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METHOD DEVELOPMENT AND VALIDATION OF RELATED SUBSTANCES IN ASENAPINE TABLETS BY REVERSE PHASE HPLC

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ABSTRACT

A novel RP-HPLC Quantification method was developed for estimation of Asenapine known impurities like its N-oxide, Des methyl, Des Chloro and Amide which, were separated on an Inertsil C_8 column (250.0 mm x 4.60 mm; 5.0µ). Mixture of phosphate buffer (pH 6.8), water & acetonitrile as a mobile phase with a rate of flow 1 ml/minute was used; λ max at 220 nm. The developed method was validated all the parameters like linearity, specificity, limit of quantification (LOQ) and detection (LOD), precision, accuracy, ruggedness, filter variation, robustness & solution stability.

KEYWORDS: Asenapine, inertsil C_8 column, reverse phase HPLC, method development & validation, related substances.

INTRODUCTION

Asenapine is di benzoxepinopyrrole [Trans - 5 - chloro -2, 3, 3a, 12b – tetra hydro – 2 – methyl - 1H - dibenz (2, 3:6, 7) oxepino - (4, 5 -c) pyrrole (Z) - 2 - butenedioate (1:1)] with unique receptor pharmacology and is available as a fast-dissolving tablet for sublingual administration. It has potent dopaminergic (D1–D4), serotonergic (5 - HT2A, 5 -HT2C, 5-HT6 and 5 - HT7), adrenergic (α1 and α2) & histaminergic (H1) activity.[1] Many techniques have been reported quantitave estimation including Spectrophotometric[2] liquid chromatographic[3] stress degradation by RP-HPLC[4] Stability indicating liquid chromatographic.[5-7]

Since no method has been developed for the separation and estimation of impurities in Asenapine tablets and the drug is being marketed in domestic and international market the present study by the author describes a rapid, accurate and precise RP – HPLC method for the estimation of known related impurities, i.e., N-Oxide impurity((3aRS,12bRS)-trans-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenz[2,3:6,7]-oxepino[4,5-c]pyrrole N-Oxide), Desmethyl Impurity((3aRS,12bRS)-trans5-chloro-2,33a,12b-tetrahydro-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole oxalate), Deschloro Impurity ((3aRS, 12bRS)-2-methyl-2,3,3a,12b-tetrahydro-1Hdibenz[2,3:6,7]-oxepino[4,5-c]pyrrole oxalate) and Amide impurity((3aRS,12bRS)-trans-1l-chloro-2,3,3a,l2b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7] oxepino [4,5-c]pyrrole-l-one) present in Asenapine tablets. The method was validated as per ICH guidelines (ICH, 2005).

Experimental

Chromatographic Conditions

Agilent 1200 series HPLC consisting pump, Auto sampler, VWD & photo diode array detector, thermostatted column compartment connected with Open lab and EZ Chrom software connected with a Inertsil C_8 250x4.6mm, 5.0µm.

MATERIALS AND METHODS

Asenapine pure drug and impurities, Acetonitrile (HPLC Grade), water (HPLC Grade), Di-potassium hydrogen orthophosphate, orthophosphoric acid 85% pure Triethylamine were AR grade from SD Fine Chem., was used in the present study. The tablet formulations purchased from local market Hyderabad, India.

Mobile Phase

Accurately weigh 3.48g of Di-potassium hydrogen ortho phosphate in 1000ml of water, add 0.5ml Triethylamine adjust pH 6.8 with dilute phosphoric acid. Filter the solution through 0.22µ nylon filter and sonicate to degas it. The buffer was used as mobile phase preparation A, Acetonitrile and water in the ratio (90:10 v/v) used as mobile phase mobile preparation B, the solutions were mixed 38:62 v/v respectively filtered through 0.22µ membrane filter and were degassed and Asenapine and its impurities were eluted in a isocratic flow. The flow rate of the mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 40ºC and the detection of the drug was carried out at 220nm with an injection volume of 10µl.

Diluent

Prepare a filtered and degassed mixture of methanol and water (50:50 v/v).

Standard Preparation

Weigh accurately Asenapine maleate working standard equivalent to 20 mg of Asenapine into 100 ml volumetric flask, add 60 ml of diluent and dissolve, further make up the volume with Diluent. Further dilute 5 ml to 50 ml with diluent.

For 5 mg Test Preparation

Transfer 10 tablets into 25 ml volumetric flask, add 15 ml of diluent and sonicate to dissolve for about 20 min further make up the volume with diluent. Filter through 0.45 micron Nylon filter.

For 10 mg Test Preparation

Transfer 10 tablets into 50 ml volumetric flask, add 30 ml of diluent and sonicate to dissolve for about 20 min, further make up the volume with diluent. Filter through 0.45 micron Nylon filter.

For 5 mg Placebo Preparation

Transfer placebo powder present in 10 tablets into 25 ml volumetric flasks, add 15 ml of diluent and sonicate to dissolve for about 20 min, further make up the volume with diluent. Filter through 0.45 micron nylon filter.

For 10 mg Placebo Preparation

Transfer placebo powder present in 10 tablets into 50 ml volumetric flasks, add 15 ml of diluent and sonicate to dissolve for about 20 min, further make up the volume with diluent. Filter through 0.45 micron nylon filter.

Impurities Calculation for 5mg/10mg Tablets

$$\textbf{\% of Any Impurity} = \frac{\text{mpurity Area x Std .Wt x } 5 \text{ x } 25 \text{ x } 1}{\text{Avg. STD Area x } 100 \text{ x } 50 \text{ x } 10 \text{ x Label amount}} \text{x } 0.711 \text{ x Std. Potency}$$

% of Total Impurities = Sum of % Individual impurities,

0.711 is the conversion factor of Asenapine maleate equivalent to Asenapine.

RESULTS AND DISCUSSION

System Suitability and Precision

System suitability was evaluated from the standard solution by injecting six times into the HPLC. The parameters measured were peak area, retention time and asymmetry factor, the observations were tabulated in Table 1.the observed results were within the limits indicates the system is suitable and a typical chromatogram of Asenapine standard chromatogram shown in fig 1.

Placebo and Impurities Interference

Interference from placebo and impurities was carried out by preparing the following specificity samples. Performed related substances on Placebo equivalent to the amount present in test preparation and injected into the chromatography. By preparing and inject impurities at 0.5% of test concentration. By preparing active sample as per test concentration. By spiking the active sample with individual known impurities at 0.5% of test concentration. The above samples were injected and observed for any interference from blank and placebo at the retention time of analyte and known impurity peaks. This was further demonstrated by determining the peak purity of analyte and known impurity peaks. The results of the peak purity values of analyte and known impurities were tabulate in Table 2. Since no interference of blank, placebo and known impurities was observed at the retention time of analyte. Individual impurity peaks are separated from the analyte peak. Peak purity of analyte peak and known impurity peaks are greater than 0.99, so the method is specific for Asenapine tablets. The chromatograms of blank, placebo and spiked impurities with Asenapine shown in (fig 2a-c).

Limit of Quantitation and Detection

The limit of quantitation (LOQ) and detection (LOD) were conducted on the basis of signal to noise ratio method. Different concentrations of impurities with sample solution were injected, LOQ established the values which give the signal to noise ratio about 10.0, for LOD of impurities were established which give the signal noise ratio about 3.0; the results of both LOQ & LOD values were tabulated in Table 3 & 4 and the chromatograms shown in (fig 3a,b) respectively.

Precision at Limit of Quantitation Level

Each solution of the six LOQ preparations injected, the % RSD values of impurities with sample were calculated, the values were less than 10.0 indicates precise at LOQ level, and the results were tabulated in Table 5.

Linearity and Detector Response

The linearity of detector response for impurities was demonstrated by prepared solutions of Asenapine and its impurities over the range of LOQ to 200% level of target concentration (0.5% of test concentration) and the detector response was found to be linear and the correlation coefficient was more than 0.998, proves Asenapine and its impurities are linear, the results were tabulated in Table 6. The chromatogram of (100%) linearity solution is shown as in (fig 4).

Establishment of RRT's and RF Values for Impurities

The RRT's and RF values were calculated from the linearity levels of 50%, 100% and 200% i.e., 0.25%, 0.5% and 1.0% of test concentration.

$$\text{N-Oxide Impurity (RRF)} = \frac{\text{N-Oxide Imp area} \times \text{Asenapine wt} \times \text{Asenapine purity}}{\text{Area of Asenapine} \times \text{N-Oxide Imp wt} \times \text{N-Oxide Impurity}}$$

$$\text{Desmethyl Impurity (RRF)} = \frac{\text{Desmethyl Imp area} \times \text{Asenapine wt} \times \text{Asenapine purity}}{\text{Area of Asenapine} \times \text{Desmethyl Imp wt} \times \text{Desmethyl Imp purity}}$$

$$\text{Deschloro Impurity (RRF)} = \frac{\text{Deschloro Imp area} \times \text{Asenapine wt} \times \text{Asenapine purity}}{\text{Area of Asenapine} \times \text{Deschloro Imp wt} \times \text{Deschloro Imp purity}}$$

$$\text{Amide Impurity (RRF)} = \frac{\text{Amide imp area} \times \text{Asenapine wt} \times \text{Asenapine purity}}{\text{Area of Asenapine} \times \text{Amide imp wt} \times \text{Amide imp purity}}$$

RF Results

$$\text{Response Factor (RF)} = \frac{1}{\text{RRF}}$$

RRT's

$$\text{RRT} = \frac{\text{Impurity RT}}{\text{Asenapine RT}}$$

The RRT's and RF values were calculated and the results were tabulated in Table 7.

PRECISION

Six sample preparations representing a single batch were injected, the each impurity area were determined and the precision was evaluated, the %RSD of each impurity results was less than 10.0 indicates the method is precise, the results are tabulated in Table 7.

Intermediate Precision

The ruggedness of the method was injected six preparations of a single batch sample by different analyst (analyst-2), different column (column-2) and different instrument (instrument-2). The %RSD of each impurity was calculated; the results were less than 10.0. consider the precision results for analyst-1, column-1 and system-1, the mean % RSD values of both precision and intermediate calculated, the results were less than 15.0 shows the method is rugged and the results were tabulated in Table 7.

Accuracy

The accuracy of the test method was prepared recovery samples (i.e. test sample with known quantities of N-Oxide Impurity, Desmethyl Impurity, Deschloro Impurity and Amide Impurity) at the level of LOQ, 50%, 100%, 200%, 300% and 400% of target concentration (i.e. 0.5% of test concentration).as the recovery results were found between 90 to 110% the method is accurate for the estimation of Asenapine 5/10mg tablets and its impurities over the range of LOQ to 400% level of target concentration and the results were tabulated in Table 8.

Robustness

The solution stability

The sample solution prepared and injected initially, after 24 hours and 48 hours. The difference between initial, 24hrs and 48hrs of individual impurity less than 0.04% and total impurities less than 0.1% indicates the solution is stable up to 48hrs and the results were tabulated in Table 9.

Filter Variation

The filter variation was injected the test solution of centrifuged and filtered through 0.45µ nylon filter and 0.45µ PVDF filter and the difference between filtered portions of individual impurity less than 0.04% and total impurities were less than 0.1% w.r.t to centrifuged sample shows no effect of filter variation and the results were tabulated in Table 9.

Effect of Column Temperature, Buffer pH, Mobile phase composition, Extraction and Flow Variation

The test preparation was injected under normal condition (i.e. as such condition) and of the altered conditions column temperature 40±5°C, flow rate 1±0.2ml, buffer pH 6.8±0.2, mobile phase composition ±2%, extraction time of analyte 15min, 20min and 25 min, the difference between individual for all changed conditions is less than 0.04% and total impurities for all changed conditions is less than 0.1% proves the method is robust and the results were tabulated in Table 9.

Table: 1 System suitability results

System suitability	Result
RSD for three Standard injections (NMT 5.0%)	0.43
Theoretical plates (NLT 3000)	12381
Asymmetry (NMT 2.0)	1.37

Table: 2 Retention times and peak purity results of analyte with impurities

S. No.	Compound Name	Peak Purity	RT (Individual)	RT (Spiked sample)
1	Asenapine	1.00000	16.44	15.97
2	N-Oxide Impurity	1.00000	4.69	4.72
3	Desmethyl Impurity	1.00000	5.27	5.38
4	Deschloro Impurity	1.00000	9.83	10.03
5	Amide Impurity	1.00000	12.29	12.29

Table: 3 LOQ results

S. No	Name of the Component	S/N Ratio	% level of component concentration in ppm
1	Asenapine	10.30	0.150
2	N-Oxide Impurity	9.73	0.074
3	Desmethyl Impurity	10.46	0.074
4	Deschloro Impurity	9.52	0.131
5	Amide Impurity	9.65	0.103

Table: 4 LOD results

S. No	Name of the Component	S/N Ratio	% level of component concentration in ppm
1	Asenapine	2.78	0.045
2	N-Oxide Impurity	3.37	0.022
3	Desmethyl Impurity	2.93	0.022
4	Deschloro Impurity	2.56	0.039
5	Amide Impurity	2.65	0.031

Table: 5 Precision at LOQ results

S. No	N-Oxide Impurity	Desmethyl Impurity	Deschloro Impurity	Amide Impurity	Asenapine
1	17184	21456	31969	46483	46233
2	17399	21941	32791	45545	46854
3	18092	22233	32058	46896	45748
4	18241	21822	32766	46895	47454
5	18485	21801	32171	46713	47316
6	17706	21361	32979	46310	46656
Avg:	17851	21769	32456	46474	46710
SD:	506.4	320.36	437.9	510.4	647.67
% RSD:	2.84	1.47	1.35	1.10	1.39

Table: 6 Linearity results

	Asenapine	N-Oxide Impurity	Desmethyl Impurity	Deschloro Impurity	Amide Impurity
Correlation coefficient	0.9998	0.9998	0.9998	1.0000	0.9999
Slope	318230	241133	287100	246533	458164
Y-Intercept	82257.39	5804.49	1884.57	2387.4	12881.67
Residual sum square	2.2455×10^{10}	8.6946×10^{9}	1.5638×10^{12}	2.7944×10^{11}	9.4578×10^{9}
Residual standard deviation	74925	47367	65872	22541	48626

Table: 7 Precision, intermediate precision and RRT & RF results

Parameter	N-Oxide Impurity	Desmethyl Impurity	Deschloro Impurity	Amide Impurity
Precision(n=6)				
Asenapine 5mg Tablets	2.72	1.21	1.15	1.95

Asenapine 10mg Tablets	1.86	2.29	1.07	1.02
Intermediate Precision(n=6)				
Asenapine 5mg Tablets	1.31	2.74	3.23	0.42
Asenapine 10mg Tablets	1.26	1.26	1.83	0.63
Mean method precision and Intermediate precision				
Asenapine 5mg Tablets	3.86	2.08	3.27	3.42
Asenapine 10mg Tablets	0.51	0.48	0.5	0.51
RRT&RF Values				
RRT values	~0.27	~ 0.32	~ 0.58	~ 0.84
RF values	1.14	0.98	1.14	0.67

n is the number of repetitions

Table: 8 Accuracy results

Asenapine Tablets 5/10mg

Spike Level	Amount added(ppm)	Mean Amount recovered(ppm)	% Mean Recovery
Recovery of N-Oxide impurity			
LOQ level	0.074	0.071	94.93
50%	5.47	5.4633	99.81
100%	10.95	10.89	99.5
200%	21.89	22.88	104.52
300%	32.84	34.1467	103.99
400%	54.73	51.96	94.95
Recovery of Desmethyl impurity			
LOQ level	0.074	0.073	98.53
50%	6.2	5.91	95.3
100%	12.4	12.007	96.8
200%	24.8	26.11	105.28
300%	37.2	39.333	105.74
400%	62	58.073	93.67
Recovery of Deschloro impurity			
LOQ level	0.131	0.134	102.06
50%	6.33	6.397	101.13
100%	12.65	12.467	98.55
200%	25.3	25.400	100.39
300%	37.95	37.740	99.45
400%	63.25	60.683	95.94
Recovery of Amide impurity			
LOQ level	0.1	0.095	92.7
50%	5.15	5.253	102.09
100%	10.29	10.483	101.87
200%	20.58	21.640	105.15
300%	30.87	31.747	102.84
400%	51.45	47.420	92.17

Table: 9 Robustness of solution stability, filter varation, column temperature, buffer pH, mobile phase composition and flow variation results

Condition		%N-Oxide impurity	%Desmethyl impurity	%Deschloro impurity	%Amide impurity	%Total impurities
Normal (i.e as such condition)		0.13	0.07	0.02	0.01	0.59
Flow changed to 0.8ml/min		0.14	0.1	0.02	0.01	0.65
Flow changed to 1.2ml/min		0.11	0.05	0.02	0.01	0.57
Column Temperature changed to 35°C		0.11	0.09	0.01	0.01	0.56
Column Temperature changed to 45°C		0.14	0.06	0.01	0.01	0.58
Buffer pH changed to 6.6		0.15	0.09	0.01	0.01	0.64
Buffer pH changed to 7.0		0.15	0.09	0.03	0.02	0.62
Buffer ratio changed to -2%		0.11	0.06	0.01	0.01	0.65
Buffer ratio changed to +2%		0.13	0.06	0.02	0.01	0.54
Extraction time	15 min	0.12	0.08	0.02	0.01	0.62
	20 min	0.13	0.07	0.02	0.01	0.59
	25 min	0.12	0.07	0.02	0.01	0.6
Solution Stability						
Initial		0.12	0.08	0.02	0.01	0.61
After 24 Hours		0.13	0.07	0.02	0.01	0.62
After 48 Hours		0.13	0.1	0.02	0.01	0.65
Filter Variation Results						
Centrifuged		0.12	0.08	0.02	0.011	0.62
Nylon Filter		0.12	0.08	0.02	0.008	0.6
PVDF Filter		0.12	0.08	0.02	0.004	0.59

CONCLUSION

The proposed RP-HPLC method satisfies the parameters like system suitability, specificity, precision, accuracy, linearity, and robustness, ruggedness. The obtained results from the validation as per the ICH guidelines and drug stability were indicates this method is accurate, sensitive and best suitable method for determination of known and unknown impurities in Asenapine regular laboratory analysis to reduce the cost of analysis.

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