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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE DETERMINATION AND ESTIMATION OF ASENAPINE MALEATE IN BULK AND BUCCAL (EFFERVESCENT) DOSAGE FORM

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Keywords:

Asenapine Maleate, Antipsychotic, RS-HPLC, ICH-guideline

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ABSTRACT: Asenapine maleate is an atypical antipsychotic drug. It was approved by USFDA in August 2009. It is an antagonist of 5-HT dopamine and α-adrenergic receptors and a high affinity for dopamine D_2 and serotonin 5-HT_{2A} receptors. It is indicated for the treatment of various psychotic conditions like schizophrenia and bipolar disorder in adults. So it leads to the requirement of accurate and precise quantification in its bulk and buccal (effervescent) dosage form. The analytical method was developed and validated as per ICH guidelines. The proposed RS-HPLC methods fulfill the need at 100.8% accuracy with a precision of 0.25% relative standard deviation. Waters Alliance HPLC system with column Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) having UV-detector at 270 nm wavelength was used. The mobile phase having a mixture of 550 mL Acetonitrile and 450 mL of Milli-Q water and 1mL Ortho Phosphoric Acid (OPA), was used. The flow rate was set to 1.5 mL/min that gives the retention time at 4.9 min for Asenapine Maleate. The method is found linear (r^2 = 0.999 and R = 1) for a concentration range of 50 ppm to 75 ppm with zero percent interference at specificity. The robustness of the proposed method provides the back support for analysis of the sample in unfavorable laboratory conditions and instrumental variation. This method can be easily transferable to quality control laboratory and even at institution platform too.

INTRODUCTION: The chemical name of Asenapine maleate is 5-chloro-2,3,3a,12b-tetra-hydro-2-methyl-1H-dibenz(2, 3-6, 7) oxepino (4, 5-c)pyrrole[1] and the structure is as shown in **Fig. 1**. The molecular formula of the Asenapine maleate is $C_{17}H_{16}ClNO.C_4H_4O_4$. The molecular weight of the Asenapine maleate is 401.84gm/mol[2]. It is sparingly soluble in 0.1M HCl, soluble in methanol. Asenapine maleate is white to off white non-hygroscopic powder.

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Asenapine Maleate is a typical antipsychotic drug. It is an antagonist of 5-HT dopamine and α-adrenergic receptors and high affinity for dopamine D_2 and serotonin 5-HT_{2A} receptor. It was approved by USFDA in august 2009 [3]. It is indicated for the treatment of various psychotic conditions like schizophrenia and acute mania associated with bipolar disorder in adults. It also belongs to the dibenzo-oxepino pyrolle class [2]. It is also for severe post-traumatic stress disorder nightmares in soldiers as off-label use. Asenapine is a serotonin, dopamine, noradrenaline and histamine antagonist in which Asenapine possess more potent activity with serotonin receptors than dopamine [2].

Asenapine is a potent drug, and it falls in BCS (Biological Classification system) Class-II [4]. Its

potent nature and low solubility create hurdles in the development of formulation as well as its analytical method development.

The literature review reveals the estimation of Asenapine by UV-visible spectrophotometric method [5, 6,] but compare to RS-HPLC method, that method is not more reliable. HPLC method was also found[7,] but the proposed method is far superior in all aspects from peak shape to the validation data of method. Another method suggests estimation by Mass spectrometry method in human plasma that is again high cost analysis. The proposed method overcome all the issues with objective of simple, precise, accurate, robust and cost effective method. This method aimed to develop and validate a qualitative and quantitative method for estimation of Asenapine maleate in bulk and Buccal dosage form by RP-HPLC method as per ICH guidelines [8].

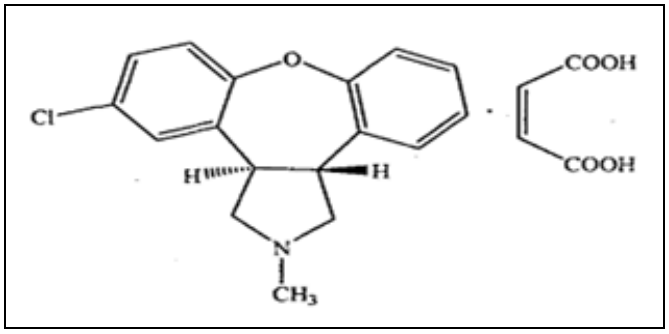

FIG. 1: ASENAPINE MALEATE

MATERIALS AND METHODS:

Chemicals and Reagents: Asenapine maleate was obtained from Sun Pharmaceutical Limited, Vadodara, Gujarat, India. Buccal Tablet (buccal effervescent tablet) was prepared at Vovantis Laboratory, Vadodara, Gujarat, India. Milli-Q water was used during whole study. Methanol and Acetonitrile were of HPLC grade (Make-Rankem).

Instruments and Chromatographic Conditions: Waters alliance and Shimadzu LC-2010HT equipped with UV-Visible detector controlled by Empower 3 software were used with column Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm), at 270 nm wavelength was used. The mobile phase having a mixture of 550 mL Acetonitrile and 450 mL of Milli-Q water and 1mL Ortho Phosphoric Acid (OPA) was used. All weighing was done on Sartorius's analytical balance. Thermo Lab made a hot air oven used in the study. The ultrasonic bath of Labman was used.

Preparation of Mobile Phase, Standard and Sample Solution: An Isocratic mobile phase was prepared by mixing 550 mL Acetonitrile and 450 mL of Milli-Q water and 1mL Ortho Phosphoric Acid (OPA) and sonicated for 15 min to degas. The same mobile phase was used as diluent. The standard stock solution was prepared by dissolving 25mg of Asenapine Maleate in 100 mL volumetric flask, and standard solution (50 ppm) was prepared by further diluting 5mL of this solution to 25 ml with diluent. Linearity solutions (seven levels) were prepared, using the standard stock solution, in the range from 25 ppm (50% of standard solution) to 75 ppm (150% of standard solution). To prepare the sample solution weighed 20 Tablets and calculated average weight. Crushed tablet to powder and transferred tablet powder equivalent to 25mg of Asenapine maleate in 100 mL flask. Added 70 mL diluent and sonicated for 15min and made up to mark with diluent. Then further diluted 5 mL of this solution to 25mL with diluent. Filter the resultant solution through a 0.45µm PVDF filter and was used as the sample solution. Placebo was prepared in the same manner as a sample with all excipients except the Asenapine maleate.

Method Validation: The RP-HPLC method was validated according to ICH Guidelines for validation of analytical procedures for different validation parameters. The method was validated for its specificity, Linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ.

RESULTS AND DISCUSSION:

System Suitability and System Precision: To ascertain its effectiveness, 10 µL of the freshly prepared standard solution was injected six times. System suitability and system precision data were calculated. The results obtained are shown in **Table 1-2**.

TABLE 1: SUMMARY OF SYSTEM SUITABILITY CRITERIA IN STANDARD SOLUTION

S. no.	Parameter	Observation (Limit)
1	The % RSD of asenapine maleate peaks for six replicate injections of standard	0.01 (≤ 2%)
2	The number of theoretical plates for asenapine maleate peak in standard solution	7227 (> 2000)
3	The tailing factor for asenapine maleate peak in standard solution	1.04 (< 2.0)

TABLE 2: SUMMARY OF PEAK AREA FOR SYSTEM PRECISION

Injection no.	Peak area (Asenapine Maleate)
1	2007124
2	2006678
3	2007174
4	2006560
5	2006720
Average peak area	2006851
SD	278.7
%RSD	0.01

Solution Stability: Solution stability was performed by analyzing standard and sample preparation periodically into the HPLC system at sample cooler temperature and Room temperature. The obtained data were summarized in **Table 3** and **Table 4**. The data shows that the standard solution was stable up to about 48 h at a sample cooler temperature (20 °C) and about 31 h at Room temperature. The sample solution was stable up to about 49 h at sample cooler temperature (20 °C) and 39 h at room temperature.

TABLE 3: STABILITY OF STANDARD AND TEST SOLUTION AT TEST METHOD TEMPERATURE (20°C)

Time (h)	Standard		Time (h)	Test	
	Area response	Cumulative % RSD		Area response	Cumulative % RSD
Initial	2006901	NA	Initial	1878356	NA
5	2009683	0.10	5	1896579	0.68
13	2016992	0.26	13	1904731	0.71
23	2008418	0.22	22	1886243	0.61
31	2011105	0.19	30	1898742	0.56
39	2020234	0.26	39	1914223	0.68
48	2030865	0.42	48	1922525	0.80

TABLE 4: STABILITY OF STANDARD AND TEST SOLUTION AT ROOM TEMPERATURE

Time (h)	Standard		Time (h)	Test	
	Area response	Cumulative % RSD		Area response	Cumulative % RSD
Initial	2006901	NA	Initial	1878356	NA
6	2014583	0.27	5	1893274	0.56
14	2009782	0.19	14	1909025	0.81
23	2023013	0.35	23	1912109	0.82
31	2028206	0.44	31	1916251	0.83
39	2122724	2.17	39	1897995	0.74
49	2032559	1.98	49	1998441	2.03

Specificity: A study to establish the interference of blank and placebo was conducted. The analysis was performed on placebo preparation and diluent as blank. As shown in **Fig. 2-5**, it clearly indicates the ability of the method in the presence of other excipients.

Linearity and Range: Inject each level into the chromatographic system and measure the peak area. Plot a graph of area versus concentration and calculate the correlation coefficient. The correlation coefficient should be not less than 0.9999. The linearity was calculated by measuring different concentration levels like 50%, 80%, 90%, 100%, 110%, 120%, and 150% for Asenapine Maleate and was shown in **Table 5** and **Fig. 6-7**.

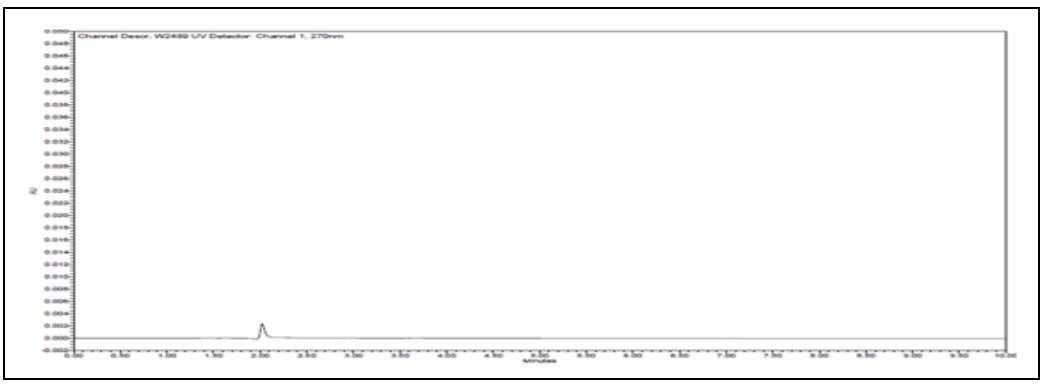

FIG. 2: CHROMATOGRAM OF BLANK

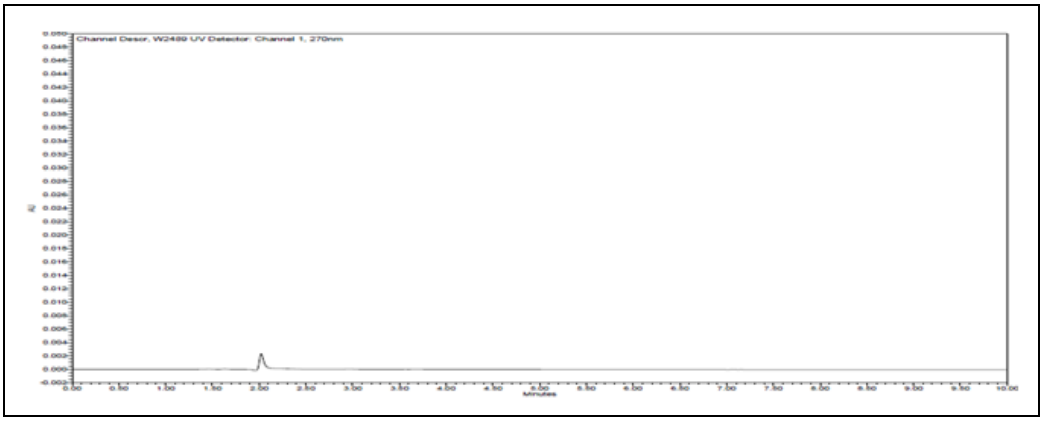

FIG. 3: CHROMATOGRAM OF PLACEBO

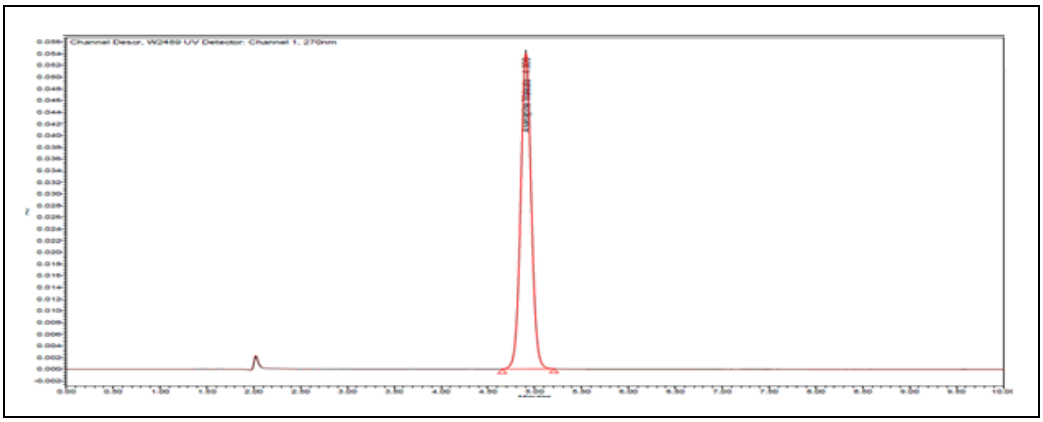

FIG. 4: OVERLAY GRAPH OF BLANK AND STANDARD

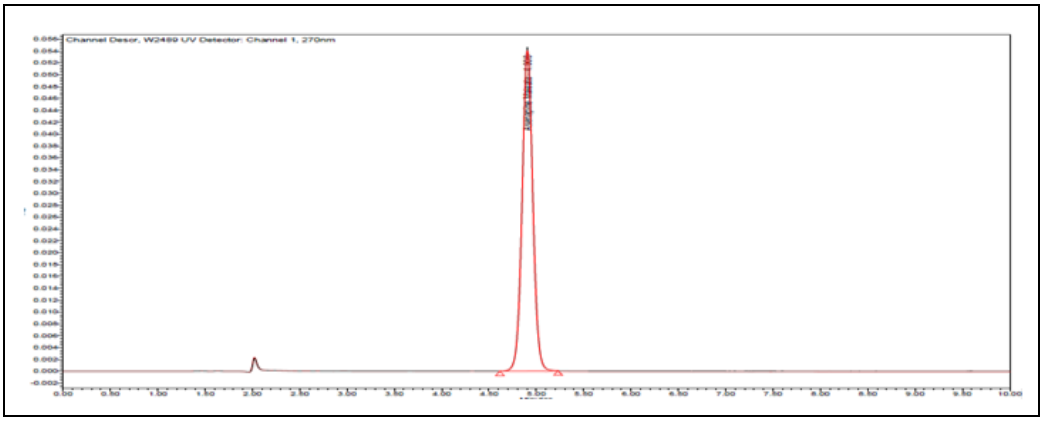

FIG. 5: OVERLAY GRAPH OF PLACEBO AND SAMPLE

TABLE 5: LINEARITY OF ASENAPINE MALEATE

S. no.	Asenapine Maleate peak response as peak area		
	Level (%)	Concentration (µg/mL)	Area response
1	50	25	957053
2	80	40	1539822
3	90	45	1718994
4	100	50	1908453
5	110	55	2093769
6	120	60	2289508
7	150	75	2856071
Correlation coefficient (R)		1.000	
Slope		37900.6407	
Intercept		14222.3210	
Regression coefficient (R^2)		0.999	

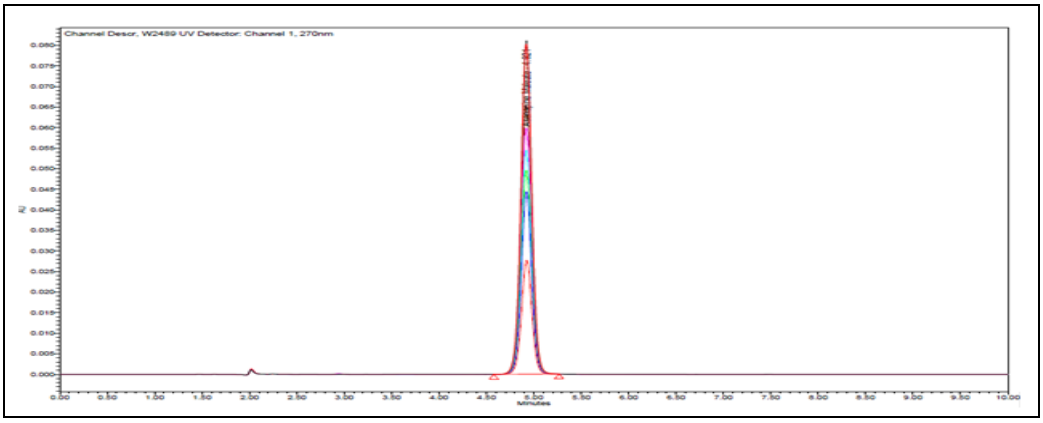

FIG. 6: OVERLAY CHROMATOGRAM OF LINEARITY

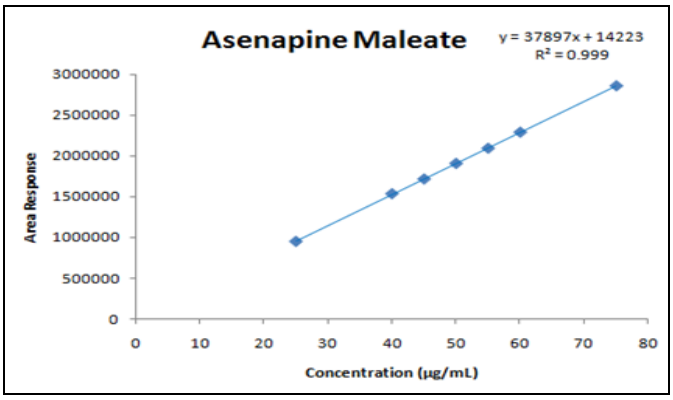

FIG. 7: LINEARITY GRAPH OF ASENAPINE MALEATE

Method Precision: To evaluate the method precision, six individual sample solution was prepared and calculate the % of Assay, as shown in **Table 6** and **Fig. 8**. The % RSD for the % Assay of six determination should not be more than 2%.

TABLE 6: SUMMARY OF RESULTS FOR PRECISION OF THE METHOD FOR ASENAPINE MALEATE

Injection no.	Assay (%w/w) (Asenapine Maleate)
1	98.73
2	99.31
3	99.10
4	98.80
5	99.13
6	98.71
Average peak area	98.96
SD	0.250
%RSD	0.25

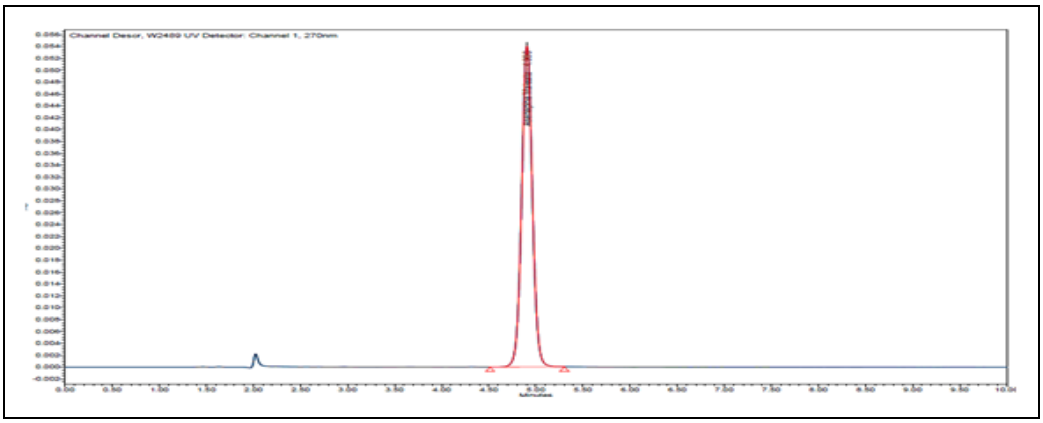

FIG. 8: OVERLAY CHROMATOGRAM OF METHOD PRECISION

Ruggedness (Intermediate Precision): Ruggedness of method was performed on a different day and on different make instruments by injecting six replicate of sample preparation. The %RSD of six replicate should not be more than 2%, and the overall % RSD should not be more than 2%, as shown in **Table 7**.

TABLE 7: RESULTS OF RUGGEDNESS DATA FOR ASENAPINE MALEATE

Preparation no.	Set-I Assay (%w/w)	Set-II Assay (%w/w)
1	98.73	98.61
2	99.31	99.37
3	99.10	97.75
4	98.80	99.20
5	99.13	99.42

6	98.71		98.35
Average	98.96		98.78
SD	0.250		0.665
%RSD	0.25		0.67
Overall average		98.87	
Over all SD		0.488	
Over all % RSD		0.49	
Instrument	Shimadzu LC 2010HT		Waters alliance

Accuracy: The accuracy of the method was determined by analyzing three solutions containing Asenapine maleate at approximately 50%, 100%, and 150% of the working concentration. Each solution was analyzed in triplicate. The % recovery results obtained are shown in **Table 8** and **Fig. 9**. The % recovery at each spike level should be not less than 98% and not more than 102% of the added amount.

TABLE 8: ACCURACY OF ASENAPINE MALEATE

Recovery level	Asenapine Maleate				
	Amount added (mg)	Amount recovered (mg)	% Recovery	Average recovery (%)	% RSD
50%	25.01	25.2601	101.6	101.4	0.21
	25.04	25.2595	101.5		
	25.11	25.2457	101.2		
100%	50.07	50.2358	101.0	100.8	0.15
	50.09	50.1768	100.8		
	50.16	50.1912	100.7		
150%	75.25	74.7599	100.0	100.1	0.12
	75.09	74.7925	100.2		
	75.20	74.7482	100.0		
Overall % Recovery				100.8	
Overall % RSD				0.61	

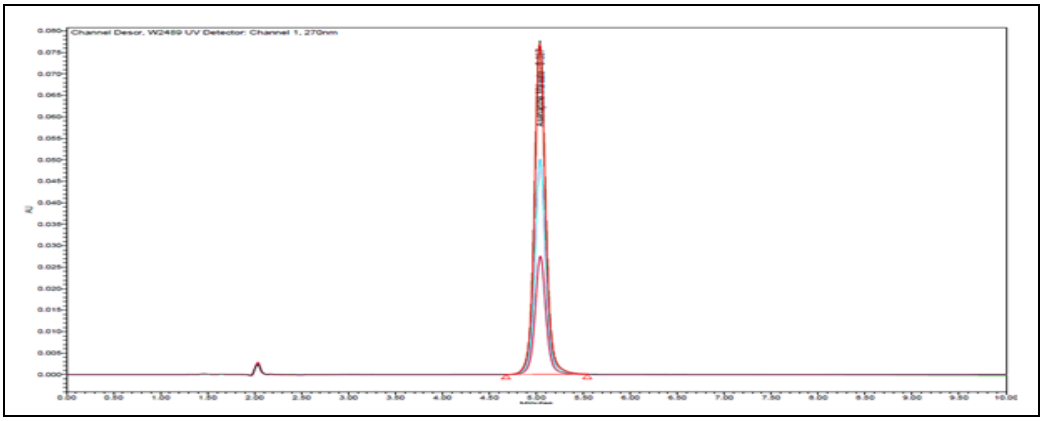

FIG. 9: OVERLAY CHROMATOGRAM OF ACCURACY DATA

Robustness: Prepared standard solution and test preparation in single as per proposed test method and performed robustness parameter by variation in chromatographic conditions like flow rate (±10.0%), column oven temperature (±5°C), wavelength (±2nm) and mobile phase composition (10% of Organic Phase). The robustness of the method is as shown in **Table 9-10**.

TABLE 9: SYSTEM SUITABILITY FOR ASENAPINE MALEATE (ROBUSTNESS PARAMETER

S. no.	Robustness Parameter	System Suitability parameter	Observations			Limits
			Test Method	Plus	Minus	
1	Flow	%RSD of six replicate injection	0.01	0.02	0.04	NMT 2
		Tailing Factor	1.04	1.20	1.23	NMT 2
		Theoretical Plates	7227	7202	7968	NLT 2000
2	Wavelength	%RSD of six replicate injection	0.01	0.01	0.03	NMT 2
		Tailing Factor	1.04	1.20	1.20	NMT 2
		Theoretical Plates	7227	7469	7517	NLT 2000
3	Column Oven	%RSD of six replicate injection	0.01	0.11	0.02	NMT 2

		Tailing Factor	1.04	1.23	1.21	NMT 2
		Theoretical Plates	7227	8215	7018	NLT 2000
4	Mobile Phase Composition	%RSD of six replicate injection	0.01	0.03	0.04	NMT 2
		Tailing Factor	1.04	1.21	1.27	NMT 2
		Theoretical Plates	7227	6558	9017	NLT 2000

TABLE 10: PRECISION DATA COMPILATION ASENAPINE MALEATE (ROBUSTNESS PARAMETER)

Robustness Parameter	Set-Robustness	Method precision Data						%RSD
		Set-1	Set-2	Set-3	Set-4	Set-5	Set-6	
Minus Wavelength	99.72	98.73	99.31	99.10	98.80	99.13	98.71	0.37
Plus Wavelength	99.79	98.73	99.31	99.10	98.80	99.13	98.71	0.39
Minus Flow Rate	99.53	98.73	99.31	99.10	98.80	99.13	98.71	0.32
Plus Flow Rate	99.61	98.73	99.31	99.10	98.80	99.13	98.71	0.34
Minus Column Temp.	99.56	98.73	99.31	99.10	98.80	99.13	98.71	0.32
Plus Column Temp.	100.07	98.73	99.31	99.10	98.80	99.13	98.71	0.48
Minus Organic	99.90	98.73	99.31	99.10	98.80	99.13	98.71	0.42
Plus Organic	98.34	98.73	99.31	99.10	98.80	99.13	98.71	0.33

CONCLUSION: A specific, precise, accurate, less time consuming, and simple method was developed for the quantitative estimation of Asenapine maleate in bulk drug and buccal formulation using RP-HPLC and validated as per ICH guidelines. The result of the analysis by the proposed method is highly reproducible and reliable. Robustness and ruggedness of method lead its application from a small college lab to a quality control department of big pharmaceutical organizations.

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CONFLICTS OF INTEREST: The authors have no conflict of interest

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