides a summary of the specificity results.

Table-4: Specificity

Solvents	RT (mins)	RRT
isopropyl amine	8.620	0.28
Acetone	8.948	0.29
Tertiary butanol	10.268	0.33
Dichloromethane	10.864	0.35
Pet Ether-I	12.415	0.40
Pet Ether-II	13.220	0.42
Pet Ether-III	14.087	0.45
Pet Ether-IV	16.061	0.51
Tetrahydrofuran	15.996	0.51
Cyclohexane	18.241	0.58
Toluene	24.983	0.80
Dimethyl acetamide	28.579	0.92
Benzene	18.175	0.58
Cyclopropyl methyl bromide	24.625	0.79
NMP	31.189	1.00

Acceptance criteria: Peaks due to Residual solvents should be adequately resolved from each other and from the diluent.

Limit of detection and limit of quantitation: According to ICH Q2(R2)[9], the limit of quantitation (LOQ) is the lowest amount that can be quantified with reasonable precision and accuracy, and the limit of detection (LOD) is the lowest amount of analyte that can be detected (but not necessarily quantified); both are defined and advised to be estimated using statistical techniques like the standard deviation/slope approach or the signal to noise method. "The lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value" is the definition of limit of detection, or LOD. "The lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy" is the definition of limit of quantification, or LOQ.

Six replicate injections with a low concentration of residual solvents were used to assess the limit of detection and quantitation. Tables 5 through 11 summarise the relative standard deviation and limit of detection and quantitation value for residual solvents.

Table-5: RSD of Limit of detection

Injection No	Area of Acetone	Area of t- Butanol	Area of Dichloromethane
1	28697	11431	35513
2	29299	10556	43486
3	27627	11622	40462
4	29343	11538	39591
5	27286	10723	39707
6	26931	12242	33698
Mean	**28197**	**11352**	**38743**
SD	**1052.17**	**622.11**	**3549.21**
% RSD	**3.73**	**5.48**	**9.16**

Table-6: RSD of Limit of detection

Inj.No	Area PET Ether-I	Area PET Ether-II	Area PET Ether-III	Sum of All Fractions
1	7037	6415	19718	33170
2	7481	6907	18927	33315
3	8507	7033	18749	34289
4	9291	6927	19705	35923
5	9333	6048	18836	34217
6	9076	6613	18360	34049
Mean	**-**	**-**	**19049**	**34161**
SD	**-**	**-**	**-**	**983.31**
% RSD	**-**	**-**	**-**	**2.88**

Table-7: RSD of Limit of detection

Inj. No	Area of tetrahydrofuran	Area of Cyclohexane	Area of Toluene	Area of Dimethyl acetamide
1	23517	16599	18366	4551
2	24601	16402	18135	4556
3	22987	16403	18505	5295
4	24809	17018	18209	4365
5	23331	16535	18785	4176
6	23158	16434	18624	3412
Mean	**23734**	**16565**	**18438**	**4393**
SD	**775.45**	**235.47**	**248.79**	**612.43**
% RSD	**3.27**	**1.42**	**1.35**	**13.94**

Table-8:RSD of Limit of Quantitation

Injection No	Area of Acetone	Area of t- Butanol	Area of Dichloromethane
1	82314	35498	75063
2	83026	35984	77566
3	82486	35600	76202
4	81831	35865	77984
5	80858	34723	73284
6	82265	37089	70961
Mean	**82130**	**35793**	**74646**
SD	**733.35**	**773.36**	**2683.59**
% RSD	**0.89**	**2.16**	**3.60**

Table-9: RSD of Limit of Quantitation

Inj.No	Area PET Ether-I	Area PET Ether-II	Area PET Ether-III	Sum of All Fractions

1	21294	16732	42938	80964
2	24615	12888	43120	80623
3	21826	16285	41869	79980
4	23382	17400	36857	77639
5	22958	15624	41068	79650
6	24109	16821	20198	61128
Mean	**-**	**-**	37675	**76664**
SD	**-**	**-**	**-**	**7699.27**
% RSD	**-**	**-**	**-**	**10.04**

Table-10:RSD of Limit of Quantitation

Inj. No	Area of tetrahydrofuran	Area of Cyclohexane	Area of Toluene	Area of DMA
1	65995	47316	53123	9048
2	67366	48366	54811	8442
3	66599	48188	54986	8482
4	67631	47999	53798	8256
5	66121	47362	53588	8121
6	67042	48284	55546	8167
Mean	**66972**	**47918**	**54309**	**8419**
SD	**666.12**	**465.29**	**940.57**	**340.30**
% RSD	**1.00**	**0.97**	**1.73**	**4.04**

Table-11: Limit of Detection and Quantitation

Solvents	LOD		LOQ	
	Conc. ppm	Conc. %	Conc. ppm	Conc. %
Acetone	5.25	1.00	15.22	2.91
t-Butanol	5.06	1.01	15.09	3.01
Dimethyl methane	12.54	20.56	29.07	47.66
Pet Ether	1.02	3.44	3.04	10.26
Tetrahydrofuran	3.01	4.11	9.00	12.30
Cyclohexane	3.01	0.77	8.99	2.31
Toluene	5.08	5.68	15.25	17.06
Dimethyl acetamide	22.05	15.90	54.81	39.51

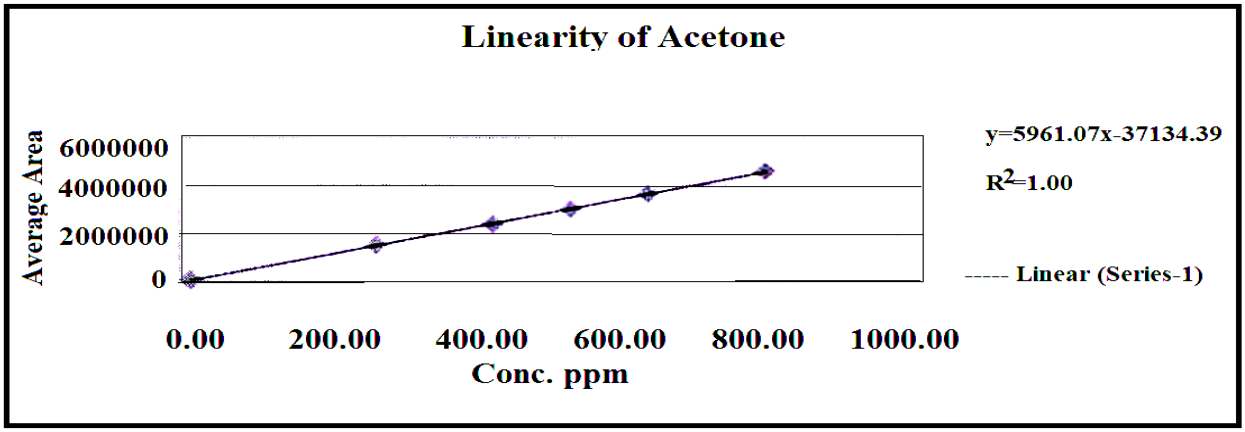
Acceptance criteria: NMT 30.0% should be the RSD of six replicate injections of the limit of detection solution. NMT 15.0% should be the RSD of six duplicate injections of the quantitation solution limit.

Linearity: The ability of the analytical process to produce test results that are directly proportionate to the analyte concentration in the sample, or by a precise mathematical translation, falls under the category of linearity. Verify proportional response to ensure that the instrument signal can be used to accurately calculate concentration. Provide evidence for the method's range by illustrating the permissible range for linearity, accuracy, and precision. By examining residual solvent ranging from LOQ to 150% (LOQ, 50%, 80%, l00%, 120%, and 150%) of the specification limit, the linearity of the GCHS method was shown. Tables and graphs provide a summary of the results.

Table 12: Linearity for Acetone

Level	Conc. ppm	Area-1	Area-2	Area-3	Avg. Area

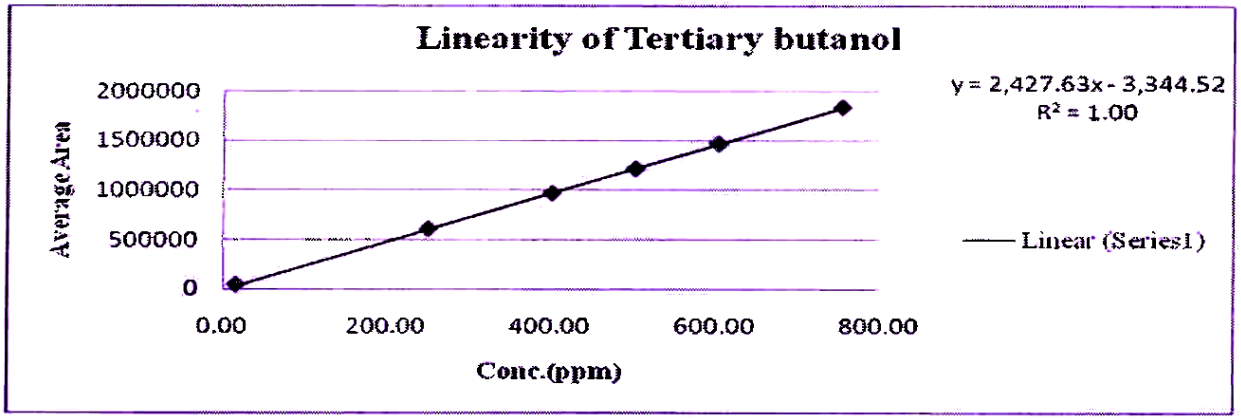
I (LOQ)	15.42	65866	62959	66683	65048
II (50%)	261.45	1538341	1539201	1533982	1537175
III (80%)	418.32	2437904	2419234	2418283	2425140
IV (100%)	522.90	3034775	3059082	3078466	3057441
V (120%)	627.48	3728826	3722972	3664302	3705367
VI (150%)	784.35	46833453	4682275	4623508	4663079



Graph-1: Linearity plot for Acetone

Table 13: Linearity for Tertiary Butanol

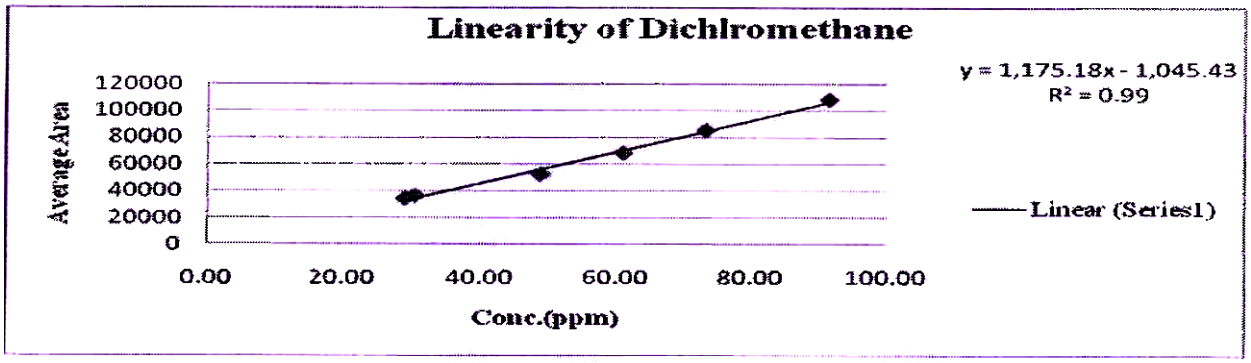
Level	Conc. ppm	Area-1	Area-2	Area-3	Avg. Area
I (LOQ)	15.09	38364	38593	39017	38658
II (50%)	250.99	605453	603239	608735	605809
III (80%)	401.59	965594	963076	958223	965809
IV (100%)	501.99	1205092	1207003	1120363	1210819
V (120%)	602.38	1468821	1475587	1442607	1462338
VI (150%)	752.98	1845854	1828473	1815210	1829846



Graph-2: Linearity for Tertiary Butanol

Table 14: Linearity for Dichloromethane

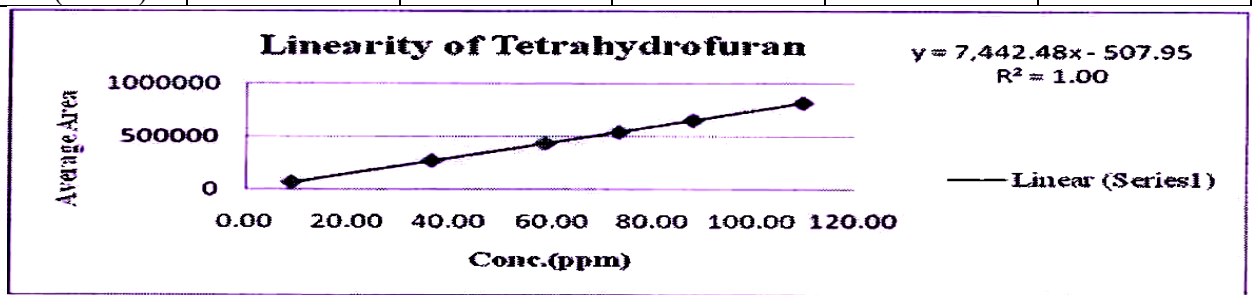
Level	Conc. ppm	Area-1	Area-2	Area-3	Avg. Area
I (LOQ)	29.07	35125	33945	34783	34618
II (50%)	30.50	36662	36096	37334	36697
III (80%)	48.80	52921	53262	51900	52694
IV (100%)	61.00	67664	67844	69839	68449
V (120%)	73.21	86475	86498	83887	85620
VI (150%)	91.51	109109	108474	107214	109266



Graph-3: Linearity for Dichloromethane

Table 15: Linearity for Tetrahydrofuran

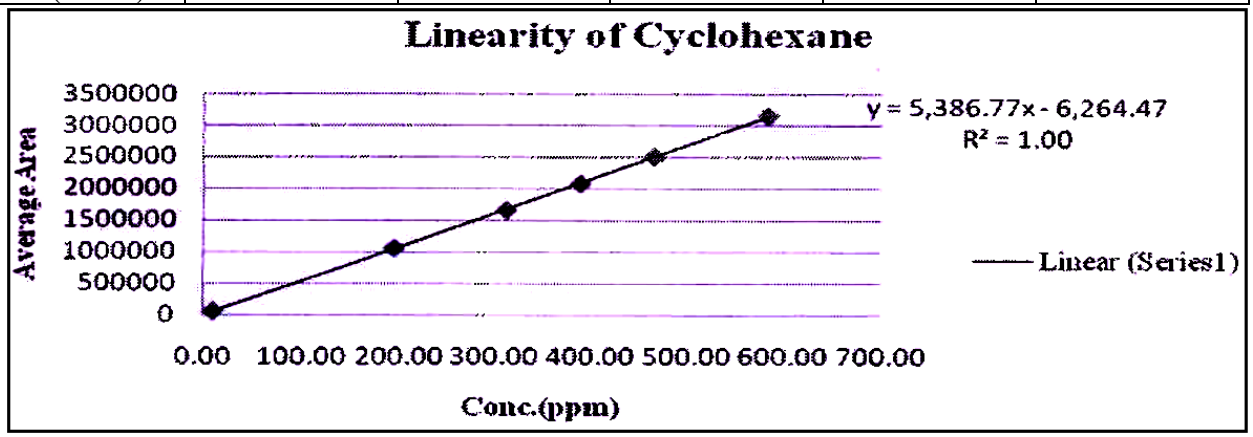
Level	Conc. ppm	Area-1	Area-2	Area-3	Avg. Area
I (LOQ)	9.00	67357	67507	67636	67500
II (50%)	36.62	271606	271054	274162	272274
III (80%)	58.59	436333	436713	431508	434851
IV (100%)	73.24	540349	538872	547768	542330
V (120%)	87.89	653584	658639	648289	653504
VI (150%)	109.86	825777	818622	812342	818914



Graph-4: Linearity for Tetrahydrofuran

Table 16: Linearity for Cyclohexane

Level	Conc. ppm	Area-1	Area-2	Area-3	Avg. Area
I (LOQ)	8.99	47792	47344	47414	47517
II (50%)	194.30	1046420	1051284	1053475	1050393
III (80%)	31089	1664898	1649195	1645975	1653356
IV (100%)	388.61	2060595	2069562	2095296	2075151
V (120%)	466.33	2508072	2516916	2478760	2501249
VI (150%)	582.91	3176390	3150531	3122893	3149938



Graph-5: Linearity for Cyclohexane

Table 17:Final Results

1. System suitability	**Component**	**% RSD**					
	Acetone	1.41					
	Tertiary butanol	1.74					
	Dichloromethane	3.63					
	Pet Ether	1.66					
	Tetrahydrofuran	1.17					
	Cyclohexane	1.12					
	Toluene	1.44					
	Dimethyl acetamide	2.00					
2. Specificity	Peak due to each residual solvent should be adequately resolved from other solvents and from the diluent.						
3. Limit of Detection and Limit of Quantitation	**Component**	**LOD**			**LOQ**		
		ppm	%	%RSD	ppm	%	%RSD
	Acetone	5.25	1.00	3.73	15.22	2.89	0.91
	Tertiary butanol	5.06	1.01	5.48	15.09	3.99	2.18
	Dichloromethane	12.54	20.56	9.16	29.07	47.70	3.59
	Pet Ether	1.02	3.44	2.88	3.04	1.28	10.04
	Tetrahydrofuran	3.01	4.11	3.27	9.00	12.28	1.01
	Cyclohexane	3.01	0.77	1.42	8.99	2.29	0.98
	Toluene	5.08	5.68	1.35	15.24	17.15	1.74
	Dimethyl acetamide	22.05	15.90	13.94	5479	39.49	4.05
4. Linearity & Range	**Component**	Correlation coefficient(r)			% y-intercept		
	Acetone	1.00			-1.2145		
	Tertiary butanol	1.00			-0.2759		
	Dichloromethane	1.00			-1.5270		
	Pet Ether	1.00			-1.1739		
	Tetrahydrofuran	1.00			-0.0938		
	Cyclohexane	1.00			-0.3020		
	Toluene	1.00			0.1580		
	Dimethyl acetamide	1.00			16.9059		
5. Accuracy	% Recovery obtained is between 85.1 I to 128.26 for all residual solvents at LOQ level % RSD of recovery at LOQ level is between 0. 1 8 to 5 .92 for all residual solvents. %o Recovery obtained is between 87.75 to 109.12 for all residual solvents at 50 % to 150% level. % RSD of recovery at 50 o/o to l50Yo level is between 0.21 to 2.07 for all residual solvents.						

CONCLUSION

To guarantee conformity with ICH criteria, the devised approach was validated. It was investigated how Betaxolol Hydrochloride behaved under different stress scenarios. It was discovered that the approach was straightforward, resilient, accurate, precise, and selective. Consequently, this technique can be applied to both routine testing and stability study of the medicinal ingredient Betaxolol Hydrochloride. Every statistical result met the requirements for acceptance. The technique may be useful for detecting contaminants in pharmaceutical formulations as well as for routinely assessing the quality of Betaxolol Hydrochloride in bulk drug manufacturing facilities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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