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# Determination of degradation products and process related impurities of asenapine maleate in asenapine sublingual tablets by UPLC

Nitin Kumar<sup>1,2</sup>, D Sangeetha<sup>2</sup> and L Kalyanraman<sup>1</sup>

Department of Analytical Research and Development, IPDO, Dr Reddy's Laboratories, Hyderabad-500 072, India

<sup>2</sup>Department of Chemistry, School of Advanced Sciences, VIT University, Vellore, Tamilnadu, India

E-mail : dsangeetha@vit.ac.in

**Abstract.** For determination of process related impurities and degradation products of asenapine maleate in asenapine sublingual Tablets, a reversed phase, stability indicating UPLC method was developed. Acetonitrile, methanol and potassium dihydrogen phosphate buffer with tetra-n- butyl ammonium hydrogen sulphate as ion pair (pH 2.2; 0.01 M) at flow rate of 0.2 ml/min were used in gradient elution mode. Separation was achieved by using acquity BEH Shield RP18 column (1.7  $\mu$ m, 2.1 mm $\times$ 100 mm) at 35  $^{\circ}$ C. UV detection was performed at 228 nm. Subsequently the liquid chromatography method was validated as per ICH. The drug product was exposed to the stress conditions of acid hydrolysis, base hydrolysis, water hydrolysis, oxidative, thermal, and photolytic. In oxidative stress and thermal stress significant degradation was observed. All the degradation products were well separated from analyte peak and its impurities. Stability indicating nature of the method was proved by demonstrating the peak purity of Asenapine peak in all the stressed samples. The mass balance was found >95% for all the stress conditions. Based on method validation, the method was found specific, linear, accurate, precise, rugged and robust.

## 1. Introduction

Asenapine maleate is available under the brand name of Saphris. It is antipsychotic drug which is used to treat schizophrenia and bipolar disease. It belongs to chemical class of Dibenzo oxepino pyrroles and chemically described as (2Z)-but-2-enedioic acid; 17-chloro-4-methyl-13-oxa-4-azatetracyclo[12.4.0.0<sup>2,6</sup>.0<sup>7,12</sup>]octadeca-1(14),7,9,11,15,17-hexaene. C<sub>17</sub>H<sub>16</sub>ClNO.C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> is the molecular formula and 401.84 is the molecular weight. [1, 2]

There are few published methods for quantification of asenapine in the bulk drug and formulated drug product [3, 4, 5, and 6]. No analytical method is available in Pharmacopeia for estimation of asenapine [7, 8]. One HPLC method was published for estimation of impurities in Asenapine using a solid core C8 column. Few other published methods are also there for the quantification of impurities and asenapine maleate by RP-LC. In addition to the reported impurities in these methods, one



additional impurity was observed in our drug product during stability studies. [9, 10] This impurity was identified as Fumaric Acid. Asenapine is a maleate salt. It has some free maleic acid. At elevated temperatures, maleic acid converts in to fumaric acid. Fumaric acid impurity increases during stability studies of drug product. There is not reported method for determination of fumaric acid impurity in the asenapine maleate and its formulated drug product.

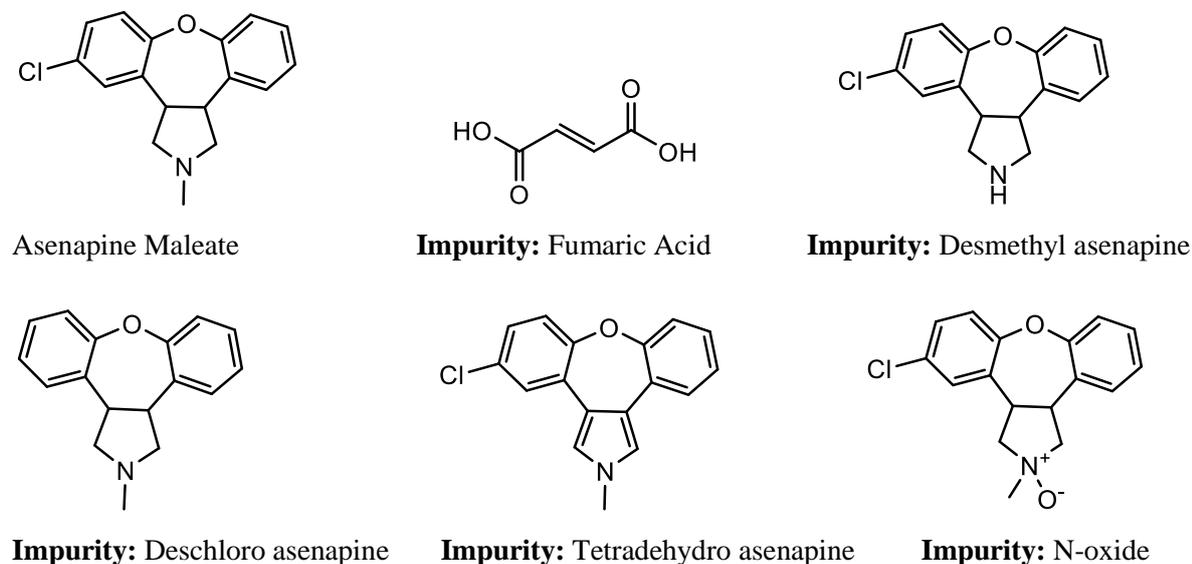
The present paper describes a liquid chromatography method to estimate fumaric acid and other impurities of asenapine maleate.

The developed method can separate and quantitate three degradants and the process related impurities of asenapine maleate namely fumaric acid, desmethyl asenapine, N-oxide, cis–asenapine and deschloro asenapine. The limit for the impurities in asenapine sublingual tablet, based on maximum daily dose is 0.5% [11].

## 2. Experimental

### 2.1 Materials

Potassium dihydrogen phosphate, tetra-n- butyl ammonium hydrogen sulphate, o- phosphoric acid, methanol and acetonitrile were purchased from Merck. Methanol and acetonitrile were of gradient grade. Asenapine sublingual tablets, its impurities desmethyl asenapine, N-oxide, cis–asenapine, des chloro asenapine and asenapine maleate reference standard were provided by Dr. Reddy's, India. Fumaric acid was purchased from Sigma Aldrich, USA. Chemical structures of asenapine maleate, desmethyl asenapine, N-oxide, cis–asenapine, des chloro asenapine and fumaric acid are shown in Figure 1.



**Figure 1** Impurities structures

### 2.2 Instrumentation

Water was used for preparation of all the solutions. Water purification was performed by using Millipore Water purification system (Bedford, MA, USA). Waters Acquity UPLC was used for analysis; which was consisted of binary solvent delivery pump, PDA UV detector and an auto sampler.

### 2.3 Method

### 2.3.1 Chromatographic condition

By using Acquity BEH Shield RP18 (2.1 mm x 100 mm), 1.7  $\mu\text{m}$ , separation was achieved. Mobile phase A and B were used in gradient elution mode. Mobile phase A was potassium dihydrogen phosphate buffer (pH 2.2; 0.01 M) with tetra-n-butyl ammonium hydrogen sulphate. Mobile phase B was prepared by mixing water, acetonitrile and methanol in ratio of 10:50:40 (v/v). Gradient programme T (min) / % B: 0/5, 7/5, 22/20, 35/50, 38/50, 39/90, 44/90, 45/5, and 62/5 was applied at 0.20 ml/min flow rate. Temperature in column compartment was maintained as 35 °C. 2  $\mu\text{l}$  of each solution was injected and UV detection was performed at 220 nm.

### 2.3.2 System suitability solution preparation

Methanol and water were mixed in ratio of 50:50 (v/v) to prepare diluent. An appropriate amount of fumaric acid and desmethyl asenapine standard was dissolved in diluent to obtain both the impurities at a concentration level of 7.5  $\mu\text{g/ml}$ .

### 2.3.3 Standard preparation

Appropriate amount of asenapine maleate reference standard was dissolved in diluent to prepare standard solution containing asenapine maleate at 15  $\mu\text{g/ml}$  level.

### 2.3.4 Sample preparation

Twenty asenapine sublingual Tablets 5 mg were transferred to 100 mL volumetric flask having 60 ml diluent. Drug extraction from sample matrix was done by sonicating the solution for 20 minutes followed by centrifugation for 10 minutes at 4000 RPM. Final sample preparation contained asenapine maleate at 1405  $\mu\text{g/ml}$  level.

## 2.4 Method validation

Following validation parameters were performed for the proposed method in accordance to ICH guidelines:

### 2.4.1 System suitability

System performance was ensured by establishing the system suitability. System suitability solution was injected to ensure the resolution between desmethyl asenapine and asenapine peaks. The acceptance criteria was resolution should be not less than 2.0 between asenapine and desmethyl asenapine peaks. System precision was determined by injecting standard preparation six times. Acceptance criteria for system suitability include %RSD for asenapine peak areas as not more than 10.0%, tailing factor as less than 2.0 for asenapine peak and plate counts as greater than 5000 for asenapine peak.

### 2.4.2 Specificity/Stress study

Stress studies were conducted separately on asenapine sublingual tablets 5 mg and its placebo. The stress conditions included acid hydrolysis (2N HCl, 70 °C, 24 hrs), base hydrolysis (2N NaOH, 70 °C, 24 hrs), water hydrolysis (70 °C, 24 hrs), oxidation (3%  $\text{H}_2\text{O}_2$ , 35 °C, 1 hr), thermal (105 °C, 14 hrs), humidity (90%RH, 7 days) and photolytic (200 wh/square  $\text{m}^2$  UV light and 1.2 million lux hr visible light, 16 hrs)

The stressed samples were then analyzed by using proposed method. Peak purity was checked and mass balances were calculated for stressed samples. Placebo preparation was also injected to check the placebo interference. Placebo did not show any interference at the retention time of fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide and asenapine.

### 2.4.3 Precision

Method precision was demonstrated by doing repeatability and intermediate precision. Repeatability was performed by analyzing six sample preparation of asenapine sublingual tablets 5 mg spiked with

impurities fumaric acid, desmethyl asenapine, N-oxide at 0.25 % level (0.25 % of 1400 µg/ml asenapine maleate) and deschloro asenapine, cis-asenapine each at 0.15 % level (0.15% of 1400 µg/ml asenapine maleate). % RSD was calculated for each impurity. Intermediate precision was performed by using different scientist, different UPLC, different column, and performing the analysis on different days.

#### *2.4.4 Limit of detection (LOD) and Limit of quantitation (LOQ)*

Limit of detection and limit of quantification for asenapine and its impurities (fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide) were established based on visual method. LOD was determined by identifying concentration where peak was visible. LOQ was determined by identifying the concentration where it was quantified with desired accuracy and precision.

Precision at LOQ was performed for asenapine and its impurities. Six test preparations of asenapine sublingual tablets 5 mg placebo, containing asenapine and its impurities at LOQ level were prepared and injected into UPLC. The % RSD for six replicate preparations was calculated.

#### *2.4.5 Linearity*

Linearity was performed by determining the correlation coefficient. Six linearity solutions were prepared with different concentration levels ranging from LOQ to 150 % of target concentration (about 10.5 µg/ml for fumaric acid, desmethyl asenapine, N-oxide, about 3.2 µg/ml for deschloro asenapine and cis-asenapine and 21 µg/ml for asenapine) and injected into the UPLC system. Calibration curves were plotted between the analyte concentration and the peak areas. Correlation coefficient, y-intercept, slope, and bias at 100 % were calculated

#### *2.4.6 Accuracy*

Accuracy for asenapine and its impurities (fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide) was performed by spiking impurities on test preparation of asenapine sublingual tablets 5 mg. Samples were prepared in triplicate with five concentrations ranging from Limit of quantitation to 150 % of maximum allowed limit (LOQ, 50 %, 75 %, 100 %, 125 %, 150 % for deschloro asenapine, cis-asenapine, and LOQ, 50 %, 75 %, 100 %, 200 %, 300 % for fumaric acid, desmethyl asenapine and N-oxide) by spiking asenapine impurities on test preparation.

#### *2.4.7 Robustness*

Experiments were performed by deliberately changing the conditions. System suitability was evaluated during this study. Chromatographic parameters evaluated in this study included pH of buffer in mobile phase A from 2.0 to 2.4 ( $\pm 0.2$  units), column temperature 30 °C to 40 °C ( $\pm 5$  °C), column flow rate from 0.18 ml/min to 0.22 ml/min ( $\pm 10$  %), aqueous phase and organic phase (acetonitrile) in mobile phase B 90 % to 110 % ( $\pm 10$  %).

#### *2.4.8 Mobile phase and Solution stability*

Solution stability was performed for test and standard solutions by keeping them on bench top for 48 hrs. Samples were injected at every 24 hrs interval and the impurity levels were estimated against a fresh standard solution. Mobile phase stability was also performed by keeping the mobile phase in tightly closed condition on bench top for 48 hrs. The freshly prepared sample and standard were injected by using the stored mobile phase at every 24 hrs interval.

### **3 Results and Discussion**

#### *3.1 Method development and Validation*

##### *3.1.1 Optimization of chromatographic conditions*

Key objective of this method development was to achieve separation for fumaric acid and other impurities in a single chromatographic method. Fumaric acid, being polar in nature, was difficult to be retained. Based on UV spectra of asenapine and its impurities, the maximum absorption wavelength of

asenapine was observed as 228 nm. So 228 nm was chosen as the detection wavelength. A solution containing all the impurities (10 µg/ml) and asenapine (1400 µg/ml) was prepared in the diluent.

During initial method development, a buffer for mobile phase was chosen as dihydrogen phosphate buffer (pH 2.2, 0.01M) based on the pKa of asenapine maleate. The mobile phase was selected as dihydrogen phosphate buffer (pH 2.2, 0.01M) and acetonitrile with 0.2 ml/minute flow rate by using BEH C18 (100\*2.1mm) 1.7µm column. Linear gradient starting T (min) / % B, 0/15 to 70/85 was run. It was observed that fumaric peak was eluting in void and the desmethyl asenapine peak was not separated from asenapine. In drug product, placebo peaks were also observed, which were eluting closely with other impurity peaks. So to retain fumaric peak, initial gradient was started from T (min) / % B, 0/5. Fumaric peak was retained a bit but still was not well separated from void. To retain fumaric acid, column was changed to BEH Shield RP18 (100\*2.1mm) 1.7µm because this column gives better retention for polar analytes. By changing the column fumaric peak was separated from void but still desmethyl asenapine was not separated from asenapine. The separation of desmethyl asenapine and asenapine was achieved by adding tetra-n- butyl ammonium hydrogen sulphate in the mobile phase as an ion pair reagent.

To separate other impurities (Deschloro asenapine and N-oxide) from placebo peaks, experiments were conducted by changing the gradient program. Finally Mobile phase A, containing potassium dihydrogen phosphate (pH 2.2; 0.01M) with tetra-n- butyl ammonium hydrogen sulphate and mobile phase B consisted of water, acetonitrile and methanol in the ratio of 10:50:40 (v/v) were found suitable. Gradient programme T (min) / % B: 0/5, 7/5, 22/20, 35/50, 38/50, 39/90, 44/90, 45/5, and 62/5 with flow rate of 0.2 mL/min was finalized. Detector wavelength was chosen as 220 nm and injection volume as 2 µl. Column temperature was finalized as 35 °C.

The relative retention times for fumaric acid, deschloro asenapine, cis asenapine, asenapine, desmethyl asenapine, N-oxide were 0.10, 0.72, 0.97, 1.00, 1.03 and 1.10 respectively. The relative response factor for fumaric acid, deschloro asenapine, cis asenapine, desmethyl asenapine, N-oxide against asenapine were 1.80, 0.46, 0.79, 0.88, 1.30 respectively.

### 3.1.2 Method Validation

Method validation was performed in accordance to ICH guidelines for specificity, Precision, Accuracy, LOD/LOQ, ruggedness, linearity, and robustness. [12].

#### 3.1.2.1 System suitability

System suitability was established on the basis of resolution between asenapine and desmethyl asenapine peaks from system suitability solution, RSD (%) for asenapine peak areas from six standard replicates, USP plate count and tailing factor for asenapine peak from standard preparation. System suitability parameters were found meeting the acceptance criteria (Table 1)

#### 3.1.2.2 Specificity

Specificity studies were performed to demonstrate the stability indicating nature of method. Analysis was done as per test method for placebo samples to evaluate the placebo interference. No interference was observed in placebo at the retention time of fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide and asenapine. All forced degradation samples were injected into UPLC with PDA. All unknown impurity peaks were well separated from asenapine and known impurities peak. Degradation was observed mainly in oxidation (3% H<sub>2</sub>O<sub>2</sub>, 35 °C, 1 hr), and thermal (105 °C, 14 hrs) stress study. Oxidation leads to formation of N-oxide while fumaric acid was observed during thermal degradation.

Asenapine was found stable under acid Hydrolysis (2N HCl, 70 °C, 24 hrs), base hydrolysis (2N NaOH, 70 °C, 24 hrs), water hydrolysis (70 °C, 24 hrs), humidity (90%RH for 7 days) and photolytic (200 watt hrs/square meter, 16 hrs). Mass balance results were calculated for all stress conditions and were found >95% (Table 2)

Peak purity of asenapine was checked for all stressed samples using by Waters Empower networking software. Peak purity was passing for all stressed samples. This confirmed the stability indicating nature of method.

**Table 1** System suitability results

Parameter	Specification	Observed Value
Resolution between asenapine and Desmethyl Asenapine	$\geq 2.0$	3.7
USP Tailing	$\leq 2.0$	1.1
Plate Count	$\geq 5000$	172026
Area [RSD (%), n=6]	$\leq 10.0$	0.7

**Table 2** Forced degradation results

Stress condition (%)	% Impurity					%Net Degradation	Mass Balance
	Fum <sup>a</sup>	Desch <sup>b</sup>	Cis <sup>c</sup>	Desm <sup>d</sup>	N-oxide		
Sample Unstressed	0.0270	0.0402	ND	ND	0.0680	NA	NA
Acid hydrolysis (2N HCl, 70°C, 24 h)	0.2722	0.0461	ND	ND	0.0672	0.3608	99.5
Base hydrolysis (2N NaOH, 70°C, 24 h)	0.0250	0.0960	ND	ND	0.1923	0.1941	96.4
Oxidation (3% H <sub>2</sub> O <sub>2</sub> , 35°C, 1h)	0.0297	0.0370	ND	ND	4.8297	4.7367	98.9
Water hydrolysis (70°C, 24 h)	0.0903	0.0352	ND	ND	0.2534	0.3882	96.8
Thermal (105°C, 14 h)	0.6230	0.0320	ND	ND	0.6752	1.4100	98.7
Humidity (90%RH, 7d)	0.2660	0.0408	ND	ND	0.6454	0.8302	98.5
Photolytic (1.2 million lux hr visible light and 200 wh/square m <sup>2</sup> UV light)	0.0849	0.0406	ND	ND	0.0920	0.0490	99.7

<sup>a</sup> Fumaric acid

<sup>b</sup> Deschloro asenapine

<sup>c</sup> cis- asenapine

<sup>d</sup> desmethyl asenapine

NA: Not applicable

### 3.1.2.3 Precision

The % RSD for the content of fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide, asenapine in repeatability were less than 2.2 and in intermediate precision were less than 5.7 which meets the acceptance criteria for method precision. The % RSD values are given in Table 3.

### 3.1.2.4 LOD and LOQ

LOD and LOQ for asenapine and its impurities (fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide) were established based on visual method. LOD, LOQ and precision at LOQ values are given in Table 3.

### 3.1.2.5 Linearity

Linearity was established for fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide, asenapine, from LOQ level to about 150 % of target concentration (about 10.5 µg/ml for Fumaric acid, desmethyl asenapine, N-oxide, about 3.2 µg/ml for deschloro asenapine and cis-asenapine and 21 µg/ml for asenapine). The correlation coefficient values were more than 0.997 and bias at 100 % level was less than 5 %, for asenapine and its impurities (Table 3)

**Table 3** LOD/LOQ, Precision and Linearity

Parameter	Fum <sup>a</sup>	Desch <sup>b</sup>	Cis <sup>c</sup>	Desm <sup>d</sup>	N-oxide	Asenapine
LOD (µg/ml)	0.1547	0.4359	0.3656	0.4780	0.3796	0.4218
LOQ (µg/ml)	0.4181	0.5710	0.5716	0.5630	0.8431	0.8395
Correlation coefficient	0.999	0.999	0.999	0.999	0.999	0.999
Intercept (a)	-75.398	-202.657	192.387	950.618	529.006	-1890.140
Slope(b)	15446.554	9706.449	16916.6	18965.0	18384.6	15446.55
Bias at 100% response	0.1	1.0	0.5	1.4	0.8	4.7
Precision [RSD (%)]	0.4	2.2	1.0	0.3	0.9	1.1
Intermediate precision [RSD (%)]	0.6	5.7	1.2	0.8	0.5	NA
Precision at LOQ [RSD (%)]	1.3	5.7	8.1	1.6	3.2	1.6

<sup>a</sup> Fumaric acid

<sup>b</sup> Deschloro asenapine

<sup>c</sup> cis- asenapine

<sup>d</sup> desmethyl asenapine

### 3.1.2.6 Accuracy

The percentage recoveries of fumaric acid, desmethyl asenapine, deschloro asenapine, cis-asenapine, N-oxide, asenapine were ranging from 91.0 to 111.9 %. Chromatogram of spiked sample (at 0.15 % level for deschloro asenapine, cis-asenapine and 0.25 % level for fumaric acid, desmethyl asenapine and n-oxide) is given in Figure 2. %Recovery values are given in Table 4.

**Table 4** % Recovery of Asenapine and its impurities

Amount spiked	%Recovery <sup>a</sup>					
	Fumaric Acid	n-oxide	desmethyl Asenapine	Deschloro asenapine	cis-asenapine	asenapine
LOQ	106.1±1.9	94.7±3.5	101.5±2.3	103.7±4.5	105.1±3.9	102.8±1.0
Level-1(50%)	95.8±0.8	98.8±1.7	92.5±0.9	93.3±1.7	101.1±0.0	104.2±4.0

Level-2(75%)	100.6±1.7	94.4±0.7	104.4±0.9	91.9±1.7	99.0±0.9	110.0±0.9
Level-3(100%)	102.5±0.7	104.7±0.8	93.3±1.9	96.5±5.6	104.9±1.9	109.3±0.6
Level-4(125%/200%) <sup>b</sup>	106.2±0.2	99.8±0.8	99.1±4.8	92.2±1.1	102.9±0.8	109.1±1.7
Level-5(150%/300%) <sup>c</sup>	104.8±0.5	102.3±0.8	97.9±3.8	94.6±4.0	103.1±2.0	110.8±1.2

<sup>a</sup> Mean± RSD (%) for three determinations

<sup>b</sup> For Fumaric acid, desmethyl asenapine and n-oxide Level-4 is 200%. For deschloro asenapine and cis-asenapine Level-4 is 125%

<sup>c</sup> For Fumaric acid, desmethyl asenapine and n-oxide Level-5 is 300%. For deschloro asenapine and cis-asenapine Level-5 is 150%

### 3.1.2.7 Robustness

With deliberately changed parameters (column flow rate, column oven temperature, buffer pH, aqueous phase composition, and composition of organic solvent), all impurity peaks were well separated. No change was observed in elution order. Resolution between asenapine and desmethyl asenapine was > 3.7, USP tailing for Asenapine peak was < 1.3, USP plate count for asenapine was > 300000 and RSD of peak areas was < 2.5 % (Table 5)

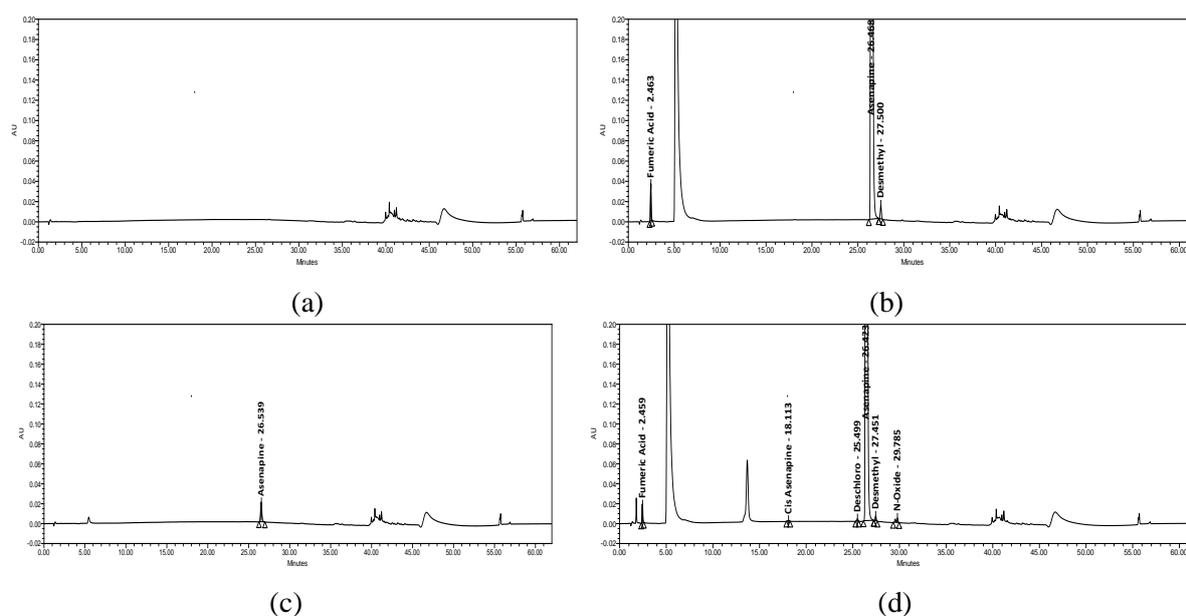
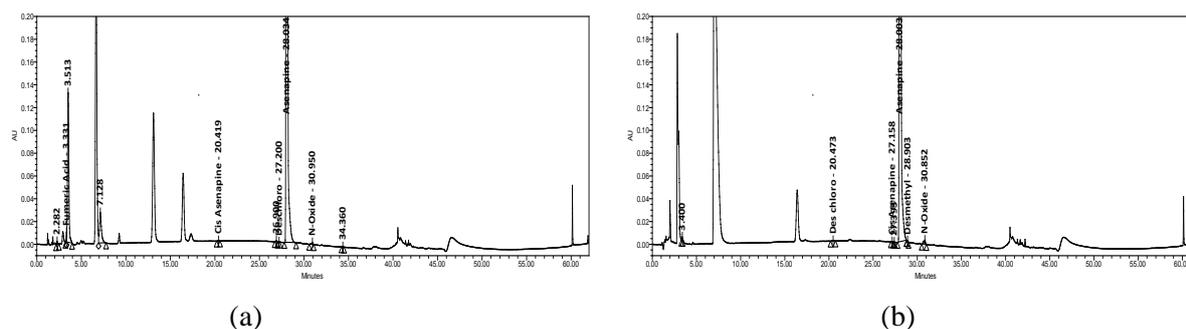


Figure 2: Representative chromatograms of Asenapine Maleate Sublingual tablets (a) Blank preparation, (b) System suitability preparation (c) Standard preparation and (d) Test Preparation spiked with impurities



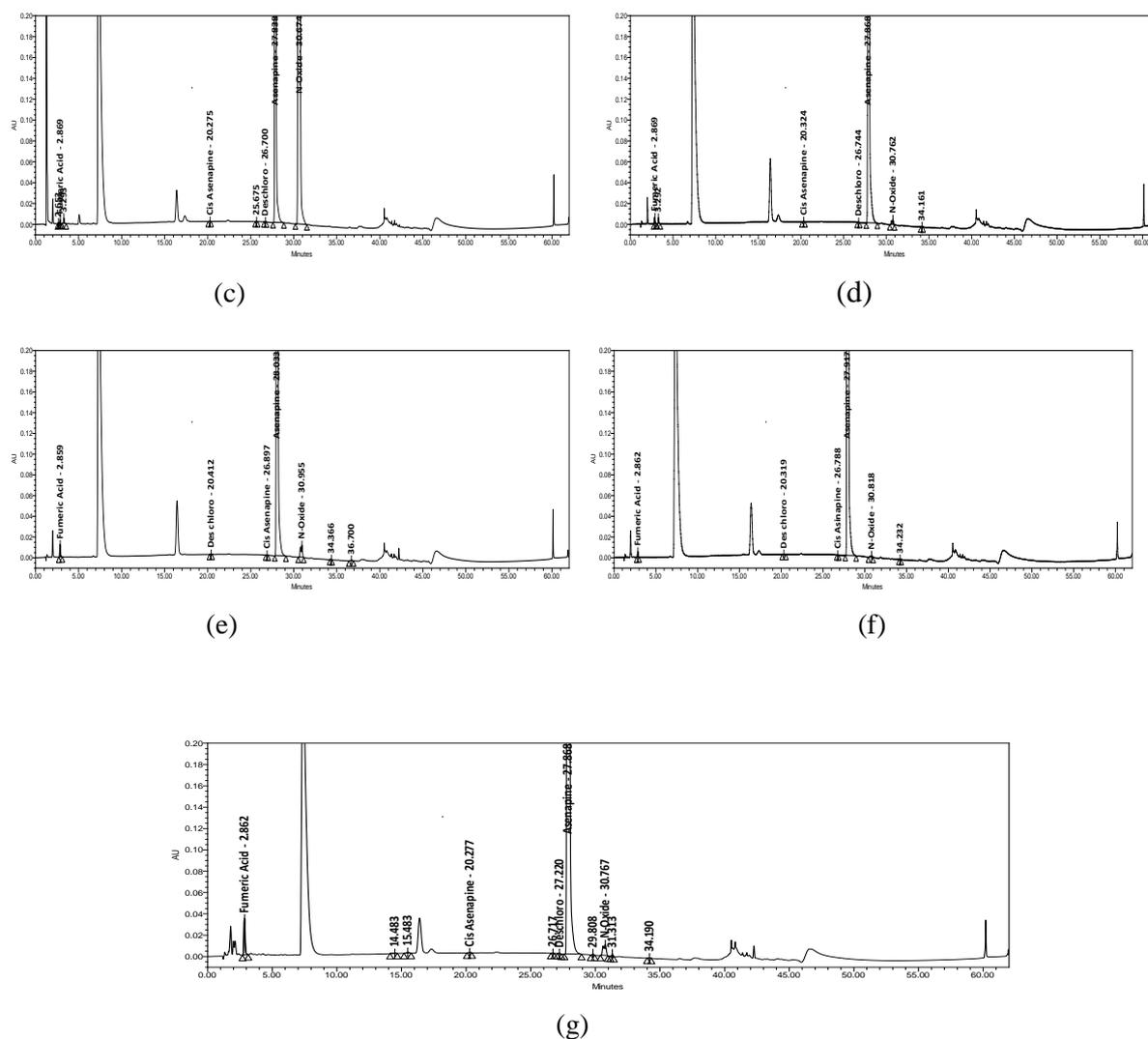


Figure 3: Representative chromatograms of Asenapine sublingual tablets (a) acid stress, (b) base stress, (c) peroxide stress, and (d) water stress (e) humidity stress (f) UV stress (g) thermal stress

### 3.1.2.8 Solution stability and mobile phase stability

Content for all five impurities did not change beyond  $\pm 15\%$  during mobile phase and solution stability. Test preparations and mobile phase are stable up to 48 hrs on bench top at room temperature

**Table 5 Robustness results**

Observed system suitability parameters				
Stress condition	Resolution between Asenapine and Desmethyl ≥ 2.0	USP Tailing ≤2.0	Plate Count ≥5000	Area (n=6) [RSD (%) ≤10.0]
Column temperature 30°C	4.6	1.2	501136	0.5
Column temperature 40°C	4.8	1.3	364800	0.5
Column flow 0.18 ml/min	4.5	1.2	447142	0.6
Column flow 0.22 ml/min	5.0	1.2	415475	0.7
Mobile phase buffer pH 2.0	4.8	1.1	431488	0.5
Mobile phase buffer pH 2.4	3.8	1.2	394231	0.4
Aqueous 90%	4.8	1.2	439778	2.5
Aqueous 110%	3.7	1.2	380742	0.4
Acetonitrile 90%	4.0	1.0	232033	0.3
Acetonitrile 110%	5.0	1.2	372369	0.9

#### 4. Conclusion

For quantitation of impurities of asenapine maleate in pharmaceutical dosage forms, a UPLC method was developed. Method was found specific, precise, accurate, rugged, robust, and linear.

#### Acknowledgements

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