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Stability indicating method development and validation of sumatriptan by using RP–HPLC method and its dosage form

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Abstract
This is a method has established to stability indicating method development and validation for sumatriptan by Reverse Phase-HPLC method. Under chromatographic conditions the Phenomenex kinetex (250*4.6 mm, 5µi.d) column has been used and 1ml/min flow rate has been maintained, The Acetonitrile 10% Methanol 10% and Tri ethyl amine 80% (adjusted pH along with orthophosphoric acid) of mobile phase has taken. So that 221 nm of wavelength was detected. A simple, precise, accurate and rapid high performance liquid chromatographic method has been developed and validated for the estimation of sumatriptan in tablet dosage forms. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 800 ng/spot. The limit of detection and the limit of quantification for the sumatriptan were found to be 63.87 and 193.54 ng, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

Keywords: Phenomenex kinetex column, Sumatriptan, RP-HPLC, Imitrix, Ortho phosphoric acid

Introduction
In 1982 Sumatriptan has been patented and in 1991 it was approved for its use in medical field. Sumatriptan, is a medication drug used in treating migraine headaches, cluster of headaches, which is getting sold as Imitrex brand along with some other brands as well. Bioavailability is seen within three hours after the ingestion. This medicine can be swallowed through mouth, into the nose or by the injection below the skin. The autoreceptors like 5-HydroxyTryptamine$_{1B}$ and 5-HydroxyTryptamine$_{1D}$, which obstructs the neurons of serotonin burning also depletion in synthesis and serotonin liberation upon activation. Activity of Adenylatecyclase is obstructed through the regulatory G proteins takes place after the binding of these receptors with sumatriptan, hence intracellular calcium increases, and intracellular events also gets affected. This results in vasoconstriction and retardation of burning sensory nociceptive (trigeminal) nerve and release of the vasoactive neuropeptide [2].

Review of literature
Rajesh Kumar Nayak, *et al.* [3] developed a paper that describes the analytical method suitable for validation of Sumatriptan by UV Spectrophotometric method.
RP Gondalia, *et al.* [4] developed a simple and specific UV/Visible spectrophotometric method for the simultaneous determination of naproxen sodium and sumatriptan succinate in tablets.
Sagar D. Solanki, *et al.* [22] developed a simple UV-Visible Spectrophotometric method for the simultaneous determination of Sumatriptan succinate (SUMA) and Naproxen sodium (NAP) in tablet dosage form.
Trinath M, *et al.* [6] developed and validated two new simple UV spectrophotometric methods for the simultaneous determination of Sumatriptan (SUM) and Naproxen sodium (NAP) in their combined dosage forms.
B Kalyanaramu, *et al.* [7] developed a simple, sensitive and cost effective visible spectrophotometric method for the estimation of sumatriptan succinate in bulk and dosage forms.
UVM. R Pourmand, *et al.* [8] developed and validated an accurate, simple reproducible and sensitive method for the determination of sumatriptan succinate.

Lokesh Singh, *et al.* [9] undertaken the present study to develop a validated, sensitive, rapid, simple and economic an isocratic HPLC method for estimating Sumatriptan succinate in tablet dosage form.

The objective of the study is to develop a simple, accurate, sensitive and rugged new analytical methods for estimation of sumatriptan in bulk and pharmaceutical tablet dosage form and to validate the proposed methods as per ICH guidelines.

Materials and Methods

Instrumentation

A Electronic Balance AY220HPLC Waters (empower-2-software) with UV/ Visible Detector, PH meter MKVI, Hamilton syringe Ultrasonicator Biotech 250, and Vaccum Filteration and Phenomenex kinetex (250* 4.6mm, 5µi.d) Column has been used. The Data processing was equipped with HPLC system through the Empower software [10].

Chemicals and Reagents

Pharmaceutically pure sample of Sumatriptan drug has obtained from Awamedica Company. Methanol, Water and Acetonitrile was obtained from the local market for HPLC.

Chromatographic Condition

The mobile phase includes 10:10:80 Acetonitrile: Methanol: Tri ethyl amine (PH-2.5 with OPA). The column used is Phenomenex Kinetex (250*4.6mm, 5 µi.d) with Isocratic elution mode of separation.1 ml/min of rate of flow has fixed. The Detector involved was Photo Diode-Array (PDA) and absorbance which has shown is good at 221nm and for further analysis it has been selected. 20µl injection volume having ambient temperature about of 10mins of run time [11-12].

Preparation of Buffer and Mobile Phase

1 ml Tri ethyl amine was dissolved into 1 lt water and 2.5 pH was adjusted along OPA. In an Ultrasonic water bath a mixed mixture of Acetonitrile 100 ml, 800 ml (80%) buffer and methanol 100 ml degassed about for 5 minutes. And now it has filtered by vacuum filtration [13-16].

Preparations of Standard solution of stock and solutions of working standard (100% solution)

10 mg of Sumatriptan has weighed accurately and transferred into volumetric flasks of 10 ml, 3/4th of Diluents are added and also sonicated for about 30 minutes. The volumetric Flasks are added with diluents and flasks were labelled as Standard Stock Solution 1ml of Sumatriptan is pipetted out from each flask of stock solution and extracted to a 10 ml measured volumetric flask and diluent has added till the mark and labelled as working standard solution [17].

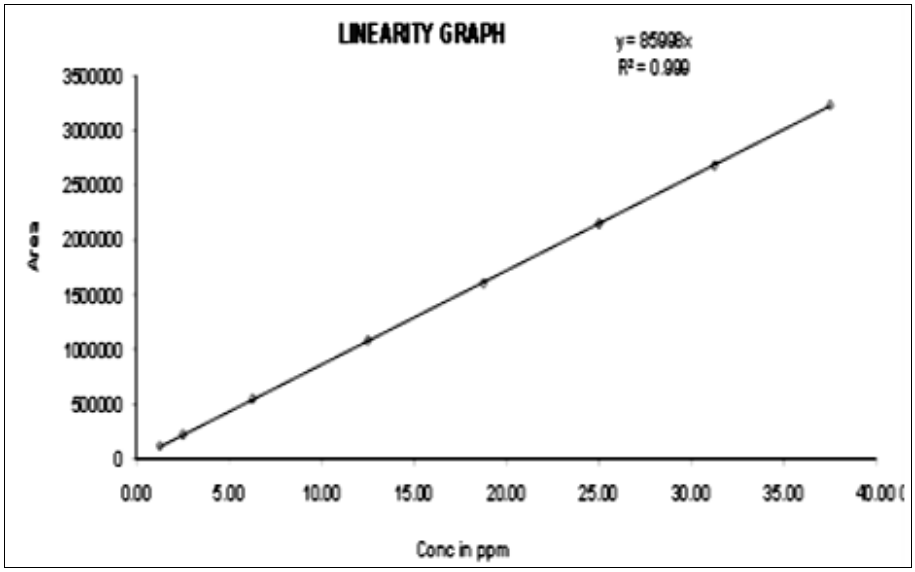
Preparations of Sample Solutions and sample working Solutions (100% solution)

5 tablets of sumatriptan were weighed and then crushed. The crushed powder was taken into a100ml volumetric flask which is equivalent to one tablet. 70 ml of diluents are added to it, these sonicated to dissolve and diluent is added to make up the volume. Further 5 ml - 50 ml is diluted with diluent. 0.45 µ Nylon syringe filter is use for filteration. To 10 ml volumetric flask 1ml filtered Sample stocks solutions are added and made up with diluent [18].

Results and Discussion [19]

1. Linearity

The concentration range of the linearity method was demonstrated for about 5-150 µg / ml concentration of the target. From the stock solution aliquots of 0.25 ml, 0.5 ml, 1.25 ml, 2.5 ml, 3.75 ml, 5 ml, 6.25 ml and 7.5 ml in a 50 ml volumetric flask and it is diluted with mobile phase upto the mark to get the concentration of 5, 10, 25, 50, 75, 100, 125, 150 ppm respectively. As per the test procedure the solutions are injected to the HPLC system. Concentration v/s Peak area. A Calibration curve has been plotted for the conc. v/s peak area. The observations of different parameters of linearity are slope, intercept, correlation coefficient was found to be24908.45985, 277063.9599, 0.9998 respectively.



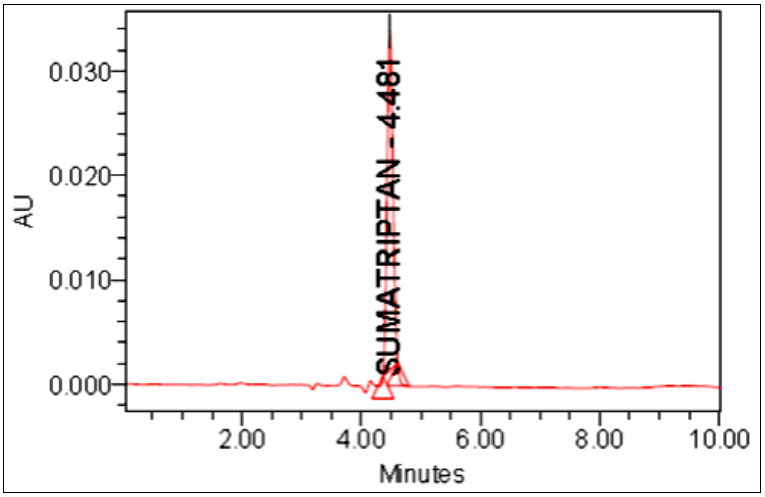


Fig 1: Linearity graph and chromatogram of sumatriptan

Table 1: Results of linearity peaks

S. No.	Conc. (µg/ml)	% linearity	Rt	Average Area	USP. plate count	USP Tailing
1.	1.25	5	4.481	223644	9005	1.144
2	2.50	10	4.487	549968	8079	1.076
3	6.25	25	4.492	1080045	8076	1.155
4	12.50	50	4.498	1601982	8192	1.159
5	18.75	75	4.499	2150780	7731	1.173
6	25.00	100	4.508	2678422	7947	1.174
7	31.25	125	4.508	3232317	7822	1.178
8	37.50	150	4.468	4149922	8557	1.170

2. Accuracy

the drug substance has been spiked on placebo in the range of 50- 150 of the Test conc. and according to specification level analyzed triplicate injections are analyzed for 50%, 100% and 150% mean % recovery, % RSD and linearity were calculated at each level. The % RSD of 50%, 100%, 150% accuracy was found to be 0.670, 0.390, 0.260 respectively.

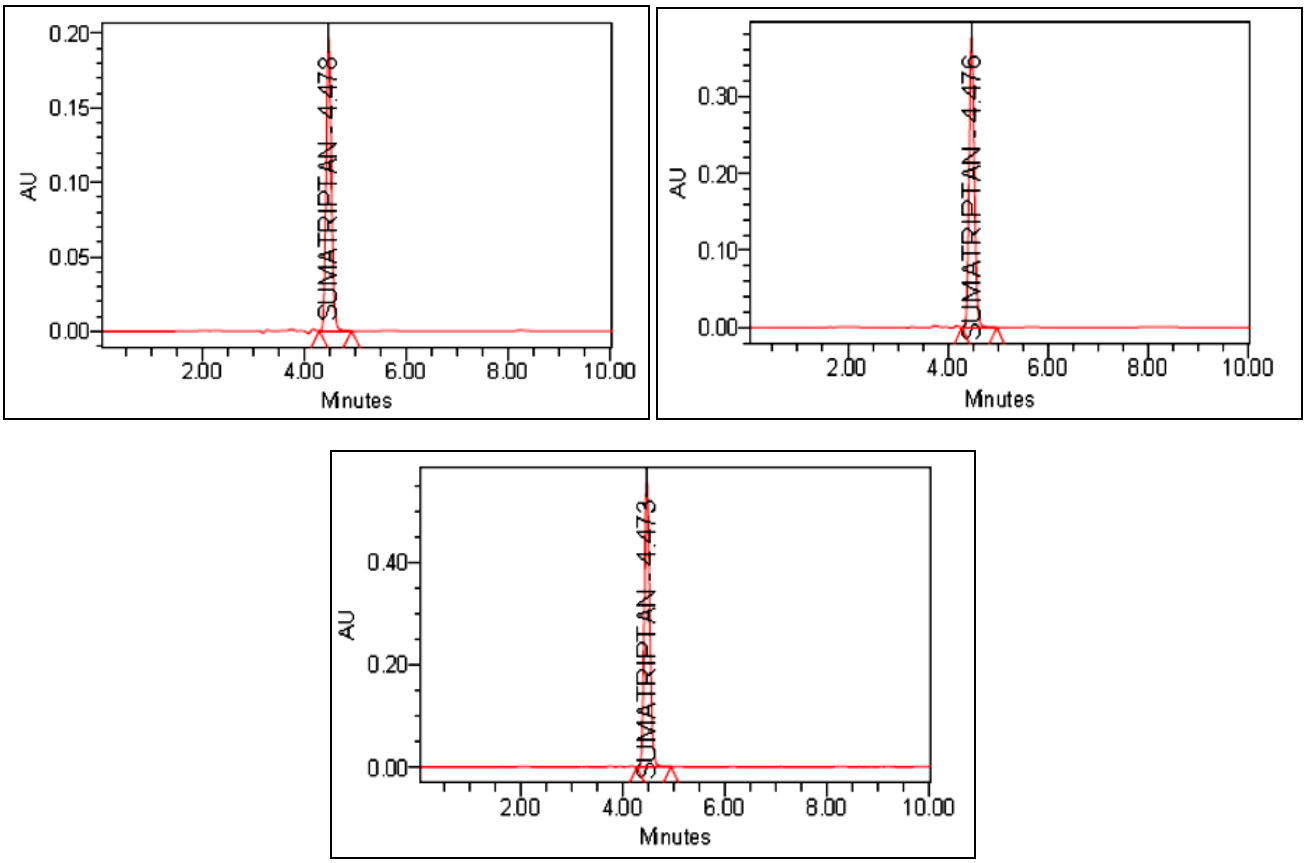


Fig 2: Accuracy 50% (4.478), 100% (4.476), 150% (4.473) chromatograms of sumatriptan

Table 2: % Recovery Results for sumatriptan

S. No.	Spike Level	RT	Avg area	Amount recovered	% Recovery	USP plate count	USP tailing
1	50 % - 1	4.478	1403564	11.9	100.9	8995	1.126
	50 % - 2	4.473	1407203	11.9	100	8583	1.142
	50% - 3	4.480	1390696	11.8	100.9	8720	1.128
2	100 % - 1	4.476	2756366	23.3	100	8745	1.141
	100 % - 2	4.475	2730164	23.1	100.8	8779	1.143
	100 % - 3	4.474	2745900	23.2	100.4	8697	1.158
3	150 % - 1	4.473	4123822	34.8	100.6	8519	1.153
	150 % - 2	4.472	4139168	35	100.3	8460	1.156
	150 % - 3	4.468	4149922	35.1	100.9	8557	1.170

3 Precision (repeatability)

A sample solution of 25 ppm is prepared from the sample stock solution and it has been injected for 6 times into HPLC system, according to test procedure. By system precision, method precision, studies was observed results that % RSD of peak area was 0.260,.0.164, 0.263 respectively. All the parameters were within the limit.

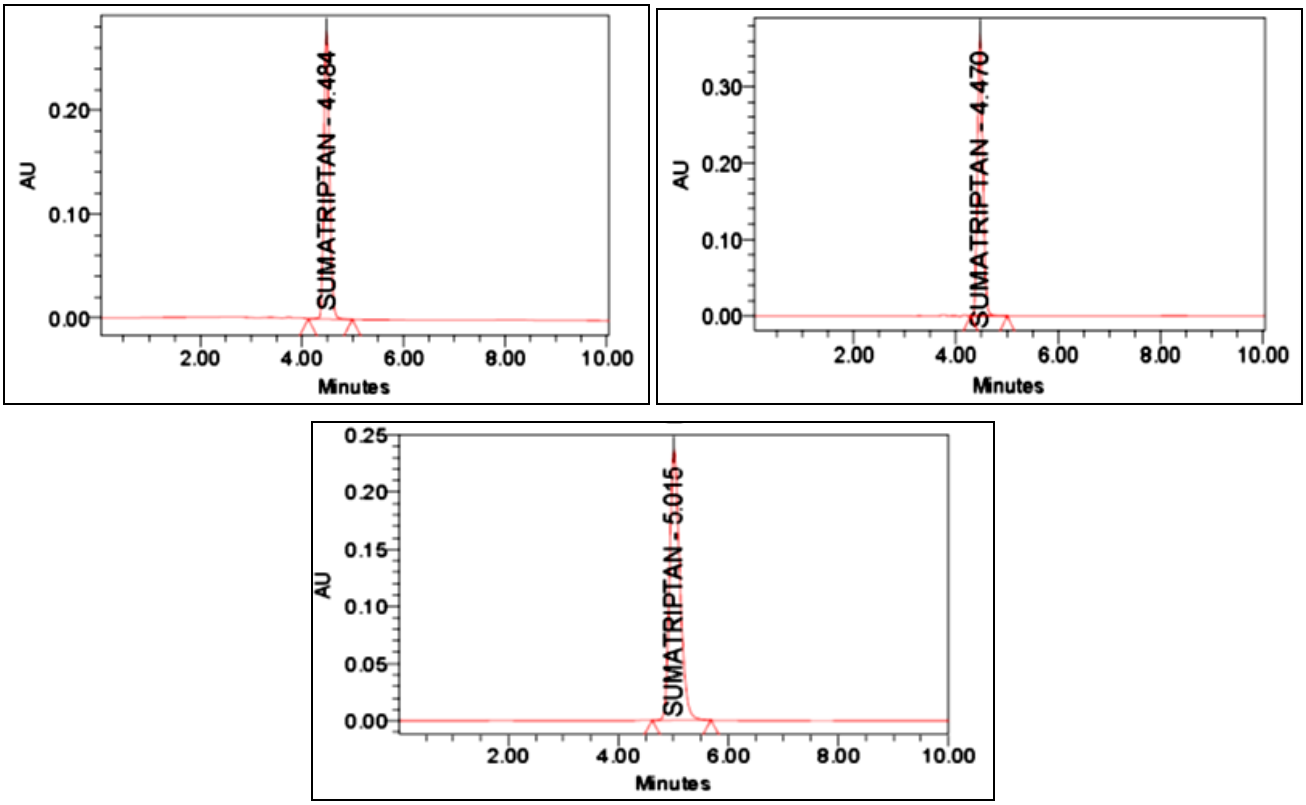
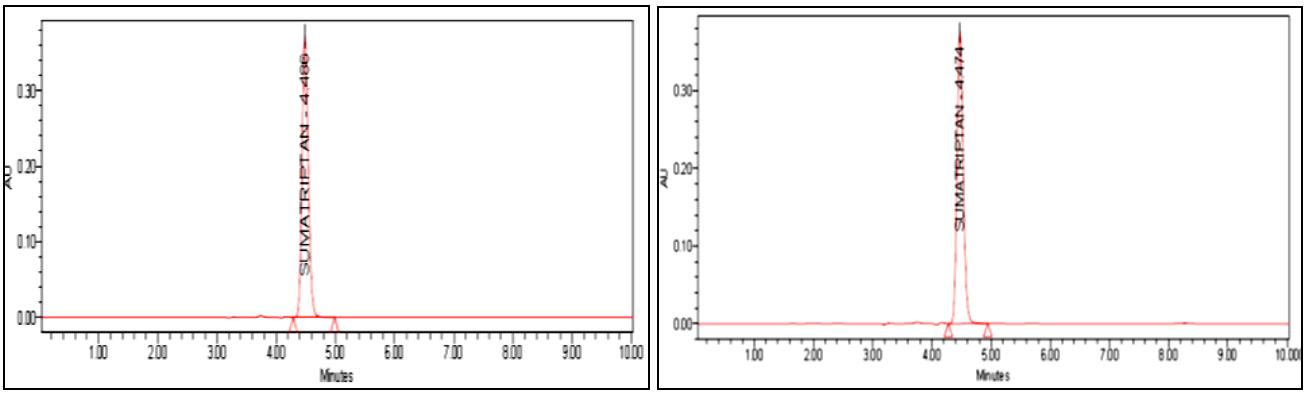


Fig 3: System precision (4.484) and method precision (4.470), intermediate precision (5.015) Chromatograms of sumatriptan

4. Specificity

A Sumatriptan Identification



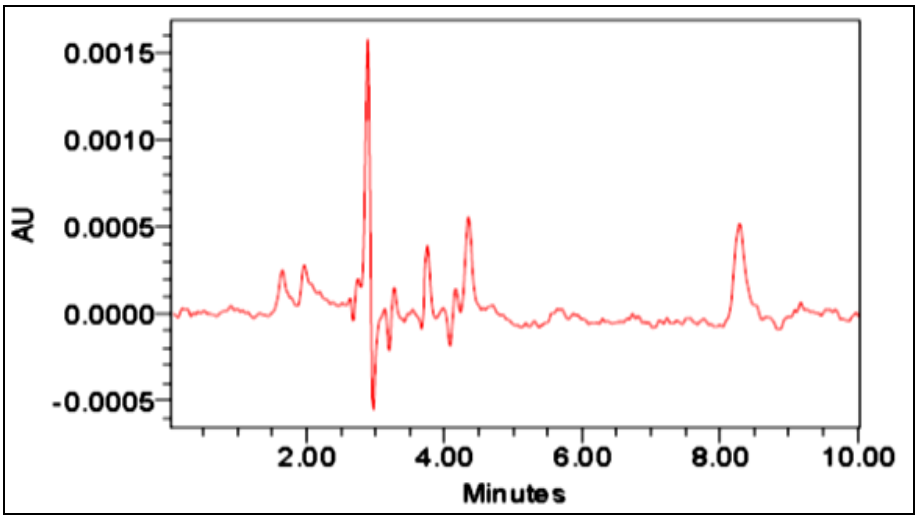


Fig 4: Standard and sample Chromatograms for identification

B Blank interference and placebo interference: No interference were found at blank and placebo at the analyte's retention time. Hence this method has found to be specific

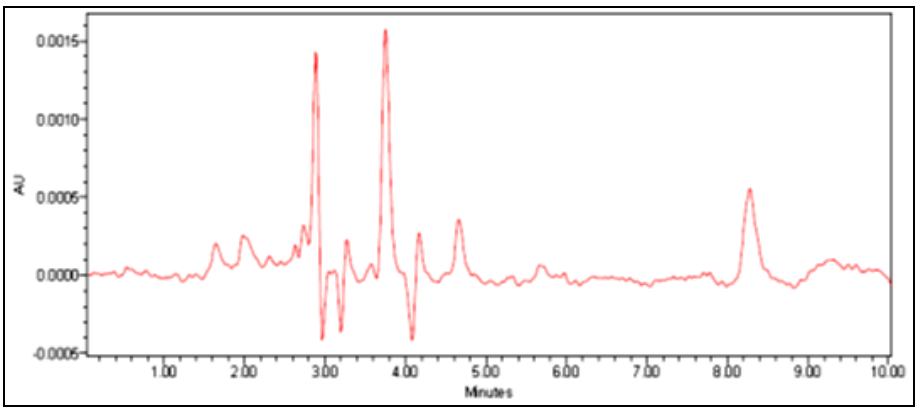


Fig 5: Blank and placebo Chromatograms

C Interference with forced degradation

Table 3: Results of forced degradation peaks

	RT	Area	Uspplate count	Usptailing
Acid	4.533	2443397	9739	1.106
Alkali	4.529	2302328	9651	1.094
Peroxide	4.488	2115622	8976	1.129
Reduction	4.517	2315896	9514	1.111
Thermal	4.477	2610726	8246	1.145
Photolytic	4.475	2605747	8328	1.146
Humidity	4.473	2606961	8485	1.145
Hydrolysis	4.473	2606366	8490	1.142

Table 4: Results of forced degradation peaks

	% Degradation	Purity angle.	Purity Threshold	Pass/ Fail
Acid (5%HCL)	9.9	0.081	1.032	Pass.
Alkali (5% NAOH)	17.5	0.097	1.043	Pass.
Peroxide (H_2O_2)	25.7	0.08	1.027	Pass.
Reduction ($NaHSO_4$)	17.	0. 078	1.035	Pass.
Thermal	9.3	0.09	1.031	Pass
Photolytic	6.2	0. 078	1.033	Pass
Humidity	6.8	0.076	1.036	Pass

5. LOD and LOQ

The limit of detection has performed for Sumatriptan is estimated to be 1.967.The Limit of quantification has performed for Sumatriptan is estimated to be 5.961

6. Robustness

Physical parameters were like flow rate, composition of mobile phase and wavelength which might differ but the responses will be under specified limit.

Table 5: Parameters of robustness

Parameter	Original Condition	Variable Condition	
		Increased	Decreased
Change in flow rate (1.0%)	1.0 ml/min.	1.2ml/min.	0.8ml / min.
Change in Mobile phase Composition (2%) buffer: ACN: methanol	80:10:10	82:9:9	78:11:11
Change in wave length (5 nm)	221 nm	226 nm	216

A. Effects of variation of flow rate: The rate of flow was kept 1ml/min after injecting standard solution preparation into the HPLC system. From standard flow plus, flow minus studies it was observed that the % RSD of flow rate was found to be 0.082, 0.086, 0.199 respectively.

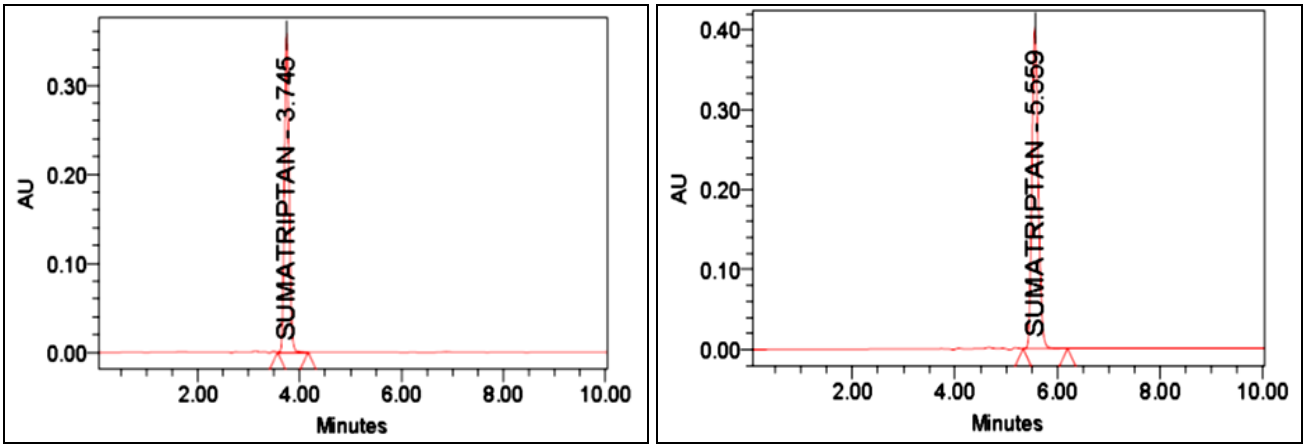


Fig 6: Flow plus and Flow minus chromatograms

Table 6: Results of flow rate peaks

S. No	Flow rate	Rt	Area	Uspplate count	Usptailing
1	1.0 ml	4.487	2674332	8563	1.156
2	1.0 ml	4.490	2678292	8488	1.141
3	1.0 ml	4.488	2674674	8670	1.155
4	1.2 ml	4.745	2282238	7899	1.127
5	1.2 ml	4.744	2286143	7886	1.132
6	1.2 ml	3.744	2283993	8239	1.118
7	0.8 ml	5..559	3465028	9642	1.175
8	0.8 ml	5..560	3477707	9637	1.180
9	0.8 ml	5..561	3466634	9745	1.172

B. Effects of variation of wavelengths: The observed % RSD of wavelength under the studies of standard (221 nm), wavelength plus (226nm), wavelength minus (216 nm) was found to be 0.082, 0.184, 0.211 respectively.

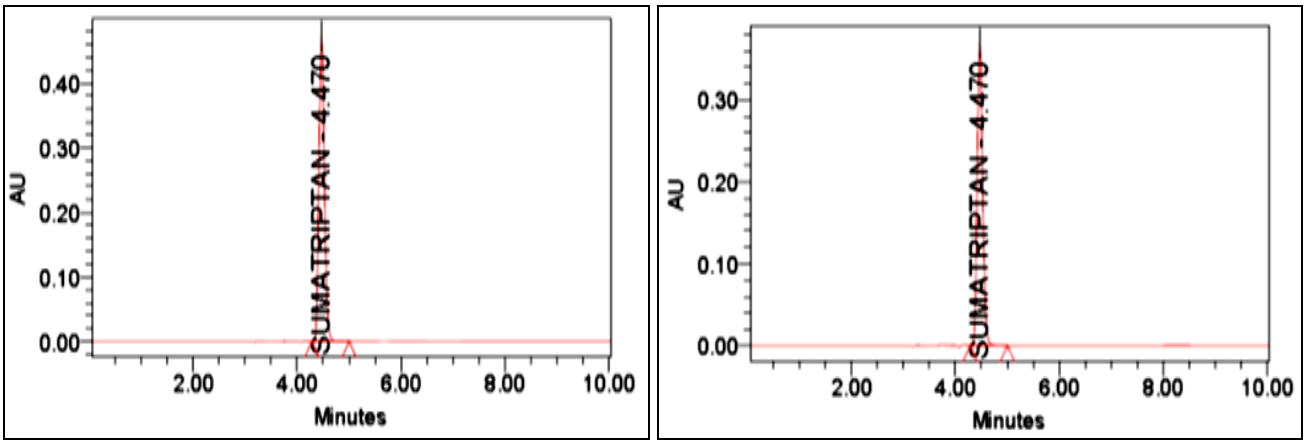


Fig 7: Wave length plus and wavelength minus chromatograms

Table 7: Results of wave length peaks

S. No	Wave length	RT	Area	Uspplate Count	Usptailing
1	221 nm	4.487	2674332	8563	1.156
2	221 nm	4.490	2678292	8488	1.141

3	221 nm	4.488	2674674	8670	1.155
4	226 nm	4.470	3475237	8477	1.152
5	226 nm	4.472	3484016	8659	1.136
6	226 nm	4.474	3487714	8622	1.139
7	216 nm	4.470	2718072	8463	1.152
8	216 nm	4.472	2726848	8642	1.137
9	216 nm	4.474	2728876	8608	1.139

C. Effects of variation of MP (mobile phase) composition: RSD % of organic phases from studies of standard (80:10:10), organic plus (78:11:11), organic minus (82:9:9) was found to be 0.082, 0.427, 0.727 respectively.

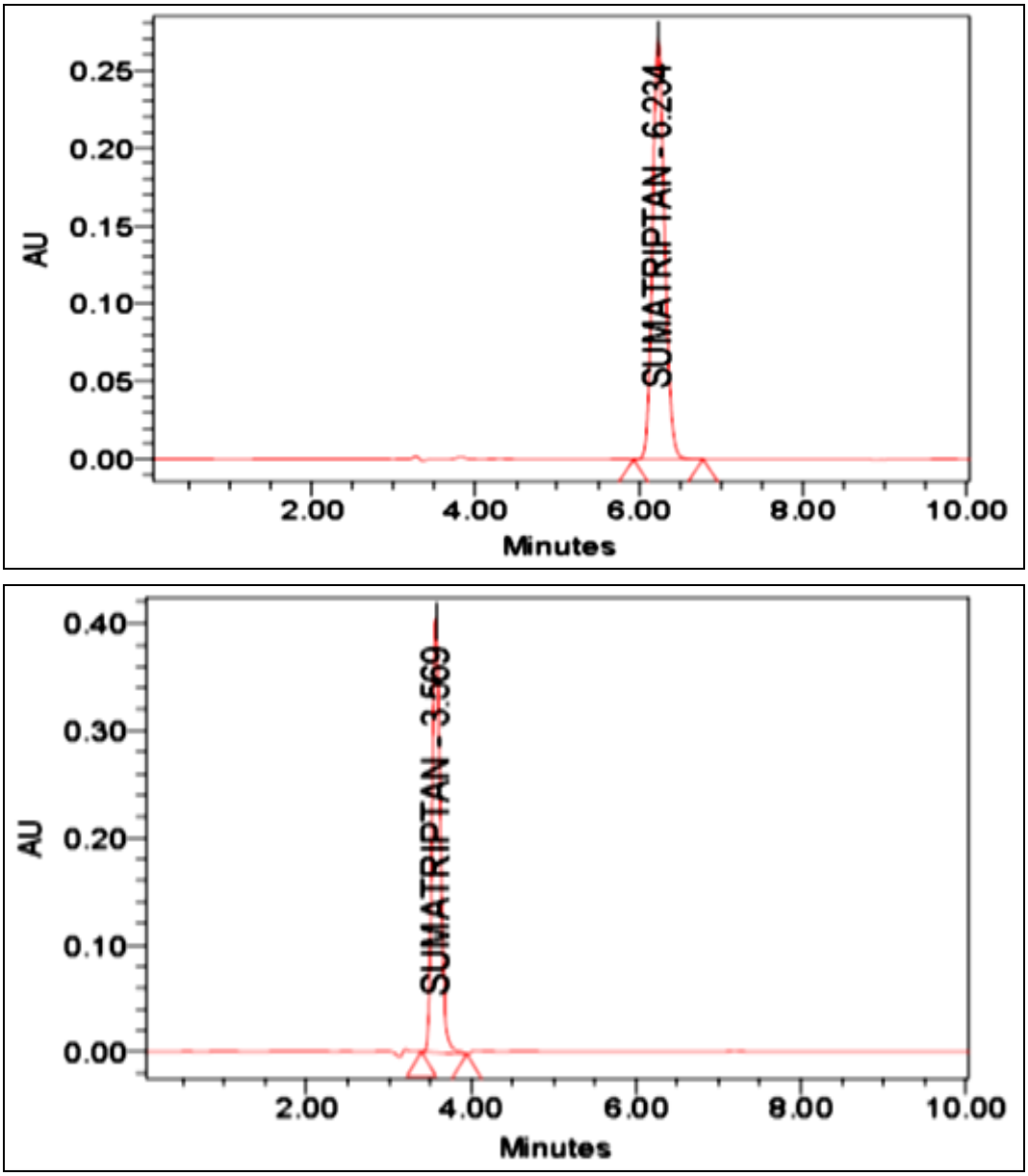


Fig 8: Organic plus and organic minus chromatograms

Table 8: Results of organic change peaks

S. No.	Mobile Phase Ratio	RT	Area	Uspplate Count	Usptailing
1	80:10:10	4.487	2674332	8563	1.156
2	80:10:10	4.490	2678292	8488	1.141
3	80:10:10	4.488	2674674	8670	1.155
4	78:11:11	3.563	2796758	6274	1.181
5	78:11:11	3.565	2819241	6195	1.189
6	78:11:11	3.569	2800794	6231	1.194
7	82:9:9	6.234	2793492	8346	1.143
8	82:9:9	6.248	2828456	8256	1.141
9	82:9:9	6.257	2792837	8066	1.137

Solution stability

The observations of % label caim of solution stability from initial, 4 hrs, 8 hrs, 12 hrs, 24 hrs are found to be 99.5, 100.3, 101.2, 102.3, 101.8 respectively and the %deviation calculated of 4 hrs, 8hrs, 12hrs, 24hrs are 0.80, 1.71, 2.81, 2.31 respectively.

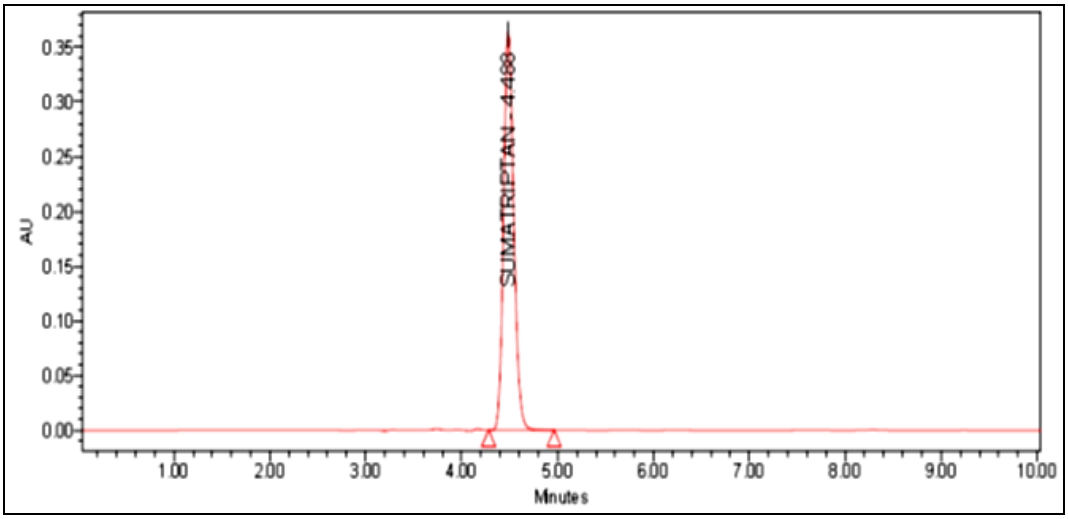


Fig 9: Solution stability initial chromatogram

Table 9: Results of solution stability peaks

S. No.	Solution Stability (hrs)	RT	Area	Uspplate count	Usptailing
1.	Initial	4.488	2695894	8516	1.158
2.	4 hrs	4.474	2717923	8487	1.144
3.	8hrs	4.475	2743702	8560	1.142
4.	12hrs	4.482	2772781	8472	1.144
5.	24 hrs	4.498	2759859	7295	1.149

Discussions [20-21]

Since importance in the quality control of drugs and drug products in recent years' determination of drugs by HPLC has gained the considerable attention. The main objective of the study has to develop an accurate rapid, simple, precise and sensitive HPLC method for analysis of sumatriptan in the bulk and its pharmaceutical dosage form by using solvent system of C_8 ODS Inertsil (250* 4.6 mm, 5µi.d) stationary phase and using solvent system of TEA: ACN: methanol in the ratio of 80:10:10.The chromatographic condition is fixed at rate of flow at 1ml/ min at 221 nm with PDA detector. By using freshly prepared solutions validation studies are carried out by as per ICH requirements.

Table 10: Validation parameters of Sumatriptan by HPLC

Parameters	Acceptance criteria's	Sumatriptan
Linearity range. Correlation, coefficient.	Correlation Coefficient $r^2 > 0.999$ or 0.995	5-150 µg / ml $.r^2 = 0.99998$
LOD	S/ N>2 or 3	1.967 µg/ ml
LOQ	S /N> 10	5.961 µg/ ml
System precision	RSD< 2%	% RSD = 0.260
Intermediate precision	RSD < 2%	% RSD = 0.263
Method precision	RSD < 2%	% RSD = 0.164
Accuracy	Recovery 98-102% (individual)	% Recovery = 100.0-100.9
Specificity	No interference from the blank, placebo & other degradation products with the main peak. Purity angle > Threshold angle.	No interference. Peak pure.
Solution stability	> 12 hr	stable upto 24 hr
Robustness	RSD is NMT 2% in modified conditions Flow plus Flow minus Organic Plus Organic Minus Wavelength Plus Wavelength Minus	Complies % RSD = 0.086 % RSD = 0.199 % RSD = 0.427 % RSD = 0.727 % RSD = 0.184 % RSD = 0.211

Conclusion

The reverse phase HPLC Method used for estimation of the sumatriptan in bulk and tablet pharmaceutical dosage forms was validated accordingly to ICH guidelines. Hence proposed method has been successfully used for routine analysis. The methods were very simple, specific and

reliable. All the results were within the limits. The developed methods have been validated as per ICH guidelines.

Consent & Ethical Approval
It is not applicable.

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Conflict of Interest
Authors have declared that there is no conflict of interest exist.

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