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Impurities profiling Method and degradation studies for Sumatriptan Succinate in Sumatriptan Succinate and Naproxen Sodium Tablets

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ABSTRACT

A simple, sensitive, and precise high performance liquid chromatographic method for the impurities profiling of Sumatriptan succinate in Sumatriptan and naproxen tablets has been developed, validated and used for the determination of impurities in commercial pharmaceutical products. The Impurities were well separated on a Waters Spherisorb ODS-1 column (250mm X 4.6mm, 5µm) by the gradient program using 0.05 M Phosphate buffer (pH 3.0), Acetonitrile and methanol at a flow rate of 1.0 mL min-1 with detection wavelength at 225 nm. The developed method was found to be specific, precise, linear, accurate, rugged and robust. LOQ Values for all the known impurities were below reporting thresholds.

Keywords: Development, Stability-indicating, Sumatriptan, Naproxen, Impurities, Combination, HPLC.

INTRODUCTION

Sumatriptan Succinate is a triptan sulfa drug containg a sulfonamide group. It is used for the treatment of migraine headaches. Chemically it is 1-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-methyl-methanesulfonamide.Succinate (Fig.1)[1]. As per the EP [2] and U.S Pharmacopeia[3], sumatriptan succinate having four impurities (Fig.1),

A number of analytical methods available to estimate the Sumatriptan active ingredient in the pharmaceutical formulation and Plasma. To the best of our knowledge, no single method which is available currently can separate and estimate all the known related compounds and degradation impurities of Sumatriptan succinate in pharmaceutical dosage form. The analytical methods mentioned in pharmacopeia for impurity determination comprises separate methodologies for Impurity-A alone and second method for remaining impurities.

Attempts were made to develop a stability indicating LC[4-8] method for estimation of related substance of Sumatriptan succinate in the Sumatriptan Succinate and Naproxen Tablets formulations.

The developed analytical method demonstrates analysis of estimation of Sumatriptan succinate impurities in presence of placebo like Naproxen and other pharmaceutical excipients with detection wavelength at 225 nm.

We also verified forced degradation of Sumatriptan succinate and Naproxen Tablets as per ICH[11-13] Conditions like acid hydrolysis, base hydrolysis, oxidation, heat , UV light and photo light. The developed method [9-10] was

found to be specific, precise, linear, accurate, rugged and robust. LOQ Values for all the known impurities were below reporting thresholds.

EXPERIMENTAL SECTION

2.1. Chemicals and reagents

Tablets and standards of Sumatriptan succinate and its 4 impurities namely impurity-A, impurity-B, impurity-C, impurity-D, were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile, and analytical grade KH_2PO_4, Dibutyl amine and ortho phosphoric acid were from Merck, Darmstadt, Germany. Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use. 2.2. Equipment

The waters HPLC PDA 2996 system used consists of a Quaternary solvent manager, a sample manager and a Photodiode array UV detector. The output signal was monitored and processed using empowers software. Water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolysis studies. Photo stability studies were performed in a photo stability chamber, UV light (200 watt hours / square meter), sun light (1.2 Million Lux hours) Calibrated (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

2.2. Chromatographic Conditions

The analytes were separated on (250 x 4.6 mm, 5µm) Waters Spherisob ODS-1 column with mobile phase containing a gradient mixture of solvent A and B at column oven temperature of 25^{o}C with a gradient run program at a flow-rate of 1.0 mL min^{-1}. 0.05 M potassium dihydrogen orthophosphate, 1.5ml of Dibutylamine in 1000ml Milli-Q-Water, pH adjusted to 3.0 with orthophosphoric acid was used as buffer. Buffer pH 3.0 was used as solvent A and MilliQ water, Methanol and acetonitrile in 200:150:650 v/v ratio was used as solvent B. The separation was achieved by gradient elution (T/%B) set as 0/5, 5/5, 35/10, 50/50, 55/60, 57/100, 60/100, 62/5 and 70/5. The mobile phase was filtered through a nylon membrane (pore size 0.45 µm) and degassed with a helium spurge for 10 min, before use. UV detection was performed at 225 nm. The sample injection volume was 10 µl. Diluent for test and standard preparation is prepared by mixing Buffer, Methanol and Acetonitrile in the ratio of 500: 250: 250 v/v/v respectively.

2.3. Preparation of standard Solutions

A standard stock solution of Sumatriptan succinate (0.60 mg/mL) prepared by dissolving 60 mg Sumatriptan succinate of reference standard in 100 mL diluent. Required dilutions of stock solution are done to obtain working solution of standard with a concentration of 30 µg/mL which is used for the related substance determination (Fig.4). The impurity stock solution was prepared by dissolving an accurately weighed amount of impurity A, impurity-B, impurity-C, and impurity-D in diluent, resulting in a concentration of 3 µL/mL of each impurity.(Fig.2)

2.4. Preparation of Sample Solution

The tablets of Sumatriptan succinate and Naproxen sodium are crushed into a fine uniform powder. A quantity of powder equivalent to 100 mg of Sumatriptan succinate was transferred to a 100 mL volumetric flask, 50 mL diluent was added. The mixture was then sonicated for 10 minute and diluted to volume to give a solution containing 1000 µg/mL of Sumatriptan succinate. The above solution was centrifuged at 4000rpm for 10 minutes in order to eliminate insoluble excipients and filtered through a 0.45 µm pore size Nylon 66 membrane filter and inject in HPLC system as per chromatographic conditions mention in section 2.2.(Fig.6)

2.5. Method Validation

The proposed method was validated as per ICH guidelines.

2.5.1. Specificity

(i) Placebo Interference:

A study to establish the interference of placebo was conducted.

Samples were prepared by taking the placebo equivalent to the amount of weight present in portion of test preparation as per the test method and injected into the HPLC system. Chromatograms of placebo solutions showed no peak at the retention time of the main peak and its impurities. (Fig.5)

(ii) Interference from Degradation Products:

A study was conducted to demonstrate the effective separation of degradents from Sumatriptan and its impurity peaks. Separate portions of Drug product Sumatriptan succinate and Naproxen sodium tablet 85 mg / 500 mg and placebo were exposed to following stress conditions to induce degradation.

- Refluxed with 1N HCl solution for about 1 hrs 30 minutes at 60ºC (Fig.7).
- Refluxed with 3N NaOH solution for about 9 hrs at 60ºC (Fig.8).
- Treated with 1% Hydrogen peroxide (H2O2) for about 2 hrs 30 minutes at room Temperature (Bench top)(Fig.9).
- Dry heating done at 105° C for about 12 hrs (Fig.10).
- Exposed to Sunlight for about 1.2 Million Lux hours (Fig.11).
- Exposed to UV light both at shorter and longer wavelengths for about 200 watt hours / square meter (Fig.12).
- Refluxed with purified water for about 12 hrs at 60ºC (Fig.13).

Stressed samples were injected into the HPLC system with photo diode array detector by following test method conditions. All degradent's peaks were resolved from Sumatriptan succinate and its impurity peaks in the chromatograms of all samples.

The chromatograms of the stressed samples were evaluated for peak purity of Sumatriptan succinate using Waters Empower Networking Software. For all forced degradation samples the purity angle found to be less than purity threshold.

This indicates that there is no interference and co-elution from degradents in quantification of the Sumatriptan succinate impurities in tablet. In Placebo stress study, interference was not observed at the Retention times of the Sumatriptan succinate and its impurity peaks. Thus, this method is considered to be "Stability Indicating". (Fig.2)

2.5.2. Precision

The precision of the related substance method was checked by injecting six individual preparations of test spiked with 0.30% of impurity-A, impurity-B, impurity-C and impurity-D with respect to Sumatriptan succinate Analyte concentration (1 mg/mL of Sumatriptan succinate). % R.S.D. of area for each impurity-A, impurity-B, impurity-C and impurity-D were calculated (Refer Table-1).

2.5.3. Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ for impurity-A, impurity-B, impurity-C and impurity-D were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations . LOD and LOQ were experimentally verified by injecting six replicate injection of each impurity at the concentration obtained from above values.

2.5.4. Linearity

A series of solutions of Sumatriptan succinate impurities in the concentration ranging from limit of quantification level to 500% of target concentration each impurity (0.30%) were prepared and injected into the HPLC system. Correlation coefficient Value for the slope and Y-intercept of the calibration curve was calculated. A graph was plotted to concentrations versus peak area and determining the correlation coefficient in linearity section. The correlation co-efficient was found to be more than 0.999 for Sumatriptan succinate and all impurities (Refer Table-3).

2.5.5. Accuracy

A study to evaluate method's accuracy was conducted by determining recovery of each impurity from test preparations of Sumatriptan and Naproxen sodium tablets spiked at 50%, 100%, 200%, 350% of the target concentration (0.3%) of Sumatriptan succinate impurities Sumatriptan succinate. The average recovery from 50% to 350% spike levels of Sumatriptan succinate impurities and Sumatriptan succinate were found to be within the range of 85% to 115% (Refer Table-2).

2.5.6. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between Sumatriptan succinate impurity A, impurity-B, impurity-C, and impurity-D was recorded. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, flow was changed by 0.2 units from 0.8 to 1.2 mL/min. The effect of the column temperature on resolution was studied at 20° and 30° C instead of 25° C. The effect of the percent organic strength on resolution was studied by varying acetonitrile by −10% +10%. While other mobile phase components was held constant as stated in Section 2.2.

2.5.7. Solution Stability and Mobile Phase Stability

The solution stability of Sumatriptan succinate and its impurities in the related substance method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 48 hours. Content of Sumatriptan succinate impurity A, impurity-B, impurity-C, and impurity-D were determined for every 24 hours interval up to the study period. Stability of the mobile phases was also carried out for 48 hours by injecting the freshly prepared sample solutions for every 24 hours interval. Content of Sumatriptan succinate impurity A, impurity-B, impurity-C, and impurity-D were checked in the test.

RESULTS AND DISCUSSION

3.1. Method Development and Optimization

The main objective of the chromatographic method was to achieve the development of single method for all four impurities and possible degradents by products of placebo and drug substances and it is a combination drug product method should able to separate Naproxen and its impurities and degradents. Impurity C has similar chemical structure as that of Sumatriptan, method has to show the good resolution between these two.

It was found that use of buffer prepared by adjusting the pH of 0.05M potassium dihydrogen phosphate to 3.0 with orthophosphoric acid (solvent A: buffer (100%) and solvent B: HPLC Grade water, Methanol and Acetonitrile in the ratio of 200 : 150 : 650 (v/v) respectively with column temperature was maintained at 25° C and gradient elution (T/%B) was set as 0/5, 5/5, 35/10, 50/50, 55/60, 57/100 , 60/100,62/5 and 70/5 shown better separation between Sumatriptan, Impurity-C and this gradient helped elution of highly nonpolar impurity-A within the gradient program.

This optimized method was verified for the separation of all possible degradents by subjecting sample and placebo to forced degradation study as per ICH Guidelines. In all the stress condition studies, purity angle value is observed lesser than purity threshold and purity flag was not observed (As per Empower software) for the Sumatriptan peak and for all four impurities.

All the degradents were well separated from Analyte peak and impurities peaks. Drug product was observed sensitive at high stress conditions like Acid, base, oxidation and dry Heat.

Under acid conditions, Sumatriptan is protonated on the oxygen atom of the methyl sulfonamide group. The first steps involve a typical ester hydrolysis during which water attacks the sulfur atom. Since sulfate is a good leaving group, this results in the release of a sulfuric acid molecule. Concurrently, nucleophilic attack by a methoxy anion causes the regeneration of aromaticity resulting in the formation of the degradation Product Impurity-A.

Sumatriptan is having tertiary amine group and secondary amine group, so it is highly susceptible for oxidation to form oxidative impurities. Oxidation of Sumatriptan seems to proceed through two distinct pathways. The first is formation of the N-oxide on the dimethylamino group. Since all of the compounds containing two or three oxygen atoms possess an unmodified dimethylamino group, it appears that once this N-oxide is formed, no further oxidations occur. However, if the first oxidation occurs on the indole ring at N-1, C-2, C-4 or C-7, then additional oxidations will occur within the ring system leading to a mixture of products containing either products were identified.

Since molecule is prone to oxidation, in presence of Alkali removal of proton from molecule is very easy. In alkali stressed study, Sumatriptan is under gone oxidative degradation and formed Impurity-D.

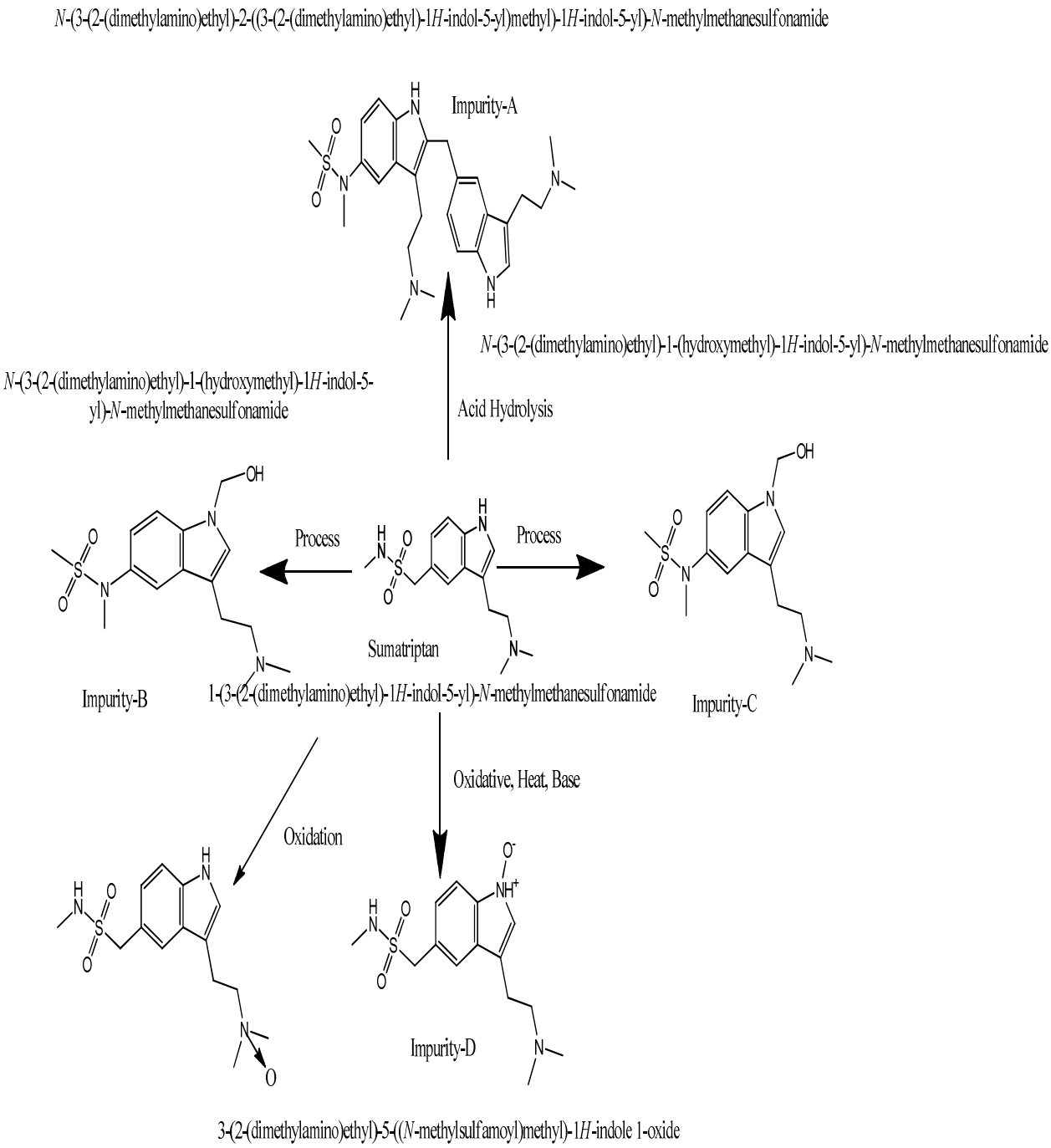

Figure-1: Degradation pathway of Sumatriptan

Fig 2: Typical chromatogram of Spiked Test

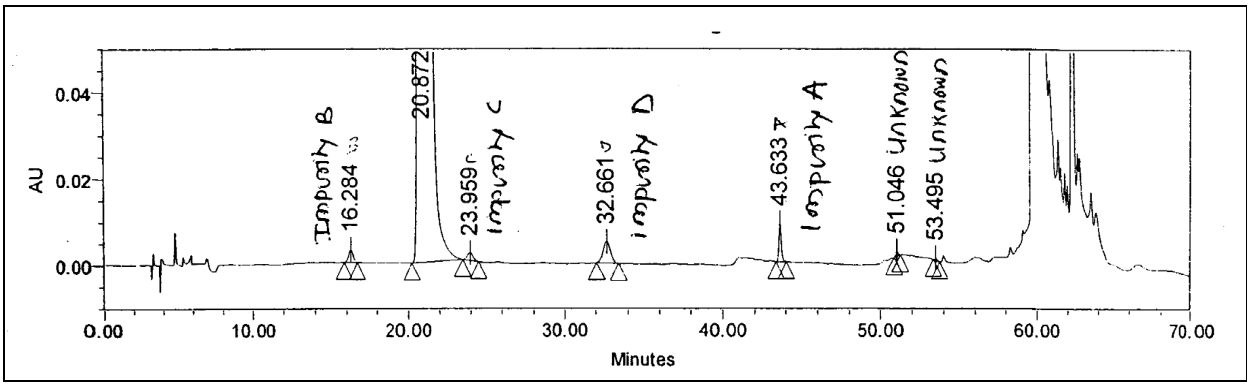

Fig 3: Typical chromatogram Blank preparation (Diluent)

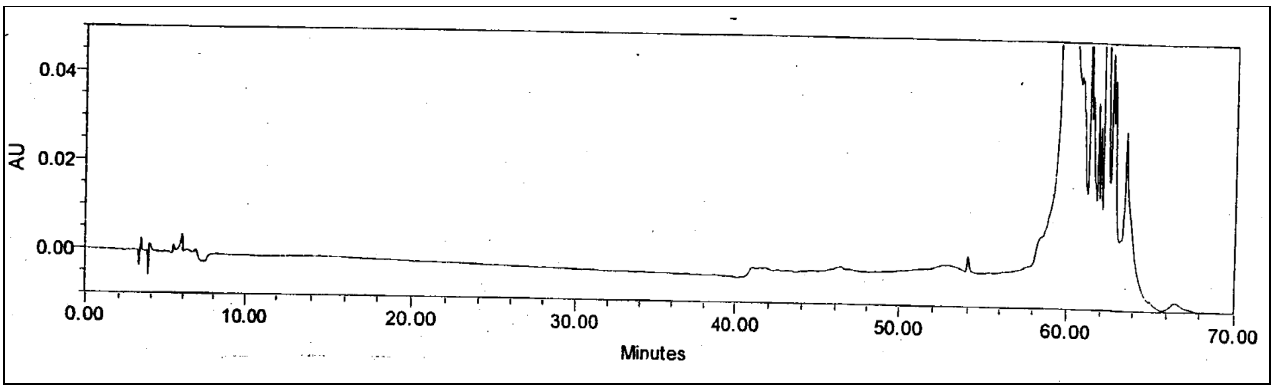

Fig 4: Typical chromatogram Standard preparation

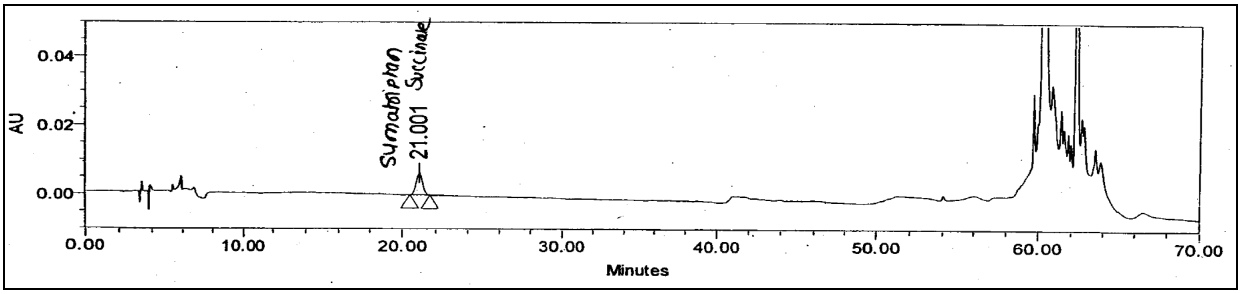

Fig 5: Typical chromatogram Placebo preparation

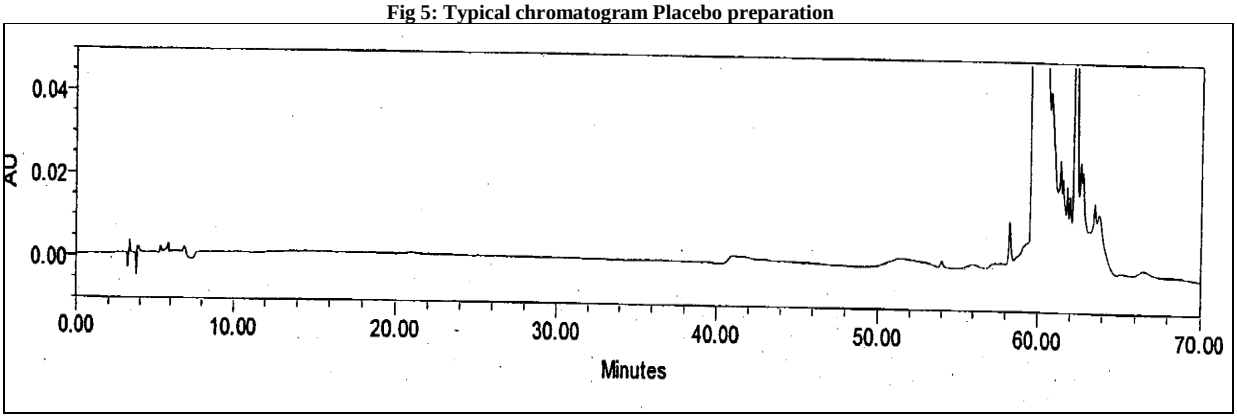

Fig 6: Typical chromatogram Test preparation

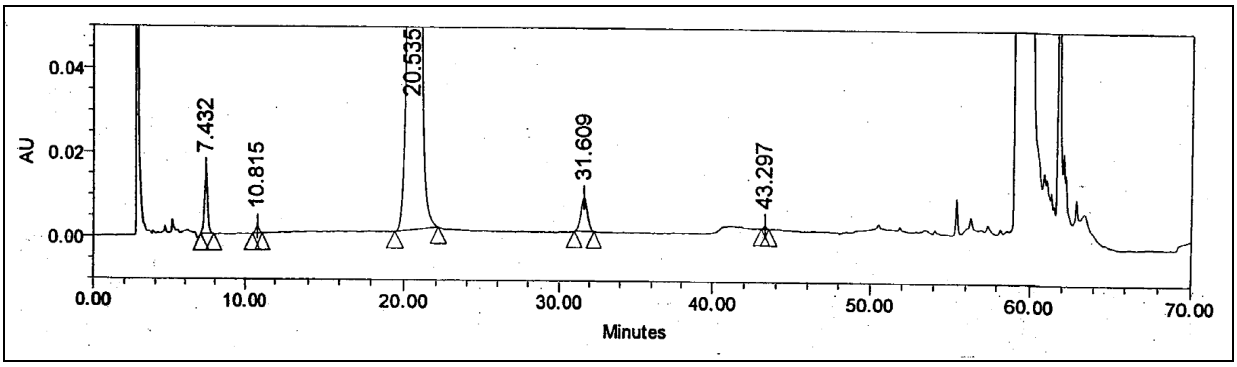

Fig 7: Typical chromatogram Forced degraded Test chromatogram – Acid degradation

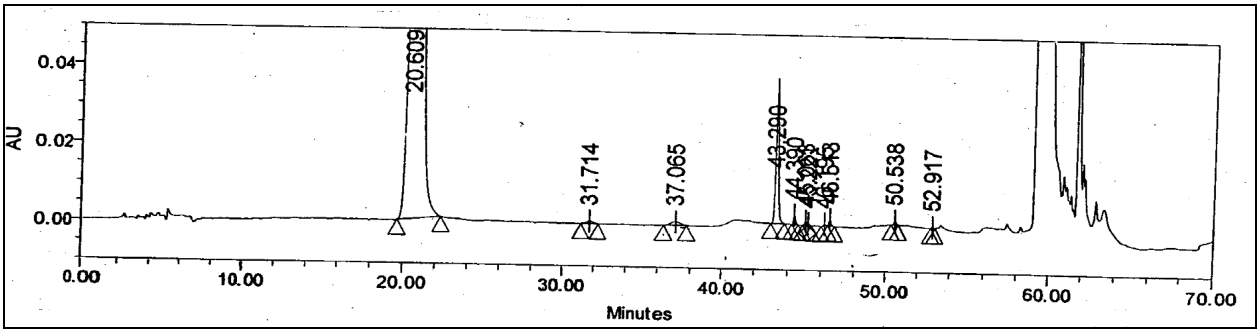

Fig 8: Typical chromatogram Forced degraded Test chromatogram – Base degradation

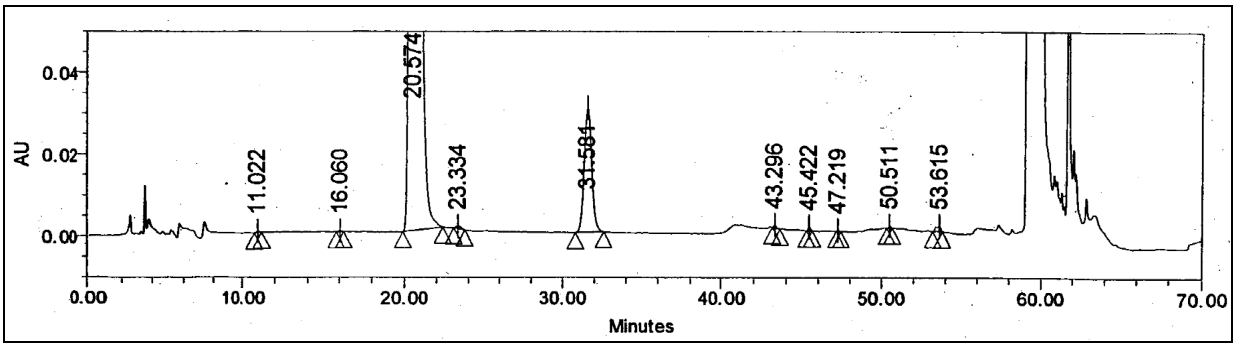

Fig 9: Typical chromatogram Forced degraded Test chromatogram – Peroxide degradation

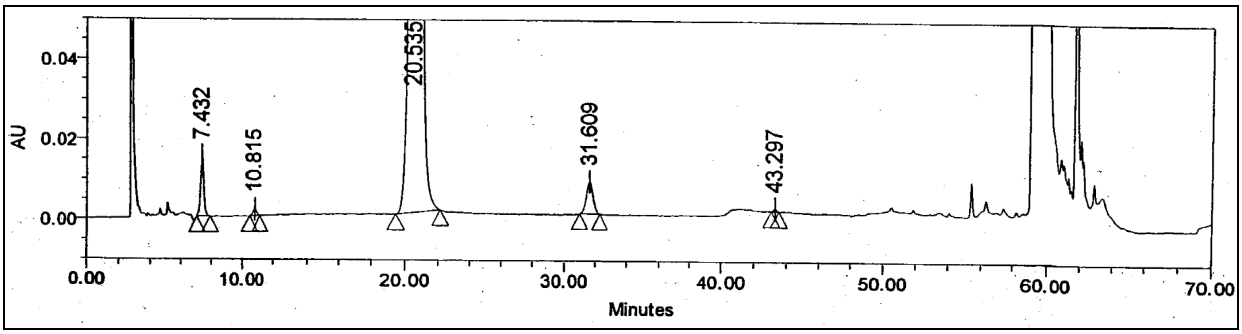

Fig 10: Typical chromatogram Forced degraded Test chromatogram – Thermal degradation

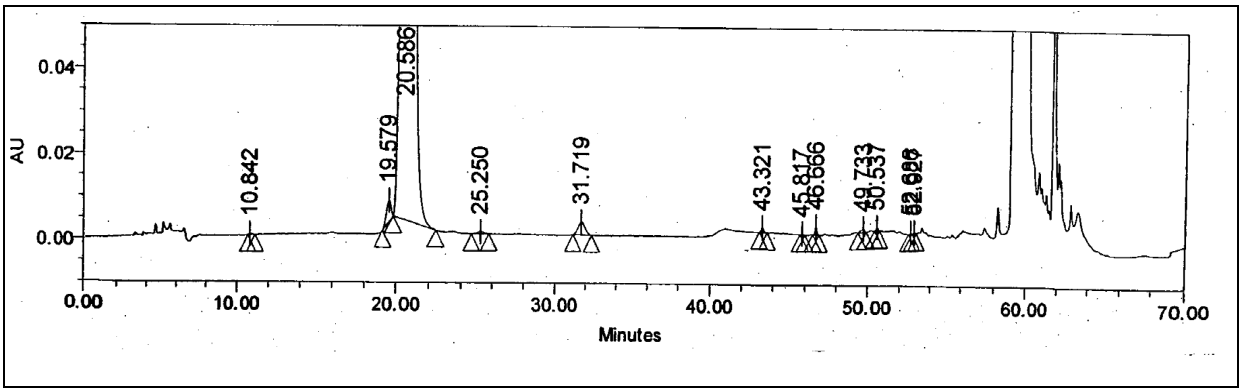

Fig 11: Typical chromatogram Forced degraded Test chromatogram – Photolytic degradation (sun light)

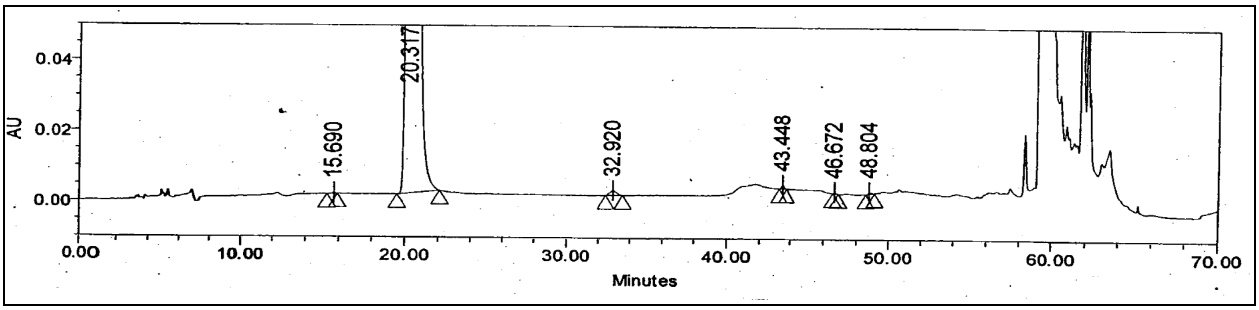

Fig 12: Typical chromatogram Forced degraded Test chromatogram – Photolytic degradation (UV light)

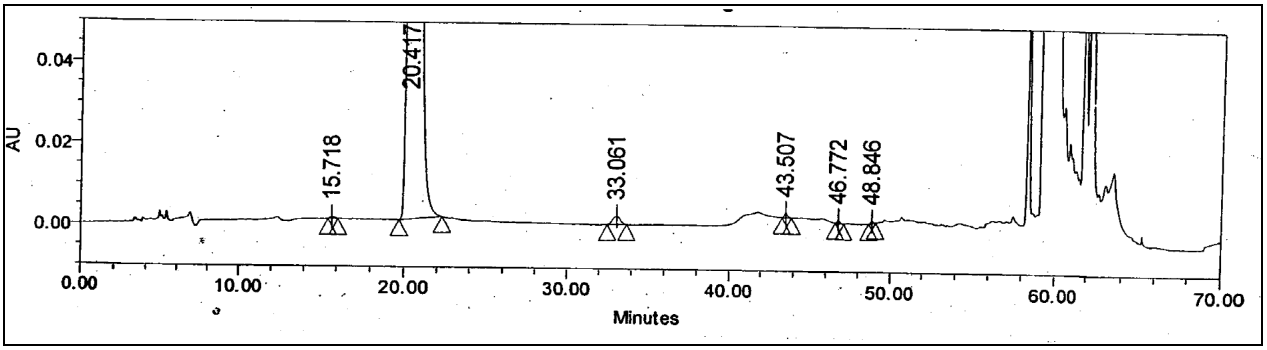

Fig 13: Typical chromatogram Forced degraded Test chromatogram – Aqueous (Water) degradation

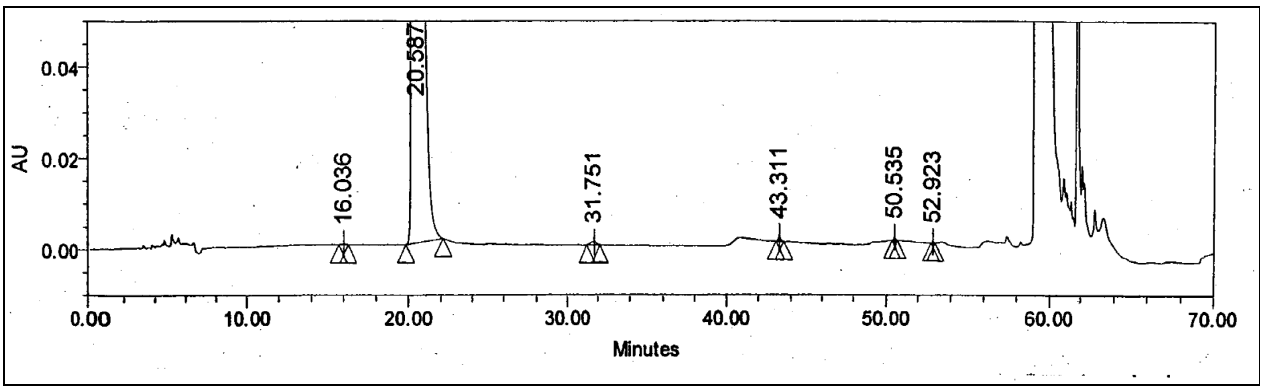

Table-1: Repeatability

Sample No.	Sumatriptan succinate Impurities					
	Impurity-A		Impurity-B		Impurity-C	
	RRT	% Impurity	RRT	% Impurity	RRT	% Impurity
1	2.09	0.259	0.78	0.095	1.15	0.082
2	2.10	0.266	0.78	0.096	1.15	0.082
3	2.10	0.263	0.78	0.095	1.15	0.078
4	2.10	0.255	0.78	0.097	1.15	0.081
5	2.10	0.268	0.78	0.102	1.15	0.081
6	2.09	0.264	0.78	0.103	1.15	0.078
AVG	2.10	0.263	0.78	0.098	1.15	0.080
%RSD	0.2	1.8	0.0	3.7	0.0	2.3

Sample No.	Sumatriptan succinate Impurities	
	Impurity-D	
	RRT	% Impurity
1	1.56	0.248
2	1.57	0.243
3	1.57	0.238
4	1.57	0.245
5	1.57	0.240
6	1.57	0.243
AVG	1.57	0.243
%RSD	0.3	1.5

TABLE 2: ACCURACY

Impurity-A

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (Recovered)	Mean % Recovery
1	LOQ	0.1753	0.17	93.2
2	LOQ	0.1753	0.16	
3	LOQ	0.1753	0.16	
1	50%	0.974	0.88	91.7
2	50%	0.974	0.94	
3	50%	0.974	0.86	
1	100%	1.948	1.85	94.3
2	100%	1.948	1.85	
3	100%	1.948	1.81	
1	200%	3.896	3.58	90.4
2	200%	3.896	3.52	
3	200%	3.896	3.47	
1	350%	6.818	6.19	90.3
2	350%	6.818	6.15	
3	350%	6.818	6.13	

Impurity-B

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (Recovered)	Mean % Recovery
1	LOQ	0.1498	0.15	100.1
2	LOQ	0.1498	0.15	
3	LOQ	0.1498	0.15	
1	50%	0.4994	0.49	100.1
2	50%	0.4994	0.5	
3	50%	0.4994	0.51	
1	100%	0.9988	1.03	101.8
2	100%	0.9988	1.01	
3	100%	0.9988	1.01	
1	200%	1.9976	2.03	99.9
2	200%	1.9976	1.97	
3	200%	1.9976	1.99	
1	350%	3.4958	3.39	96.2
2	350%	3.4958	3.35	
3	350%	3.4958	3.35	

Impurity-C

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (Recovered)	Mean % Recovery
1	LOQ	0.2427	0.25	103
2	LOQ	0.2427	0.25	
3	LOQ	0.2427	0.25	
1	50%	0.5515	0.57	100.9
2	50%	0.5515	0.56	
3	50%	0.5515	0.54	
1	100%	1.103	1.11	102.8
2	100%	1.103	1.14	
3	100%	1.103	1.15	
1	200%	2.206	2.42	109.7
2	200%	2.206	2.42	
3	200%	2.206	2.42	
1	350%	3.8605	4.3	109.9
2	350%	3.8605	4.24	
3	350%	3.8605	4.19	

Impurity-D

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (Recovered)	Mean % Recovery
1	LOQ	0.2987	0.29	100.4
2	LOQ	0.2987	0.31	
3	LOQ	0.2987	0.3	
1	50%	0.9956	0.97	97.1
2	50%	0.9956	0.96	
3	50%	0.9956	0.97	
1	100%	1.9912	1.96	100.1
2	100%	1.9912	2	
3	100%	1.9912	2.02	
1	200%	3.9824	4.07	98.5
2	200%	3.9824	3.88	
3	200%	3.9824	3.82	
1	350%	6.9692	7.04	100.1
2	350%	6.9692	6.89	
3	350%	6.9692	7	

Sumatriptan succinate

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (Recovered)	Mean % Recovery
1	LOQ	0.2086	0.21	100.7
2	LOQ	0.2086	0.21	
3	LOQ	0.2086	0.21	
1	50%	1.49	1.54	102.7
2	50%	1.49	1.52	
3	50%	1.49	1.53	
1	100%	2.9799	3.04	102.5
2	100%	2.9799	3.05	
3	100%	2.9799	3.07	
1	200%	5.9598	5.92	99.6
2	200%	5.9598	5.92	
3	200%	5.9598	5.97	
1	350%	10.4297	10.43	99.9
2	350%	10.4297	10.43	
3	350%	10.4297	10.41	

TABLE 3: LINEARITY
Impurity – A :

Sl. No.	Spike level	Concentration 'µg/ml'	Peak Area
1	LOQ	0.1845	6015
2	50%	0.9963	36080
3	100%	1.9926	74294
4	200%	3.9852	140870
5	400%	7.9704	281582
6	500%	9.93	358799

Impurity-B

Sl. No.	Spike level	Concentration 'µg/ml'	Peak Area
1	LOQ	0.1497	10500
2	50%	0.4989	35908
3	100%	0.9979	70904
4	200%	1.9958	141576
5	400%	3.9916	283613
6	500%	4.9895	360361

Impurity-C

Sl. No.	Spike level	Concentration 'µg/ml'	Peak Area
01	LOQ	0.2413	15358
02	50 %	0.5104	35555
03	100 %	1.0208	67057
04	200 %	2.0416	127550
05	400 %	4.0832	262066
06	500 %	5.1040	340164

Impurity-D

Sl. No.	Spike level	Concentration 'µg/ml'	Peak Area
01	LOQ	0.2699	18270
02	50 %	0.9897	67110
03	100 %	1.9795	140504
04	200 %	3.9589	291257
05	400 %	7.9199	589379
06	500 %	9.8974	750507

Sumatriptan succinate

Sl. No.	Spike level	Concentration 'µg/ml'	Peak Area
01	LOQ	0.2093	13441
02	50 %	1.4949	92120
03	100 %	2.9898	190918
04	200 %	5.9796	383990
05	400 %	11.9592	774584
06	500 %	14.9490	985436

Name of Impurity	Coefficient of correlation (r)
Impurity –A	0.999
Impurity –B	0.999
Impurity –C	0.999
Impurity –D	0.999
Sumatriptan succinate	0.999

CONCLUSION

The developed HPLC method for related substances of Sumatriptan succinate in Sumatriptan Succinate and Naproxen Tablets is precise, accurate, linear, robust, rugged and specific. This method can be used instead of two separate methods as mentioned in the U.S. Pharmacopeia. The developed method was validated as per ICH Guideline.The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of Sumatriptan succinate.

The degradation of Sumatriptan succinate under acid, base, heat, oxidation and UV irradiation conditions was studied using HPLC. Structures of the degradation products were known impurity-A and Impurity –D. It was found that the drug was stable to exposure of acid, base, oxidation and UV irradiation at ambient temperature, but unstable under acidic and basic conditions when heated to 60°C. Under oxidative conditions, a number of oxygenated products were predicted .

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