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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF POTENTIAL IMPURITIES OF SUMATRIPTAN AND NAPROXEN SODIUM IN FIXED DOSE COMBINATION

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ABSTRACT

The current publication describes about development, optimization and validation of simultaneous estimation of impurities present in Sumatriptan and Naproxen sodium tablets using High-Performance Liquid Chromatography (HPLC). Perchlorate buffer (contains 1.0 mL/L of Perchloric acid and 4g/L of sodium perchlorate) is used as Elution phase~A along with acetonitrile as Elution phase~B. Step gradient mode elution technique is opted with a flow rate of 1.0 mL/min with ACE 5 C18 PFP 5µ,250 x 4.6mm column. All the probable impurities are well resolved at a satisfactory level, showing resolution more than 1.2 in sensitive robustness conditions for closely eluting peaks. The projected method is appropriate for quantification of SUM and NAP related known and unknown impurities that originate from the life cycle of the combination drug product. Selected quantification wavelength of 230 nm is found suitable for quantification purpose. Impurities show satisfactory responses and did not find any placebo interference at this working wavelength. Linearity data depicts a linear relationship with a coefficient of correlation greater than 0.99. The projected method is validated as per compendia recommendation as mentioned in ICH. The developed method is highly useful for quantification of impurities in Sumatriptan and Naproxen sodium tablets in a single method.

Keywords: Sumatriptan, Naproxen Sodium, Validation, Stability Indicating, Fixed-dose Combination, Impurities.

INTRODUCTION

Sumatriptan and Naproxen sodium tablets is a combination drug product used for the treatment of migraine head ache attacks in adults. TREXIMET tablets have a label claim of 85mg Sumatriptan which is equivalent to 119mg of Sumatriptan succinate (SUM) and 500 mg of Naproxen sodium (NAP). SUM is used for the management of migraine headache. NAP belongs to the arylacetic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs). It is an anti-inflammatory medicine. It also reduces stiffness caused by bursitis, arthritis and gout attacks[1-5].

SUM is a white to off-white powder, which is freely soluble in aqueous solvents like water. The chemical name is "3-[2-(dimethylamino)ethyl]-N-methyl-1H-indole-5-methanesulfonamide-butanedioic acid". The empirical formula is $C_{14}H_{21}N_3O_2S•C_4H_6O_4$, with a molecular weight of 413.5 (Fig.-1). NAP is a white-to-creamy white crystalline solid, freely soluble in water at neutral pH. The chemical name is"(S)-6-methoxy-a-methyl-2-naphthaleneacetic acid, sodium salt". The empirical formula is $C_{14}H_{13}NaO_3$, representing a molecular weight of 252.23(Fig.-2).

A meticulous review on literature reveals that few testing procedures are available using HPTLC[6], Spectrophotometric[7-8] and LC/MS[9] techniques for quantification of SUM and NAP in different formulation products. Some methods are also available for simultaneous estimation of SUM and NAP

using HPLC[10-12] and UPLC[13] techniques. Few methods are published to determine either SUM or NAP impurities in fixed-dose combination[14-16].

Few reports are available for quantification of SUM and NAP impurities in SUM and NAP combination by UPLC method[17]. The reported publication confirms the estimation of impurities in the presence of SUM and NAP drug substances only but not in the drug product. The specificity of the unknowns generated during the life cycle of the product cannot be predicted completely in the published method. Hence considering the above facts a common HPLC method was developed for simultaneous quantification of impurities for this combination drug product and subsequently validated as per compendia requirement[18-22].

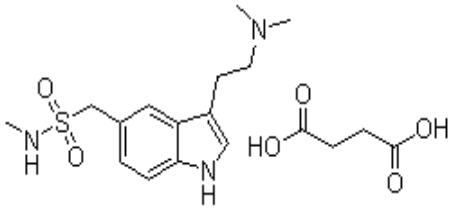


Fig.-1: SUM Structure

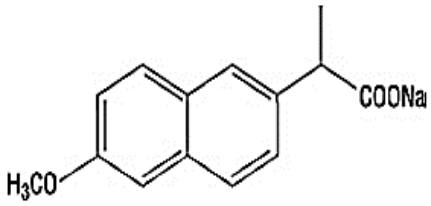
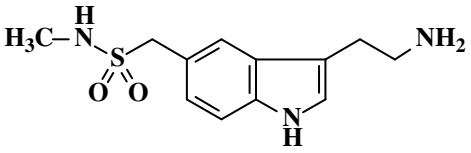
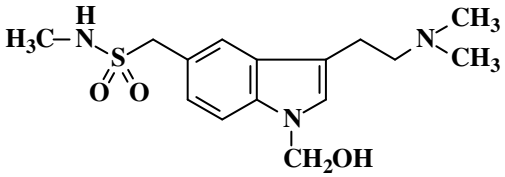


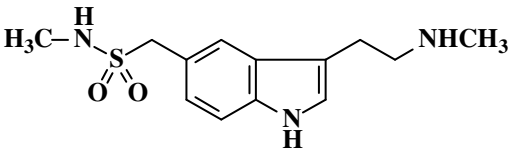
Fig.-2: NAP Structure



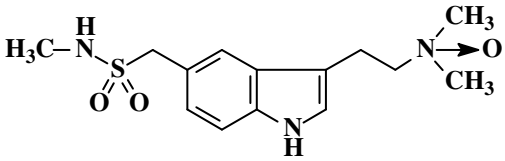
SUM impurity ~1"[3-(2-Aminoethyl)-1H-Indol-5-YL]-N-methylmethane sulfonamide"



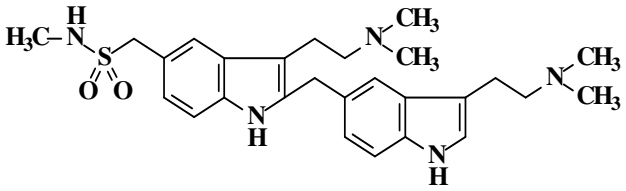
SUM impurity~2" [3-[2-(Dimethylamino) ethyl]-1-(hydroxymethyl)-1H-Indol-5-YL]-N-Methylmethanesulfonamide"



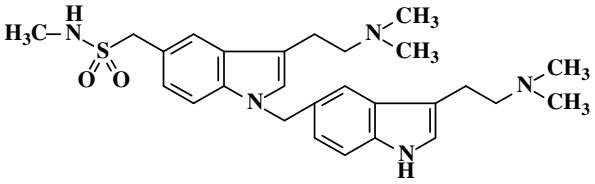
SUM impurity~3 "[3-[2-(Methyl amino) Ethyl]-1H-Indol-5-YL]-N-Methyl methane sulfonamide (OR) [3-[2-(methyl amino) Ethyl]-1H-Indol-5-YL]-N-methyl methane sulfonamide"



SUM impurity~4"[3-[2-(Dimethyl Amino N-Oxide) Ethyl]-1H-Indol-5-YL]-N-methyl methane sulfonamide (OR) [3-[-2(Dimethyl Amino-N-Oxide) Ethyl]-1H-Indol-5-YL]-N-methyl methane sulfonamide"



SUM impurity~5"Sumatriptan-2,5-Dimer:[3-[2-(Dimethylamino)Ethyl]-2-[[3-[2-(Dimethyl amino)ethyl]-1H-indol-5-YL]methyl]-1H-indol-5-YL]-N-methylmethane sulfonamide"



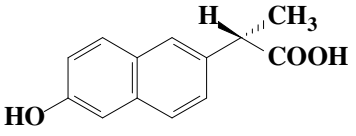
SUM impurity~6"Sumatriptan-1,5-Dimer:[3-[2-(Dimethylamino) Ethyl]-1-[[3-[2-(DimethylAmino) Ethyl] -1h-Indol-5-YL]methyl]-1H-indol-5-YL]-N-methylsulfonamide"

Fig.-3: SUM Related Impurities

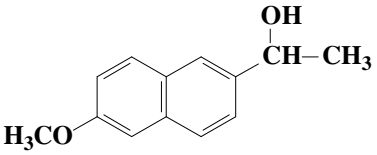
Since SUM and its by-products (impurities) are polar in nature, it is difficult to develop a method on HPLC using conventional buffers generally used in liquid chromatography. Hence the choice is to

develop a method using High-performance liquid chromatography (HPLC) adopting ion pair buffers is the modest way to retain polar peaks followed by separation of potential impurities of different polarity index.

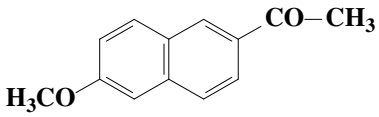
This paper presents development strategy and validation activity for the impurities that may be formed from SUM and NAP respectively. Forced degradation studies reveal that all potential impurities that are formed during different stress conditions are well separated. Hence developed procedure can be claimed as stability demonstrating method and meets the ICH requirement parameters. The possible impurities that are formed from the SUM are SUM impurity~1, SUM impurity~2, SUM impurity~3, SUM impurity~4, SUM impurity~5 and SUM impurity~6 (Fig.-3). For NAP is NAP impurity~1, NAP impurity~2, NAP impurity~3, NAP impurity~4, NAP impurity~5 and NAP impurity~6 (Fig.-4).



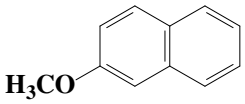
NAP impurity~1"(2*S*)-2-(6-Hydroxy naphthalene-2-YL) propanoic acid"



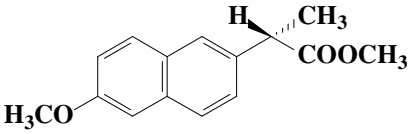
NAP impurity~2"(1*RS*)-1-(6-Methoxy naphthalene-2-YL) ethanol"



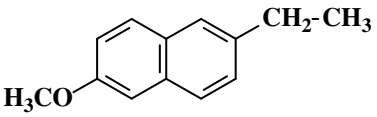
NAP impurity ~3"1-(6-Methoxy naphthalene-2-YL) ethanone"



NAP impurity~4"2-Methoxy naphthalene"



NAP impurity~5 "Methyl (2*S*)-2-(6-Methoxy naphthalene-2-YL) propanoate"



NAP impurity~6"2-Ethyl-6-Methoxy naphthalene"

Fig.-4: Naproxen Sodium Related Impurities

EXPERIMENTAL

Instruments

HPLC system of waters alliance make is used, which contains quaternary gradient pump system, Auto sampler, column oven compartment and PDA detector for detection. Empower 2 software was used as interphase. The column used was ACE 5 C18 PFP, 5µ, 250 mm × 4.6 mm.

Chemicals

Sumatriptan succinate standard, Naproxen sodium standard, SUM and NAP impurities, Sumatriptan and Naproxen sodium tablets (TREXIMET tablets) were funded by Aurobindo Pharma Limited. HPLC grade Acetonitrile and remaining chemicals like Monobasic Potassium phosphate, Sodium Perchlorate monohydrate, Perchloric acid (≈ 70%), and Orthophosphoric acid (≈ 88%) of analytical reagent grade chemicals were taken from Merck Millipore. Water for preparation was taken from Evoqua water purifier.

Method Development Along with Optimization of the Method

The current paper describes strategic method development work which is capable enough to separate all possible potential impurities that appear during the process and different degradation conditions. No compendial methods being cited in any pharmacopoeia for simultaneous determination of impurities in

this drug product combination. Polarity index of SUM shows highly polar in nature when compared to NAP. It is difficult to retain or separate polar eluents in liquid chromatography. The choice of separation for these impurities is likely dependant on a selection of ion pairs used in Elution phase preparation or use aqua-based technology columns. Using ion pair reagent is a better choice to separate polar impurities, since these reagents are widely available with different chemical properties. Hence ion-pair reagents have been chosen for buffer preparation. Initially, trials were initiated with a mixture of sodium Perchlorate monohydrate and Perchloric acid ion pair buffer. The reason to select this ion pair is due to low viscous nature, easy to handle and compatible to use for gradient elution. Since this FDC product contains different polarity properties for potential impurities, it is mandated to use gradient elution mode to get a shorter run time. Perchlorate buffer (contains 1.0 mL/L of perchloric acid and 4g/L of sodium perchlorate) as Elution phase~A along with acetonitrile as Elution phase~B is used with step gradient elution. In all trials, 1.0mL per minute flow rate shows promising separations and hence same the flow was kept in an optimized method.

The column used in chromatography for trial purpose is ACE PFP C18, 5µ, 250 x 4.6mm. The reason to select this column is ACE PFP C18 is a unique column which is a combination of C18 & PFP mechanisms and is having more advantage than either phase alone for separation. Since SUM and NAP contain structurally similar impurities which can elute very close to respective drug components, an advantage can be taken in terms of selectivity to get proper resolution by taking this unique column phase. The same column was finalized for chromatography.

Trials have been taken using a proposed diluent which contains a mixture of pH 3.6 phosphate buffer along with acetonitrile in the proportion of 55:45. In this diluent, all impurities were well dissolved and found stable in the proposed diluent. To check the individual impurity retention times, all impurity mixture is prepared at a proposed specification limit assigned to each impurity and injected using a PDA detector. Impurities related to SUM and NAP shows wavelength maxima at about 230 nm. Hence for quantification for all impurities, a wavelength of 230 nm is opted. 10 µL injection volume showed satisfactory responses to each impurity at the proposed test concentration of 212.5µg/mL for SUM 1250µg/mL for NAP. In the finalized test conditions all impurities are well separated with a reproducible area for each impurity.

Finalized Chromatographic Conditions

Elution phase~A is mixture 4g/L of sodium perchlorate and 1.0 mL/L of perchloric acid along with acetonitrile as Elution phase~B. Step mode gradient programme Table-1 with a flow of 1.0 ml/min opts. The column temperature is kept at 30°C with 230 nm wavelength for detection using 10µL injection volume. The proposed column is ACE PFP C18. 5µ, 250 x 4.6mm. The diluent is a mixture of pH 3.6 phosphate buffer (pH adjusted with dilute phosphoric acid) along with acetonitrile in the proportion 55:45 opts.

Table-1: Step Mode Gradient Programme

Time (minutes)	Flow (mL)	% Elution phase~A	% Elution phase~B
0.0	1.0	90	10
15.0	1.0	70	30
25.0	1.0	60	40
30.0	1.0	45	55
35.0	1.0	20	80
40.0	1.0	20	80
41.0	1.0	90	10
50.0	1.0	90	10

Retention times of each impurity and Suitability of chromatographic system Values observed in the finalized method mentioned in Table-2.

Standard Solution Preparation

SUM and NAP standard solutions were prepared at a concentration of 1.07 and 1.25µg/mL respectively. Standard solutions were prepared using diluent and impurity stock solutions were prepared using 5mL of acetonitrile initially followed by further dilution with diluent.

Table-2: Suitability of Chromatographic System Values

Name of Drug/Impurity	Retention times in minutes	USP # ~ Resolution	USP # ~ Tailing factor	USP # ~Theoretical plates
SUM Impurity~1	10.95	--	0.99	66154
SUM Impurity~2	11.38	2.53	1.01	74506
SUM Impurity~3	11.68	1.71	1.02	72288
SUM	12.26	3.11	1.10	63289
SUM Impurity~4	13.58	6.92	1.00	89746
SUM Impurity~5	18.56	26.29	1.02	155302
SUM Impurity~6	19.60	5.48	1.05	176898
NAP Impurity~1	21.36	8.81	1.01	171997
NAP Impurity~2	29.17	34.71	1.01	237571
NAP	31.45	8.84	1.09	205429
NAP Impurity~3	34.05	11.36	1.03	597026
NAP Impurity~4	36.64	15.87	1.00	992973
NAP Impurity~5	36.93	1.88	1.04	894848
NAP Impurity~6	39.77	17.16	1.02	835675

Sample Solution Preparation

Crushed to a fine powder using not less than 10 tablets. Sample powder equivalent to 42.5mg of Sumatriptan and 250 mg of Naproxen is taken in dry and clean 200mL volumetric flask. To this add one-third volume of diluent and exposed to sonication for about 20 minutes. Mix intermittently to get homogeneity of the test solution. After sonication, keep the solution aside to attain room temperature and make up to the volume with diluent and mix thoroughly. Transfer about 15 mL of sample solution from flask to centrifuge tube and centrifuge for about 5 minutes at 5000 rpm. Filter the solution through 0.45µ syringe filter. Final sample concentration is about 212 and 1250 µg/mL of SUM and NAP respectively. TREXIMET tablets are available in 85 mg strength for Sumatriptan and 500 mg for Naproxen. These tablets were taken up for complete method validation purpose.

Method Validation

Method validation covered for specificity, stress degradation, Precision for both inter and intraday (ruggedness), method sensitivity for LOD along with LOQ determination, Linearity, range, accuracy and robustness studies as suggested in ICH.

Specificity and Stress Degradation

Specificity was performed by spiking the impurities in the test sample as per the proposed specification limit as proposed in Table-3 and loaded into the system.

Table-3. Specification limit of impurities

Name of the component	Specification Limit in %	Specification Limit in µg/mL
SUM impurity~1	0.2	0.43
SUM impurity~2	0.5	1.06
SUM impurity~3	0.5	1.06
SUM impurity~4	0.5	1.06
SUM impurity~5	0.5	1.06
SUM impurity~6	0.2	0.43
NAP impurity~1	0.1	1.25
NAP impurity~2	0.1	1.25
NAP impurity~3	0.1	1.25
NAP impurity~4	0.1	1.25
NAP impurity~5	0.1	1.25
NAP impurity~6	0.1	1.25

Samples were prepared and stressed under different degradation conditions. For Acidic exposure, the sample was exposed to 1M HCl and heated to 85°C for about 3 hours. For alkaline degradation, the sample was exposed to 1M sodium hydroxide and heated to 85°C for about 3 hours. Both acid and alkali exposed samples were neutralized before injecting into HPLC. For oxidative stress condition, the sample was added with 5% H_2O_2 and exposed for about 15 minutes. For Thermal stress study, whole tablets were exposed to 105°C for about 120 hours and the sample was prepared for testing. For Humidity stress, sample powder is exposed to 90%RH at 25°C for 120 Hours and later stressed sample was prepared for testing. For Photolytic stress, tablet powder is exposed to 10K Lux for 120 Hours along with UV 200 $W.Hr.m^{-2}$. This stressed sample is loaded into HPLC after test preparation.

Precision
Sample powder is taken for analysis and prepared six individual test preparations spiked with impurities at the proposed specification level and loaded into HPLC. Observed results were calculated for % w/w and % RSD is assessed for each impurity. Similarly, the ruggedness of the experiment was repeated with another lot column, different day and alternative system.

Sensitivity
LOD along with LOQ values were determined by loading a series of injections ranging from (1 to 150) % of the proposed specifications. Using impurity stock solutions, precision was performed based on the predicted concentrations derived from the linearity curves.

Linearity and range
Linearity along with range was demonstrated by loading a series of injections ranging from observed LOQ level of each impurity to 150% to the proposed specification limit. Slope, coefficient of correlation and Y-intercept were individually calculated for every individual impurity from the linearity curves.

Accuracy
Impurity stock solution was prepared and accuracy was proved by spiking them to the control sample at proposed LOQ concentration, at 50%, 100%, and 150% level. Based on % w/w observed results, calculated the accuracy values. Each spiking procedure was done in triplicate preparations.

Solution Stability
To establish solution stability, standard and sample solutions were periodically injected at room temperature at different time intervals. Values at different time points were extrapolated against initial freshly injected solutions of standard and sample.

Robustness
Robustness was performed by deliberately changing the proposed methodology and assessed. The flow rate was changed ± 10.0% (0.9mL and 1.1mL per minute), temperature ± 5°C (25°C and 35°C), gradient composition ± 2 absolute and wavelength ± 5 nm (225nm and 235 nm). In each experiment, one parameter has deliberately changed by keeping the remaining parameters intact.

RESULTS AND DISCUSSION

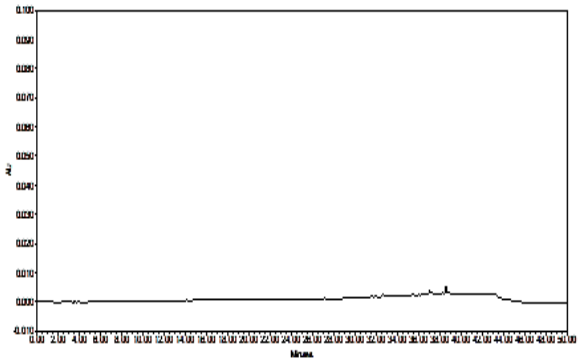
Specificity and Stress Studies
There was no interference observed from diluent and placebo solutions. Degradation data suggests the following behavior in each condition.

Acid Stress Condition
SUM shows slight increase in SUM impurity-5. NAP undergoes degradation significantly and found NAP impurity~1, NAP impurity~2, NAP impurity~4 and unknown impurities.

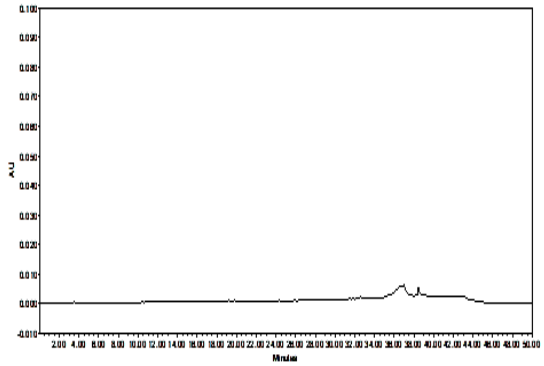
Base Stress Condition
SUM shows slight increase in SUM impurity~4 and NAP found to be stable in base degradation conditions. There are no unknown peaks seen for both SUM and NAP.

Peroxide Stress Condition

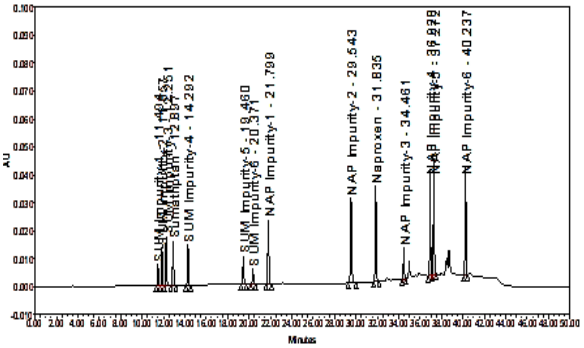
SUM shows an increase in trend for SUM impurity~4 whereas NAP is found stable. There are no unknown peaks seen for both SUM and NAP.



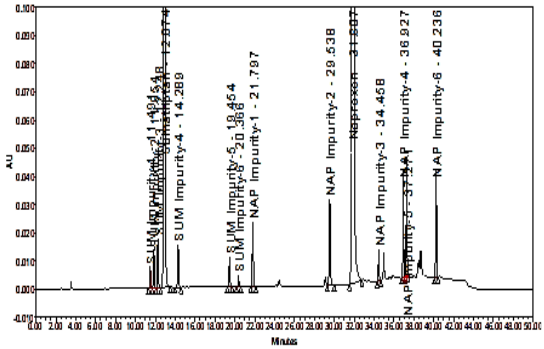
5a. Diluent



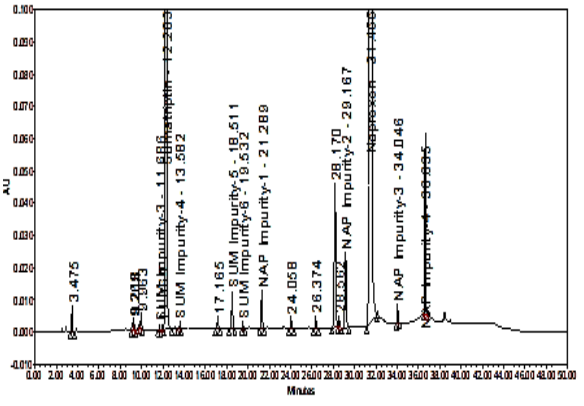
5b. Placebo



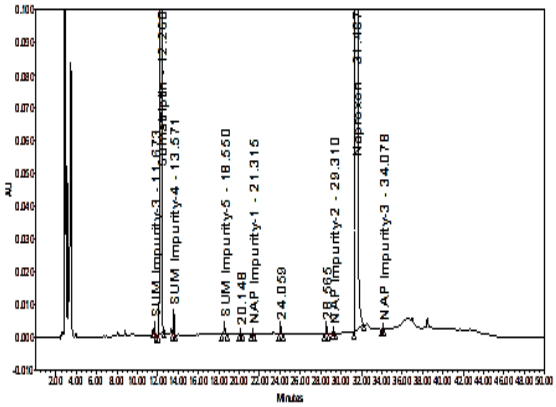
5.c. Standard solution (All impurity mixture)



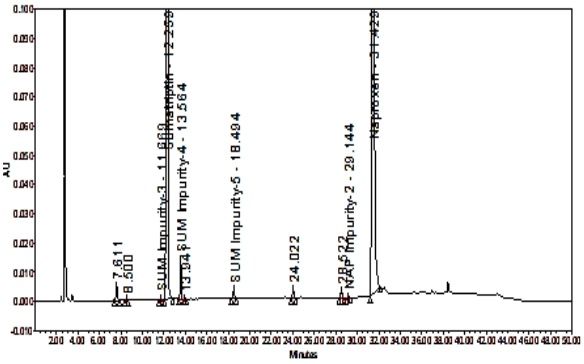
5d. Impurities Spiked Sample solution



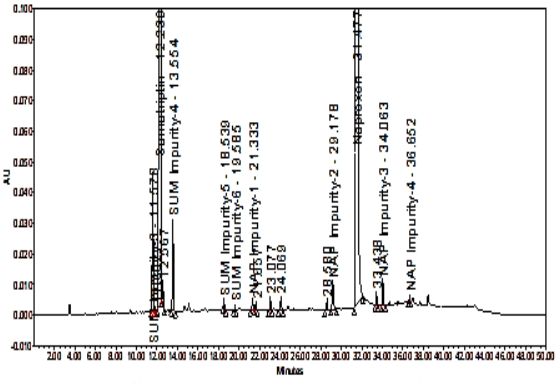
5e. Acid Stressed Sample



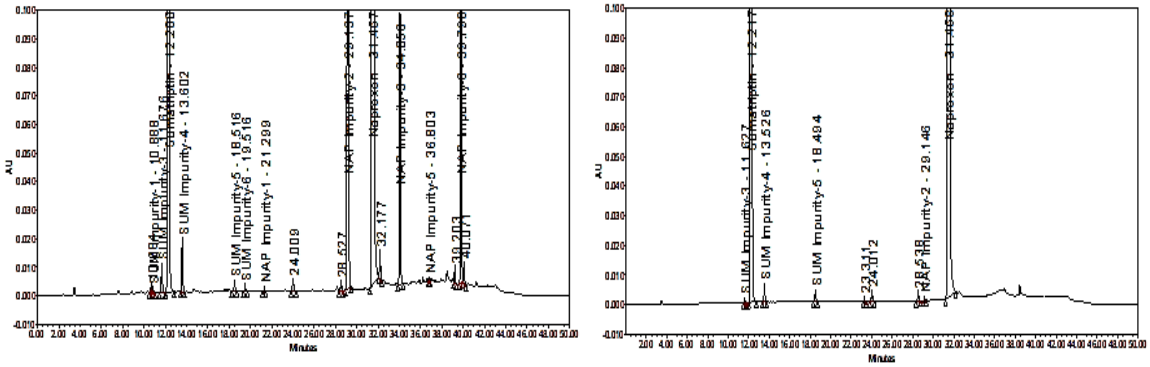
5f. Base Stressed Sample



5g. Oxidation Stressed Sample

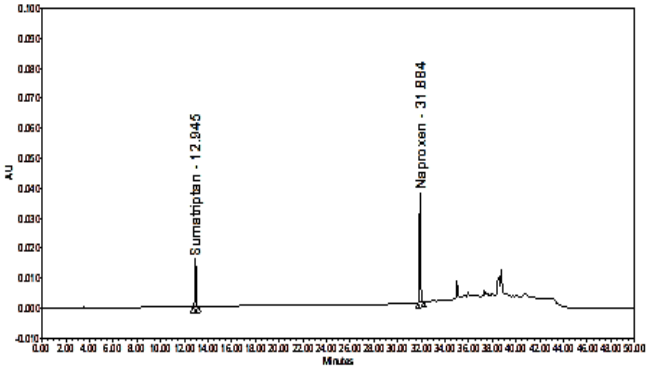


5h. Thermal Stressed Sample



5i. Photolytic Stressed Sample

5j. Humidity Stressed Sample



5k.Diluted Standard

Fig.-5: Representative Chromatograms

Thermal Stress Condition

SUM shows increased values for SUM impurity~3 and SUM impurity~4. NAP shows slight increase in NAP impurity~2 and NAP impurity~3. No major unknown peaks are not generated either for SUM or NAP.

Photolytic Stress Condition

SUM shows an increase in values for SUM impurity~3 and SUM impurity~4. Whereas NAP degraded significantly and observed NAP impurity~2, NAP impurity~3, and NAP impurity~6.There are no unknown peaks increased from SUM and NAP.

Humidity Stress Condition

SUM shows a slight increase in values for SUM impurity~4 and NAP are found stable. For comprehensive-stress degradation data refer to Table-4 and Fig.-5.

Table-4: Comprehensive Stress Degradation Data

Stress parameter	Exposure interval	% Degradation		Major peaks observed
		SUM	NAP	
Acidic exposure (1M HCl / 85°C)	180 minutes	1.94	2.90	SUM impurity~5, NAP impurity~1,2,4 & NAP Unspecified
Base exposure (1M NaOH / 85°C)	180 minutes	0.68	0.15	SUM impurity~4
Oxidation (5% H_2O_2 / 60°C)	60 minutes	1.07	0.15	SUM impurity~4
Heat exposure (105°C)	120 hours	3.81	0.72	SUM impurity~3 & SUM impurity~4, NAP impurity~2 & 3

Humidity (90%Rel.Humidity@25°C)	120 hours	0.43	0.14	SUM impurity~4
Photolytic (10K Lux along with UV 200 W.Hr.m^{-2}).	7 days	1.59	8.26	SUM impurity~ 3&4, NAP impurity~2,3 & 6

Precision

For precision along with intermediate precision (Ruggedness) data refer Table-5.

Table-5: Precision along with Ruggedness results

Name of the component	Method precision (%RSD)	Intermediate precision (%RSD)
SUM impurity~1	0.5	0.5
SUM impurity~2	0.4	0.5
SUM impurity~3	0.2	0.4
SUM impurity~4	0.4	0.5
SUM impurity~5	2.9	4.1
SUM impurity~6	1.0	1.5
NAP impurity~1	0.2	0.7
NAP impurity~2	1.0	1.4
NAP impurity~3	0.8	0.4
NAP impurity~4	0.7	0.7
NAP impurity~5	2.0	1.1
NAP impurity~6	0.4	0.4

Sensitivity

For LOD along with LOQ concentrations, refer Table-6.

Table-6: LOD Along with LOQ Values

Name of the component	LOD values (%w/w)	LOQ values (%w/w)
SUM impurity~1	0.006	0.020
SUM impurity~2	0.008	0.025
SUM impurity~3	0.008	0.026
SUM impurity~4	0.008	0.026
SUM impurity~5	0.007	0.024
SUM impurity~6	0.006	0.021
SUM	0.008	0.026
NAP impurity~1	0.0006	0.002
NAP impurity~2	0.0006	0.002
NAP impurity~3	0.0006	0.002
NAP impurity~4	0.0015	0.005
NAP impurity~5	0.0006	0.002
NAP impurity~6	0.0006	0.002
NAP	0.0006	0.002

Linearity and Range

The values observed for linearity data shows that each impurity has shown a linear relationship from proposed LOQ concentration to 150% of the proposed limit of the specification. Coefficient of correlation is found to be more than 0.995 for all impurities. For data refer Table-7a and Table-7b.

Table-7a: Linearity Table for Sumatriptan Related Impurities

Name of the constituent	Regression line equation	Linear Range(r)	Coefficient of Correlation	y-intercept	STEY X
SUM impurity~1	y = 10207x - 348	0.043-0.639	0.99933	-348	1011
SUM impurity~2	y = 78605x -176	0.053-1.603	0.99938	-176	1917
SUM impurity~3	y = 106496x -218	0.054-1.580	0.99941	-218	2502
SUM	y = 96435x -251	0.055-1.606	0.99936	-251	2390
SUM impurity~4	y = 90701x -263	0.055-1.570	0.99938	-263	2167

SUM impurity~5	y = 63047x -412	0.052-1.594	0.99945	-412	1437
SUM impurity~6	y = 73850x + 133	0.044-0.636	0.99912	133	833

Table-7b: Linearity Table for Naproxen Sodium Related Impurities

Name of the constituent	Regression line equation	Linear Range(r)	Coefficient of Correlation	y-intercept	STEYX
NAP impurity~1	y = 142230x -307	0.026-1.908	0.99941	-307	4079
NAP impurity~2	y = 213904x -2827	0.026-1.889	0.99946	-2827	5813
NAP	y = 173127x +13333	0.027-1.910	0.99724	13333	10759
NAP impurity~3	y = 52194x -191	0.063-1.886	0.99936	-191	1495
NAP impurity~4	y = 163290x -447	0.026-1.842	0.99939	-447	4591
NAP impurity~5	y = 1749479x + 6893	0.024-1.872	0.99921	6893	5844
NAP impurity~6	y = 201718x -366	0.024-1.898	0.99941	-366	5771

Accuracy

Accuracy results indicate satisfactory recovery for each impurity ranges from LOQ to 150% level. Values are found to be in the range of 90 to 110% with < 5.0% RSD in triplicate data in each concentration. For data refer Table-8a**,** Table-8b, and Table-8c and Table-8d.

Table-8a: Accuracy Values of SUM Impurities

Spiked level	SUM impurity~1		% Recovery	SUM impurity~2		% Recovery	SUM impurity~3		% Recovery
	%Spiked	%Obtained		%Spiked	%Obtained		%Spiked	%Obtained	
LOQ~1	0.043	0.045	103.4	0.053	0.055	102.3	0.054	0.055	101.1
LOQ~2	0.043	0.044	102.3	0.053	0.054	100.6	0.054	0.054	98.6
LOQ~3	0.043	0.046	105.1	0.053	0.057	106.0	0.054	0.058	105.6
50%~1	0.213	0.210	98.5	0.534	0.536	100.3	0.527	0.519	98.6
50%~2	0.213	0.211	98.9	0.534	0.536	100.3	0.527	0.522	99.2
50%~3	0.213	0.211	99.1	0.534	0.541	101.3	0.527	0.525	99.7
100%~1	0.426	0.424	99.5	1.069	1.074	100.4	1.053	1.048	99.4
100%~2	0.426	0.425	99.7	1.069	1.076	100.6	1.053	1.050	99.6
100%~3	0.426	0.423	99.4	1.069	1.070	100.1	1.053	1.047	99.4
150%~1	0.639	0.635	99.3	1.603	1.600	99.8	1.580	1.563	98.9
150%~2	0.639	0.626	98.0	1.603	1.580	98.5	1.580	1.546	97.8
150%~3	0.639	0.636	99.5	1.603	1.605	100.1	1.580	1.569	99.3

Table-8b: Accuracy Values of SUM Impurities

Spiked level	SUM impurity~4		% Recovery	SUM impurity~5		% Recovery	SUM impurity~6		% Recovery
	%Spiked	%Obtained		%Spiked	%Obtained		%Spiked	%Obtained	
LOQ~1	0.055	0.055	100.1	0.052	0.050	96.8	0.044	0.043	98.5
LOQ~2	0.055	0.053	97.5	0.052	0.051	98.6	0.044	0.041	94.5
LOQ~3	0.055	0.052	94.6	0.052	0.055	106.7	0.044	0.043	97.6
50%~1	0.523	0.484	92.4	0.531	0.493	92.8	0.219	0.202	92.4
50%~2	0.523	0.485	92.7	0.531	0.510	95.9	0.219	0.205	93.7
50%~3	0.523	0.491	93.8	0.531	0.526	99.1	0.219	0.206	94.4
100%~1	1.047	1.073	102.5	1.063	1.038	97.7	0.437	0.418	95.7
100%~2	1.047	1.073	102.5	1.063	1.054	99.2	0.437	0.404	92.4
100%~3	1.047	1.067	101.9	1.063	0.972	91.4	0.437	0.407	93.0
150%~1	1.570	1.606	102.3	1.594	1.492	93.6	0.656	0.606	92.4
150%~2	1.570	1.574	100.2	1.594	1.471	92.3	0.656	0.620	94.6
150%~3	1.570	1.609	102.4	1.594	1.492	93.6	0.656	0.635	96.8

Table-8c: Accuracy Values of NAP Impurities

Spiked Level	NAP impurity~1		% Recovery	NAP impurity~2		% Recovery	NAP impurity~3		% Recovery
	%Spiked	%Obtained		%Spiked	%Obtained		%Spiked	%Obtained	
LOQ~1	0.026	0.024	103.7	0.026	0.028	104.8	0.063	0.064	102.0
LOQ~2	0.026	0.027	103.2	0.026	0.027	102.6	0.063	0.063	100.6
LOQ~3	0.026	0.027	102.1	0.026	0.027	103.3	0.063	0.065	103.5
50%~1	0.636	0.637	100.2	0.636	0.611	96.0	0.629	0.638	101.6
50%~2	0.636	0.634	99.7	0.636	0.610	95.9	0.629	0.648	103.1
50%~3	0.636	0.638	100.3	0.636	0.615	96.7	0.629	0.656	104.3
100%~1	1.272	1.275	100.2	1.272	1.269	99.8	1.257	1.286	102.3
100%~2	1.272	1.277	100.4	1.272	1.272	100.0	1.257	1.292	102.8
100%~3	1.272	1.272	100.0	1.272	1.267	99.6	1.257	1.287	102.4
150%~1	1.908	1.922	100.8	1.908	1.876	98.3	1.886	1.904	101.0
150%~2	1.908	1.881	98.6	1.908	1.841	96.5	1.886	1.892	100.3
150%~3	1.908	1.926	101.0	1.908	1.882	98.7	1.886	1.917	101.7

Table-8d: Accuracy Values of NAP Impurities

Spiked Level	NAP impurity~4		% Recovery	NAP impurity~5		% Recovery	NAP impurity~6		% Recovery
	%Spiked	%Obtained		%Spiked	%Obtained		%Spiked	%Obtained	
LOQ~1	0.026	0.024	91.7	0.024	0.024	100.9	0.024	0.022	90.6
LOQ~2	0.026	0.024	92.9	0.024	0.023	95.4	0.024	0.022	92.5
LOQ~3	0.026	0.025	93.7	0.024	0.023	96.6	0.024	0.023	95.4
50%~1	0.614	0.597	97.1	0.624	0.576	92.3	0.633	0.603	95.3
50%~2	0.614	0.605	98.5	0.624	0.566	90.8	0.633	0.618	97.6
50%~3	0.614	0.611	99.5	0.624	0.579	92.9	0.633	0.623	98.5
100%~1	1.228	1.228	100.0	1.248	1.220	97.8	1.265	1.257	99.4
100%~2	1.228	1.230	100.1	1.248	1.226	98.2	1.265	1.261	99.7
100%~3	1.228	1.219	99.2	1.248	1.246	99.9	1.265	1.257	99.3
150%~1	1.842	1.815	98.5	1.872	1.810	96.7	1.898	1.838	96.8
150%~2	1.842	1.817	98.6	1.872	1.822	97.3	1.898	1.871	98.6
150%~3	1.842	1.837	99.7	1.872	1.826	97.6	1.898	1.873	98.7

Solution Stability

Standard and sample solutions were injected for about 44 hours on periodical intervals at room temperature. SUM and NAP impurities did not show any increase in trend and nor detected any additional peaks. Hence it can be concluded that both standards along with sample solutions are found stable for about 44 hours at 25°C.

Robustness

Robustness data revealed that closely eluting peaks SUM impurity~1 and SUM impurity~2, SUM impurity~2 and SUM impurity~3, SUM impurity~3 and SUM peak shows resolution greater than 1.2 inflow and organic ratio conditions. The similar observation is seen even for NAP impurity~4 and NAP impurity~5. Robustness data generated on remaining conditions shows satisfactory resolution for all closely eluting peaks and response is also found to be satisfactory for each impurity. Hence developed method can be claimed as robust.

CONCLUSION

Publication is available for estimation of SUM and NAP related impurities in presence of SUM and NAP drug substances, but not in tablet matrix. In general drug substance presence in placebo matrix may react and give unknown peaks during the shelf life of the product. The ability to quantify those unknown peaks is not specified in the publication. The developed method is appropriate for quantification of SUM and NAP related known and unknown impurities that originate from the life cycle of the combination drug

product. The projected method is validated as per compendia recommendation as mentioned in ICH. Forced degradation data shows not only the product behavior stability and also presents specificity and selectivity of the method. All the probable impurities are well resolved at a satisfactory level, showing resolution more than 1.2 insensitive robustness conditions for closely eluting peaks. Selected quantification wavelength of 230 nm is found suitable for quantification purpose. Impurities show satisfactory responses and did not find any placebo interference at this working wavelength. Linearity data depicts a linear relationship with a coefficient of correlation greater than 0.99. Accuracy results prove the extraction capability of the method in the proposed diluent. Hence method can be claimed as specific, selective, precise, stability demonstrating and suitable for quantification of impurities.

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