International Journal of General Medicine and Pharmacy (IJGMP)
ISSN(P): 2319-3999; ISSN(E): 2319-4006
Vol. 3, Issue 4, July 2014, 85-98

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

IMPURITIES RELATED SULFAMETHOXYPYRAZINE, CHARACTERIZATION AND QUANTITATIVE DETERMINATION OF PROCESS BY SOME ANALYTICAL TECHNIQUES

MUKESH KUMAR GUPTA[1] & MANOHAR V. LOKHANDE[2]

[1]Department of Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidya Nagari, Jhunjhunu, Chudela, Rajasthan, India

[1]IPCA Laboratories Ltd, Chemical Research Division, Kandivli Industrial Estate, Kandivli (W), Mumbai, Maharashtra, India

[2]Department of Chemistry, Sathaye College, Vileparle (E), Mumbai, Maharashtra, India

ABSTRACT

Four impurities in Active pharmaceutical ingredient (API) Sulfamethoxypyrazine were detected by a newly developed gradient reverse phase high performance liquid chromatographic (HPLC) method. These impurities were identified by LC/MS/MS. Three of the impurities were unknowns having not been reported previously. Structural assignment of these impurities was carried out by LC/MS/MS using electro spray ionization source and an ion trap mass analyzer. Structural elucidation using nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy was facilitated by newly developed preparative isolation method. These impurities were characterized as 4-amino-N-(6-hydroxypyrazin-2-yl) benzene sulfonamide (SMP-I), 4-amino-N-(pyrazin-2-yl) benzene sulfonamide (SMP-II) & 4-amino-N-(6-methoxypyrazin-2-yl) benzene sulfonamide (SMP-III). The synthesized /isolated reference samples of the impurity compounds were used for the quantitative HPLC determination. The method was validated according to ICH guidelines with respect to specificity, precision, accuracy, linearity and robustness. Forced degradation studies were also performed for Sulfamethoxypyrazine bulk drug sample to demonstrate the stability indicating power of the newly developed HPLC method.

KEYWORDS: Sulfamethoxypyrazine, Impurities, HPLC, LCMS/MS/MS, Validation, Forced Degradation

INTRODUCTION

Sulfamethoxypyrazine, N^1-(3-Methoxypyrazin-2-yl) sulphanilamide is a long-acting sulfonamide that has been used orally in the treatment of respiratory and urinary tract infections. It is given in combination with pyrimethamine in the treatment of malaria. It has also been given in the ratio 4 parts of Sulfamethoxypyrazine to 5 parts of trimethoprim as a combination with uses similar to those of co-trimoxazole [1].

A few bio-analytical methods are reported in the literature for the quantitative determination of Sulfamethoxypyrazine (SMP) concentration in biological fluids using liquid chromatography and mass spectroscopic method [2-4]. However, so far there is no published report, describing the complete characterization of related impurities in SMP as active pharmaceutical ingredient (API). There are no reports available on the investigation using LC/MS/MS and isolation/synthesis of related substances in SMP active pharmaceutical ingredient (API).

Impurity profile of a drug substance is critical for its safety assessment and manufacturing process. It is mandatory to identify and characterize the impurities in the pharmaceutical product, if present above the accepted limits of 0.1 % [5]. The present study deals with the identification and structural elucidation of the process related impurities which were found in the product (SMP). Though, different methods of synthesis of SMP are reported, the selected route was safe, feasible & economical [6]. However, the literature survey does not give any details regarding these impurities. Impurity profiling of drugs is the most important issue in the modern pharmaceutical analysis [7-8] for developing process technology to manufacture high purity drug substance.

During process development studies, four impurities were detected in both crude and pure samples of SMP using a newly developed gradient reversed phase HPLC method. A comprehensive study was undertaken for the identification of these impurities using LC/MS/MS followed by their synthesis and further characterization by NMR. This paper also deals with the analytical method validation of a new HPLC method for quantitative determination of these impurities.

EXPERIMENTAL

Materials and Reagents

Samples of SMP API were obtained from Ipca Laboratories Ltd., Chemical Research Division, Mumbai, India. HPLC grade acetonitrile and perchloric acid (70%) were purchased from Merck India Limited. Chloroform—d^3 and dimethyl sulphoxide—d^6 (for NMR) were purchased from Aldrich Chemical Co., USA.

High Performance Liquid Chromatography

Samples were analyzed on Alliance 2690 HPLC (Waters, Milford, MA, USA) system equipped with 2487 UV detector. A Unisphere C18 column (150 mm x 4.6 mm i.d. 5 μm) was used for chromatographic separation. The mobile phase consisting of A: 1 ml perchloric acid (70%) in 1000 ml water and B: acetonitrile, with timed gradient programme T_{min}/A: B: T_0/85:15; T_{10}/85:15; T_{30}/50:50; T_{40}/85:15; T_{45}/85:15 with flow rate of 0.8 ml per minute were used. The column oven temperature was maintained at 30°C. The injection volume was 20μL and the detector wavelength was fixed at 270 nm.

Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS)

The MS and MS/MS studies were performed on LCQ Advantage (Thermo Electron, San Jose, CA) ion trap mass spectrometer. The source voltage was maintained at 3.0 kV and capillary temperature at 250°C. Nitrogen was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the various range. MS/MS studies were carried out by keeping normalized collision energy at 25-30% and an isolation width of 6 amu. The HPLC consisted of an Agilent-1100 series quaternary gradient pump with a degasser, an auto sampler and column oven. A C18 column (ProntoSIL Kromabond column 150 mm x 4.6 mm i.d. 5 μm) was used for separation. The mobile phase consisting of A: 1 ml Trifluoroacetic acid in 1000 ml water and B: acetonitrile, with timed gradient programme T_{min}/A: B: T_0/85:15; T_{10}/85:15; T_{30}/50:50; T_{40}/85:15; T_{45}/85:15 with flow rate of 0.8 ml per minute were used.

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra of the synthesized/isolated impurities were recorded on Bruker 400MHz instrument. The ^{1}H and ^{13}C chemical shift values were reported on the δ scale (ppm) relative to $CDCl_3$ (7.26 ppm).

IR Spectroscopy

The IR spectra for isolated impurities were recorded in the solid state as KBr powder dispersion using Perkin-Elmer spectrum one FT-IR spectrometer.

Preparative Liquid Chromatography

Impurities were isolated from the crude sample using Waters Auto purification system consisting of 2525 binary gradient pump, a 2487UV detector and 2767sample manager (Waters, Milford MA, USA). A Peerless Basic C18 column (150mm×21.2mm i.d., particle size 5µm) was used for the separation. The mobile phase was consisted of a mixture of water and acetonitrile in the ratio of 85:15 and was pumped at flow rate 25 ml/min. The detection was monitored at 270 nm.

Preparation of Solutions for Validation of HPLC Method

A test preparation of 500 µg/mL of SMP bulk drug sample was prepared using the diluents (mixture of 0.1% perchloric acid (75%) in water and acetonitrile in the ratio of 85:15). A stock solution of mixture of impurities was prepared by dissolving 0.5mg/ml each of SMP-I, SMP-II, SMP-III and SMP. From this stock solution a standard solution containing 0.5 µg/mL each of SMP-I, SMP-II, SMP-III and SMP was prepared. This standard solution was also used for checking system suitability parameters.

RESULTS AND DISCUSSIONS

Detection of Impurities by HPLC

HPLC analysis using the method described in Section 2.2 revealed the presence of four impurities at RRTs 0.21, 0.26, 0.55 and 1.35 with respect to principle peak. The target impurities under study are marked as Sulphanilamide (starting material), SMP-I, SMP-II, and SMP-III, respectively. The typical chromatogram of crude SMP sample highlighting the retention time of impurities is shown in Figure 1.

Identification of Impurities by LC/MS/MS

Prior to characterization work it was logical to generate the mass data for the parent drug molecule so that it can be easy to compare and conclude which process related impurities may be formed during the synthetic reaction. The spectra of SMP exhibits a protonated molecular ion peak $[M+H]^+$ 281 (figure 5a) (molecular mass of SMP is 280) in electro spray ionisation in positive mode, the most probable site of protonation was at NH_2 and NH. The MS/MS spectrum obtained for the protonated SMP molecule showed prominent peak at 156 (figure 5b) which is due to cleavage of NH-SO_2 bond giving rise to $C_6H_6NO_2S^+$ plausible fragmentation is showed (Figure 2a).

SMP-I: showed a protonated molecular ion peak $[M+H]^+$ 267 having molecular mass of 266, which under goes fragmentation to form $C_6H_6NO_2S^+$ for 156 by loss of $C_4H_5N_3O$ ion (refer figure 2b, figure 6a and 6b).

SMP-II: similarly showed $[M+H]^+$ of 251 for molecular mass of 250 and a loss of $C_4H_5N_3^{\bullet+}$ giving daughter ion of mass 156 (refer figure 2c,7a and 7b).

SMP-III: which is an isomer, showed similar fragmentation that of SMP (Figure 2c, 8a and 8b). Since all the mass fragmentation as discussed above, showed similar daughter ions of 156 for $C_6H_6NO_2S^+$, this indicated that these impurities are structurally similar. SMP-III and SMP were having same molecular mass and may be regioisomer of each

other; hence it was mandatory to confirm the structure by NMR. Hence NMR of all the impurities and the product was carried out for comparison and further confirmation of structure.

Brief Synthetic Preparation of SMP

Sulphanilamide is reacted with 2, 3-dichloropyrazine in presence of potassium carbonate, N, N-dimethylformamide and toluene under stirring at temperature at 135°C - 140°C for 10hrs. A slurry is formed which is vacuum distilled. The material obtained after vacuum distillation is acidified with acetic acid to pH 4.8 to 5.0 a precipitate is formed, which is filtered and washed with water to get pure 4-amino-N-(3-chloropyrazinyl)benzenesulphonamide (SCP). SCP obtained is treated with sodium hydroxide and methanol forming SMP which is washed with water (Figure 3a).

Isolation and Structural Elucidation of SMP-I

During the synthesis of SMP i.e. (from SCP to SMP), due to the basics condition of the reaction mass there is hydrolysis of methoxy group taking place which give rise to SMP-I impurity which is then isolated by preparative HPLC (described in Section 2.6). The chromatographic purity was found to be 95%. ^{1}H and ^{13}C NMR spectral data (refer table 1) confirmed the proposed structure. The MS/MS spectrum obtained for isolated compound of impurity using direct infusion mode was exactly same as MS/MS spectrum of SMP-I

Synthesis and Structural Elucidation of SMP-II

Since SMP-II is not isolable from the reaction mixture of the SMP synthesis, it was independently synthesized. Due to the presence of 2-chloropyrazine as an impurity in 2,3-dichloropyrazine used as raw material in synthetic route of SMP there is formation of SMP-II which remains unreacted and gets carried forward to SMP final. This impurity was prepared synthetically by using the same synthetic route as that of SMP but instead of 2,3-dichloropyrazine the starting material used was 2-chloropyrazine(Figure 3b).

The chromatographic purity was found to be 97%. ^{1}H and ^{13}C NMR spectral data confirmed the proposed structure (refer table 1). The MS/MS spectrum obtained for synthesized authentic compound of impurity using direct infusion mode was exactly same as MS/MS spectrum of SMP-II.

Synthesis and Structural Elucidation of SMP-III

2, 6-dichloropyrazine which is isomer present in 2, 3-dichloropyrazine as a impurity under goes similar reaction of SMP to form SMP-III. SMP-III is synthesized by using 2, 6-dichloropyrazine instead of 2, 3-dichloropyrazine in synthetic process of SMP (Figure 3c).

The chromatographic purity was found to be 96%. ^{1}H and ^{13}C NMR spectral data confirmed the proposed structure (refer table 1). The MS/MS spectrum obtained for synthesized authentic compound of impurity using direct infusion mode was exactly same as MS/MS spectrum of SMP-III.

Analytical Method Validation by HPLC

The validation study allowed the evaluation of the method for its suitability for routine analysis. The newly developed method for SMP and its related impurities was validated according to ICH guidelines [9]. The validation study was carried out for the analysis of SMP-I, SMP-II and SMP-III. The system suitability parameters obtained for related substance method are given in Table 2 (figure 4a).

Forced degradation studies were also performed (Acid, Base) for SMP bulk drug sample to demonstrate the stability indicating power of the newly developed HPLC method.

Specificity

Specificity is the ability of analytical method to measure the analyte response in the presence of its potential impurities and degradents. The specificity of the HPLC method was determined by injecting individual impurity samples, wherein no interference was observed for any of the components.

The chromatograms were checked for the appearance of any extra peak. Peak purity of these samples under stressed conditions was verified using a PDA detector. The purity of the principle and other chromatographic peaks was found to be satisfactory. This study confirmed the stability indicating power of the HPLC method.

Precision

The precision of the method was examined using six replicate injections of a standard solution (mixture of impurities). The relative standard deviation (R.S.D.) was calculated for response (area) of each impurity. The R.S.D.'s for SMP-I, SMP-II, SMP and SMP-III were found to be 1.48%, 1.71%, 6.37% and 1.67%, respectively. The method precision was established by analyzing samples of SMP using six different test preparations. The calculated R.S.D. of these results was found to be within acceptable limit.

Accuracy

The accuracy of the method was determined for the related substances by spiking of known amounts of an impurity in SMP bulk sample (test preparation) at levels, LOQ, 80%, 100% and 120% of the specified limit. The recoveries of impurities were calculated and are given in Table 3.

Limit of Detection and Limit of Quantification

Detection limit (DL) and quantitation limit (QL) for all impurities was estimated by signal to noise (S/N) method. The limit of detection values for SMP-I, SMP -II and SMP -III were 0.002%, 0.002%, and 0.002% w.r.t. analyte concentration (500 µg/mL), respectively. The limit of quantification values for SMP-I, SMP -II and SMP -III were found to be 0.006%, 0.006%, and 0.006% w.r.t. analyte concentration (500 µg/mL), respectively.

Linearity

Linear calibration plots for the related substance method were obtained over the calibration range i.e. LOQ, 50%, 80%, 100%, 120% and 150% at six concentration levels in triplicate. For SMP-I corresponding regression equation was $y = 28876x+348.97$, with the correlation coefficient (r) is 0.9996. For SMP-II, corresponding regression equation was $y = 52747x-0.2397$, with the correlation coefficient (r) is 0.9990. For SMP-III, corresponding regression equation was $y = 63972x+434.54$, with the correlation coefficient (r) is 0.9983.The results showed excellent correlation between the peak area and concentration of impurities.

Robustness

In all the deliberately varied chromatographic conditions (column temperature, flow rate and column make), the chromatogram for system suitability solution for related substance showed no significant change in system suitability parameters Table 4.

Solution Stability

The solution stability of SMP sample and its related impurities was carried out by leaving both solutions in tightly capped HPLC vials at 25°C for 16 hrs in an auto sampler. No significant changes were observed in the area of impurities in standard solution after 16 hours.

CONCLUSIONS

A new HPLC method was developed for separation of impurities in SMP bulk drug sample. These impurities were identified by LC/MS analysis. Characterization of the impurities was carried by synthesis/isolation followed by spectroscopic analysis. The newly developed HPLC method has been validated as per regulatory guidelines; it can be conveniently used for the quantitative determination of related substances in SMP bulk drug sample. The method was found to be specific, accurate and precise, robust and can be used for the routine analysis as well as to monitor the stability studies of the API.

ACKNOWLEDGEMENTS

The authors wish to thanks, the management of Ipca Laboratories, Mr. Ashish Haldanka, Mr. Mukeshkumar Patel and Mr. Alok Acharya for necessary facilities and co-operation.

REFERENCES

1. S. C. Sweetman, Martindel (2009) The Complete Drug Reference, 36th edn., Pharmaceutical Press, London, pp. 342.
2. M. L. Storme, F.H. Jansen, W. Goeteyn, J.F.V. Bocxlaer, (2006) Simultaneous quantitative analysis of the antimalarials pyrimethamine and sulfamethoxypyrazine in plasma samples using liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 20, 2947.
3. V. K. Dua, R. Sarin, N.C. Gupta, V.P. Sharma, (1998) Sulfalene concentrations in plasma and blood cells of Plasmodium falciparum malaria cases after treatment with metakelfin using high-performance liquid chromatography, J Chromatogr B Biomed Sci Appl. 714, 390.
4. V. K. Dua, R. Sarin, V. P. Sharma, (1991)Determination of sulfalene in plasma, red blood cells and whole blood by high-performance liquid chromatography, Journal of Chromatography. 563,333.
5. ICH Q3A (R2) Guideline, (2006) Impurities in New Drug Substances version 4.
6. V. G. Turin, G. Oranto, L. (1981) Buracchi, United States Patent 4299962,.
7. S. Gorog (Ed.), (1999) Determination of impurities in drugs, Elsevier sciences, Amsterdam.
8. S. Ahuja, (1998) Impurities evaluation in pharmaceuticals, Marcel Dekker, New York.
9. ICH Guidelines, Q2 (R1), (2005) Validation of analytical procedures: test and methodology.

APPENDICES

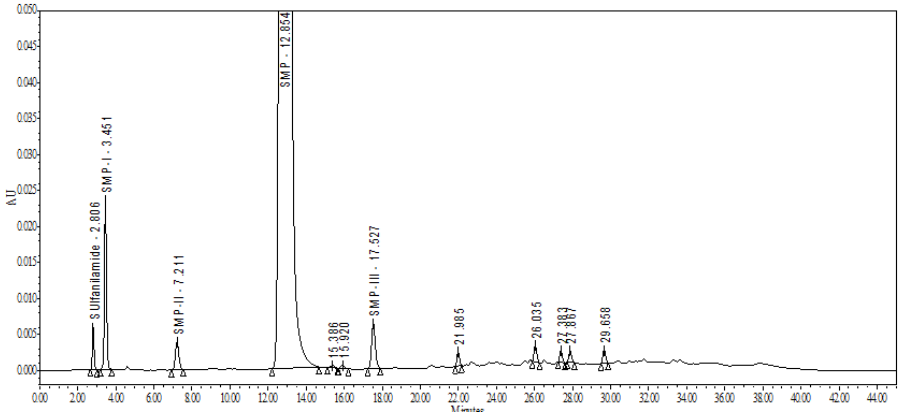


Figure 1: A Typical Chromatogram of SMP Sample

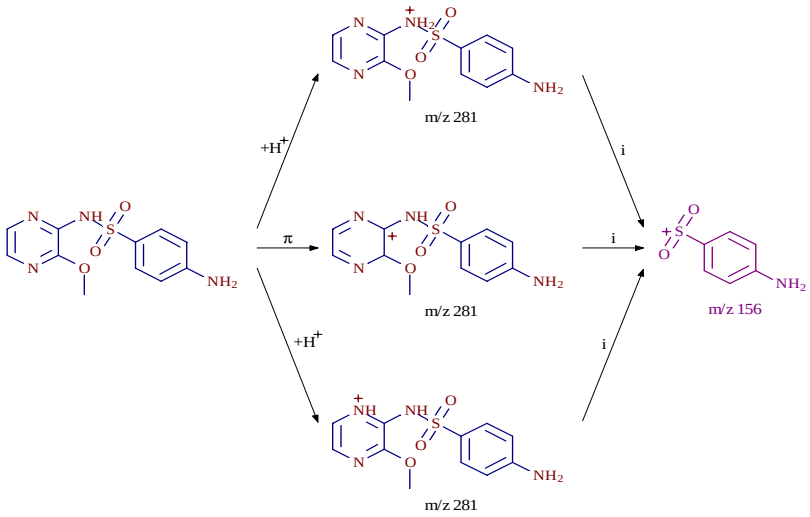


Figure 2a: Plausible Scheme for Fragmentations of SMP

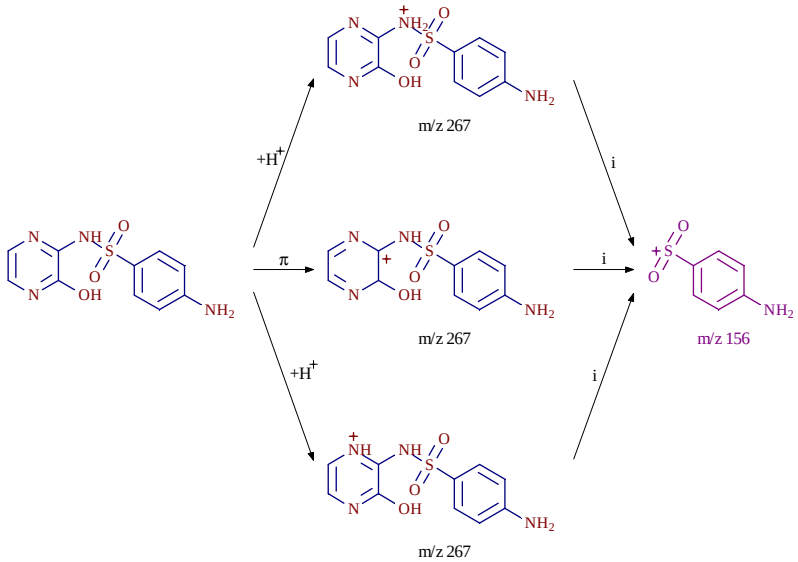


Figure 2b: Plausible Scheme for Fragmentations of SMP-I

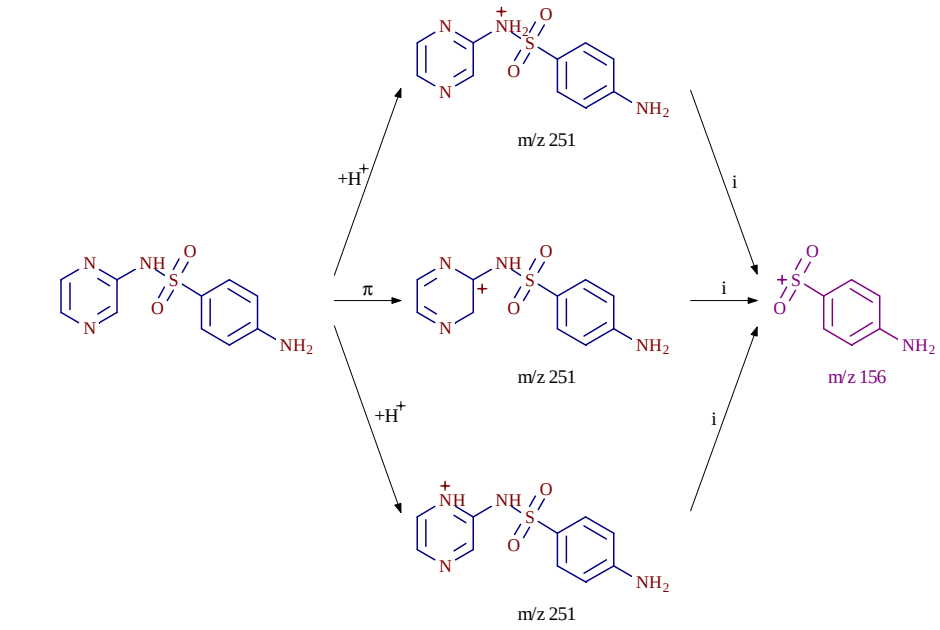


Figure 2c: Plausible Scheme for Fragmentations of SMP-II

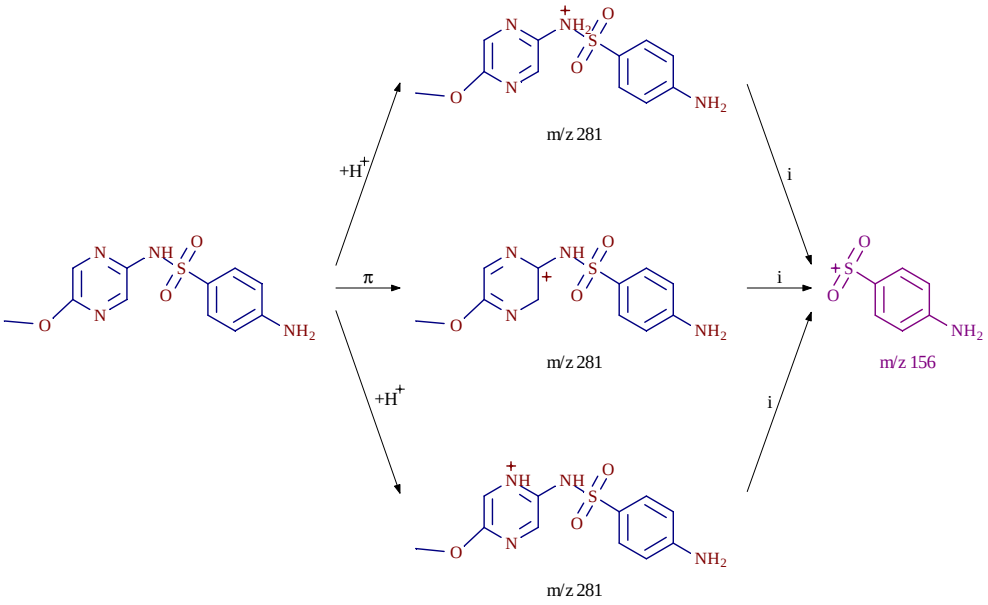


Figure 2d: Plausible Scheme for Fragmentations of SMP-III

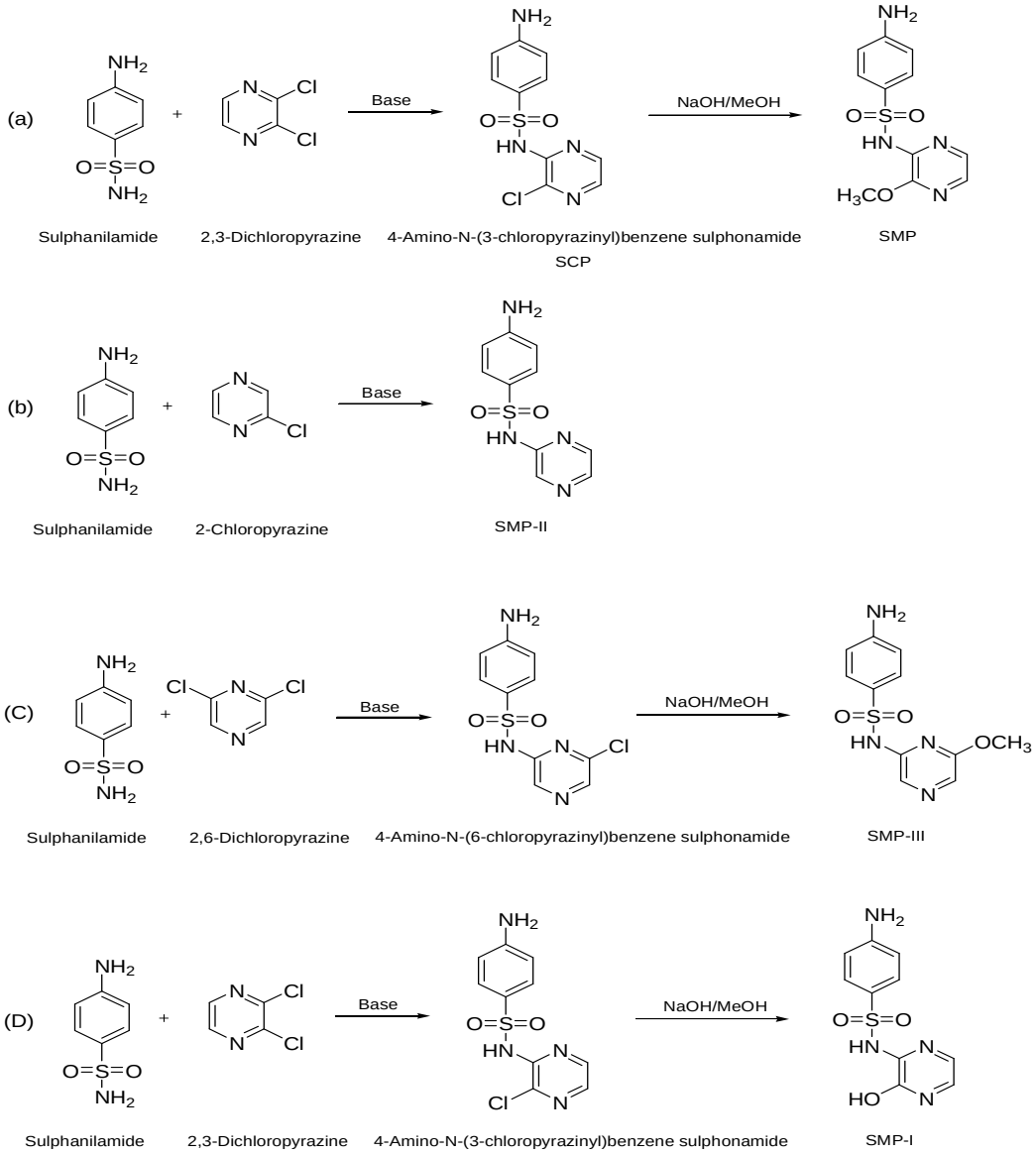


Figure 3: (a) Scheme for Synthesis of SMP, (b): Formation of SMP-II, (c): Formation of SMP-III, and (d): Formation of SMP-I

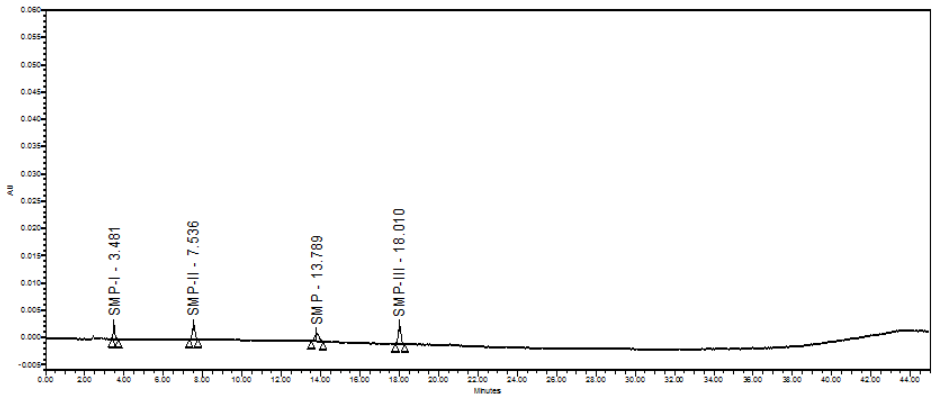
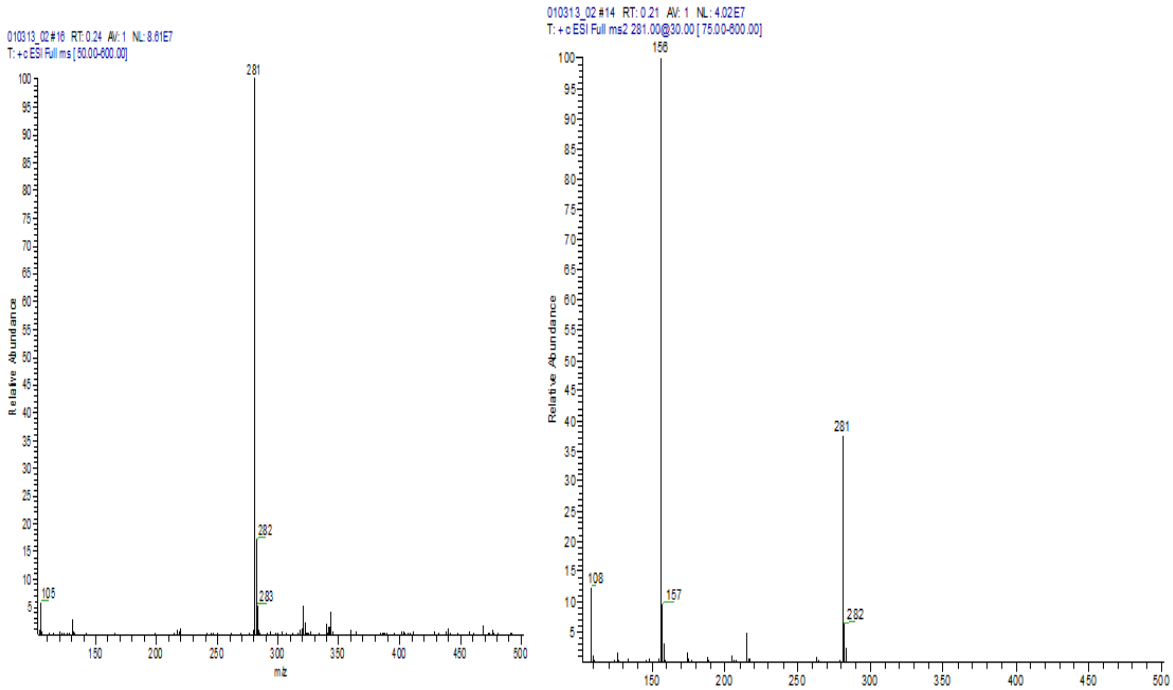
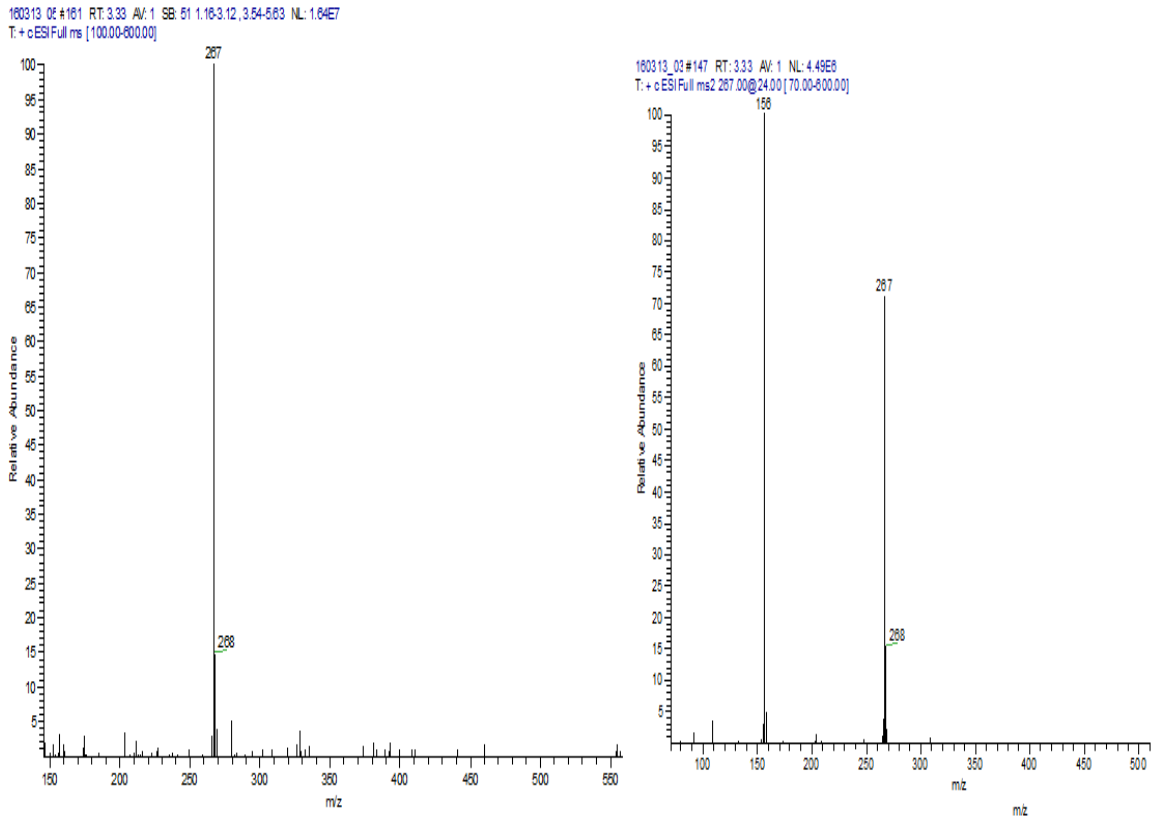


Figure 4: Chromatogram of System Suitability Solution



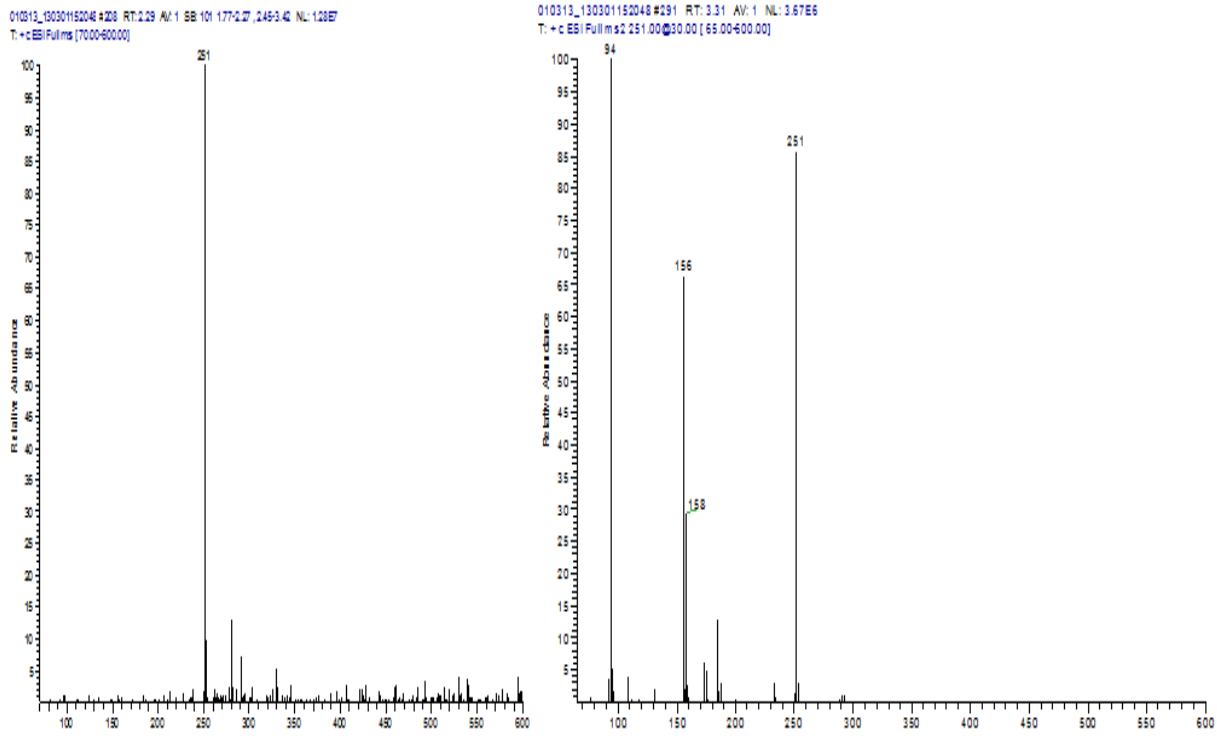
(a) **(b)**

Figure 5: (a) Mass Spectrum of SMP and (b): MS/MS Spectrum of SMP



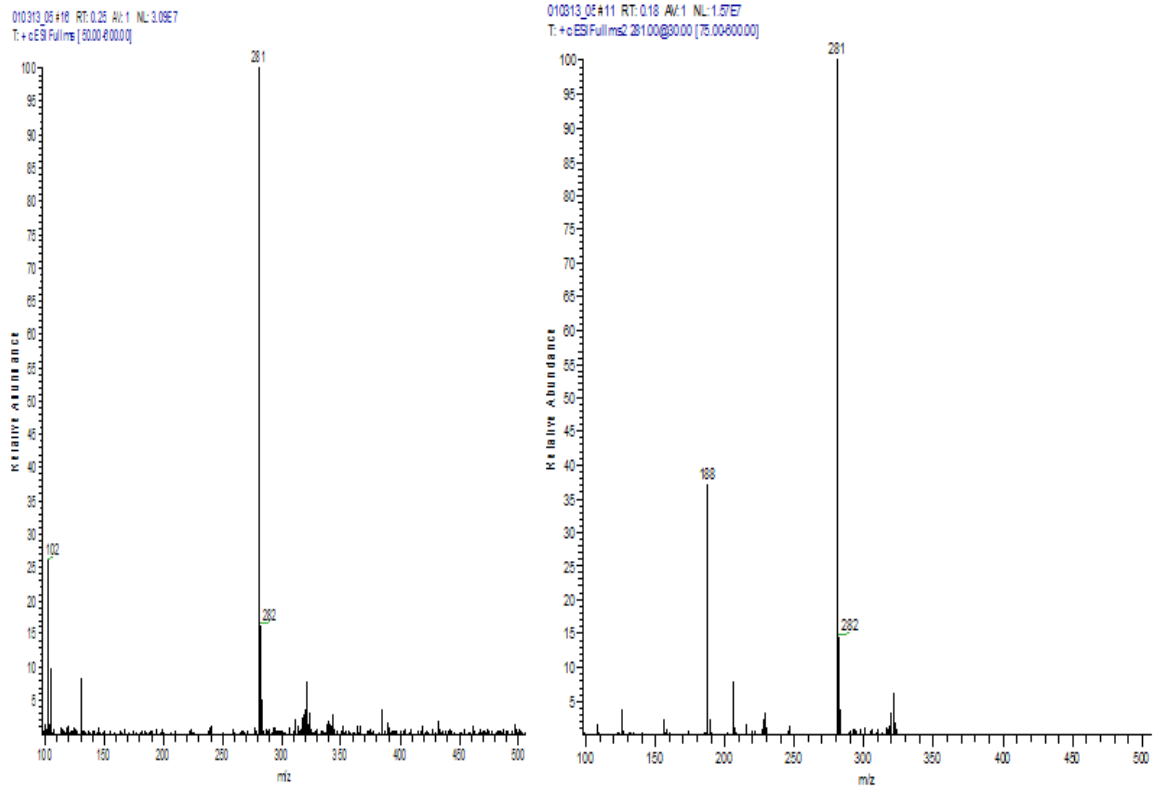
(a) **(b)**

Figure 6: (a) Mass Spectrum of SMP-I and (b) MS/MS Spectrum of SMP-I



(a) **(b)**

Figure 7: (a) Mass Spectrum of SMP-II and (b) MS/MS Spectrum of SMP-II



(a) **(b)**

Figure 8: (a) Mass Spectrum of SMP-III and (b) MS/MS Spectrum of SMP-III

Table 1: ^{1}H NMR and C-13 Assignment for SMP, SMP-I, II, and III

	SMP				SMP-I				SMP-II				SMP-III			
Position[a]	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	13C δ (ppm)	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	13C δ (ppm)	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	13C δ (ppm)	Integration	δ (ppm)	Multiplicity J (Hz)[a]	13C δ (ppm)
1	2H	6.01	brs	-	2H	4.39	brs	-	2H	6.08	brs	-	2H	6.08	brs	-
2	-	-	-	153.5	-	-	-	151.6	-	-	-	153.8	-	-	-	151.6
3, 3'	2H	6.57	d (8.8)	112.6	2H	6.61	d (8.8)	116.6	2H	6.58	d (8.8)	112.9	2H	6.56	d (8.8)	116.6
4, 4'	2H	7.67	d (8.8)	130.2	2H	7.81	d (8.8)	128.1	2H	7.58	d (8.8)	129.8	2H	7.55	d (8.8)	128.1
5	-	-	-	125.6	-	-	-	129.7	-	-	-	124.5	-	-	-	129.7
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1H	10.34	brs	-	1H	11.7	brs	-	1H	11.0	brs	-	1H	11.11	brs	-
8	-	-	-	149.9	-	-	-	152.7	-	-	-	149.0	-	-	-	154.0
9	1H	-	d (1.2)	138.8	1H	-	-	145.7	1H	7.80	-	138.8	1H	8.32	d (1.2)	123.0
10	-	-	-	-	-	-	-	-	-	7.80	-	-	-	-	-	-
11	1H	7.71	s	133.9	1H	6.65	d (4.3)	125.5	1H	-	-	134.8	1H	8.18	s	132.4
12	1H	7.71	s	133.6	1H	6.90	d (4.3)	126.5	1H	-	-	142.7	1H	8.18	s	161.1
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	1H	8.44	brs	-	-	-	-	-	-	-	-	-
15	3H	3.89	s	54.1	-	-	-	-	3H	3.73	s	-	-	-	-	55.9

s-singlet, d-doublet, brs-broad singlet.

[a] Refer the structural formula in Figure.

[b] ^{1}H-^{1}H coupling constants.

Table 2: System Suitability Report

Component	Tailing Factor	Theoretical Plates	% RSD
SMP-I	1.10	8547	1.48
SMP-II	1.02	13763	1.71
SMP	0.98	17644	1.67
SMP-III	1.06	86953	6.37

Table 3: Accuracy of Impurities

	Amount Added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	Mean
At LOQ Level				
SMP-I	0.0315	0.0279	88.67	104.22
	0.0315	0.0339	107.67	
	0.0315	0.0366	116.33	
SMP-II	0.0309	0.0342	113.67	112.45
	0.0309	0.0337	109.00	
	0.0309	0.0354	114.67	
SMP-III	0.0303	0.0333	110.00	107.78
	0.0303	0.0316	104.33	
	0.0303	0.0330	109.00	
At 80% Level				
SMP-I	0.4200	0.4012	95.53	98.48
	0.4200	0.4158	99.00	
	0.4200	0.4238	100.90	
SMP-II	0.4120	0.4098	99.48	99.38
	0.4120	0.4051	98.33	
	0.4120	0.4133	100.33	
SMP-III	0.4040	0.4073	100.83	100.86
	0.4040	0.4080	100.98	
	0.4040	0.4071	100.78	
At 100% Level				
SMP-I	0.5250	0.5144	97.98	101.17
	0.5250	0.5396	102.78	
	0.5250	0.5394	102.74	

Table 3: Contd.,				
SMP-II	0.5150	0.5081	98.66	98.28
	0.5150	0.5061	98.26	
	0.5150	0.5043	97.92	
SMP-III	0.5050	0.5075	100.48	99.87
	0.5050	0.5019	99.40	
	0.5050	0.5036	99.72	
At 120% Level				
SMP-I	0.6300	0.5844	92.76	96.78
	0.6300	0.6191	98.27	
	0.6300	0.6256	99.30	
SMP-II	0.6180	0.6018	97.38	97.56
	0.6180	0.6011	97.27	
	0.6180	0.6060	98.05	
SMP-III	0.6060	0.5996	98.95	98.61
	0.6060	0.5961	98.37	
	0.6060	0.5969	98.50	

Table 4: Robustness Study

Mix Standard Solution (Column Temperature)						
Name of Impurity	Column Temp. 27°C		*Column Temp. 30°C		Column Temp. 33°C	
	**RRT	Tailing Factor	RRT	Tailing Factor	RRT	Tailing Factor
SMP-I	0.25	1.11	0.25	1.10	1.10	1.11
SMP-II	0.55	1.03	0.54	1.02	1.02	1.03
SMP	1.00	0.98	1.00	0.99	0.99	0.99
SMP-III	1.28	0.99	1.29	1.06	1.06	1.00

Table 5

Mix Standard Solution (Flow Rate)						
Name of Impurity	Flow Rate- 0.6 ml/min		*Flow Rate- 0.8 ml/min		Flow Rate- 1.0 ml/min	
	**RRT	Tailing Factor	RRT	Tailing Factor	RRT	Tailing Factor
SMP-I	0.27	1.13	0.25	1.10	0.25	1.07
SMP-II	0.57	1.02	0.54	1.02	0.54	1.01
SMP	1.00	0.99	1.00	0.99	1.00	1.01
SMP-III	1.19	1.02	1.29	1.06	1.43	0.98

Table 6

Mix Standard Solution (Column Make Change)						
Name of Impurity	Column Temp. 27°C		*Column Temp. 30°C		Column Temp. 33°C	
	**RRT	Tailing Factor	RRT	Tailing Factor	RRT	Tailing Factor
SMP-I	0.26	1.46	0.25	1.10	0.26	1.03
SMP-II	0.55	1.55	0.54	1.02	0.55	0.92
SMP	1.00	1.34	1.00	0.99	1.00	0.92
SMP-III	1.32	1.50	1.29	1.06	1.33	0.92

*Data taken from Precision study

**RRT w. r. t. SMP