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Quantitative Nuclear Magnetic Resonance Spectroscopic Method Development and Validation of Sumatriptan Succinate in Pharmaceutical Tablet Dosage form

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Abstract : A new, simple and accurate quantitative proton nuclear magnetic resonance(qNMR) spectroscopic method was developed to determine the sumatriptan succinate in pharmaceutical tablet formulation. In this developed quantitative nuclear magnetic resonance spectroscopy method, Maleic acid was used as internal standard(IS) due to there was no overlapping of the peak to analyte peaks and deuterium oxide(D2O) was used as diluent. For the quantification of the sumatriptan succinate 4.43 ppm and 6.20ppm peaks were used as quantitative monitoring purpose to correspong to analyte sumatriptan succinate and Maleic acid internal standard(IS) respectively. The final optimized method was validated as per International Conference on Hormonisation (ICH) guidelines in terms of Specificity, Limit of detection (LOD), Limit of Quantitation (LOQ), Precision, Linearity, Accuracy ,Solution stability and Robustness. This method could be used to determination of sumatriptan succinate in bulk and pharmaceutical tablet dosage forms.

Keywords : Sumatriptan succinate, Quantitative nuclear magnetic resonance(qNMR) ,Internal standard, Method Validation.

Introduction

Sumatriptan succinate is belong to triptane class and used to treat Migraine[1-6] and cluster headaches. Sumatriptan succinate was chemically3-[2-(dimethylamino)Ethyl]-N-methylindole-5-methanesulfonamide. In July 2009, the US FDA approved a single-use jet injector formulation of sumatriptan.

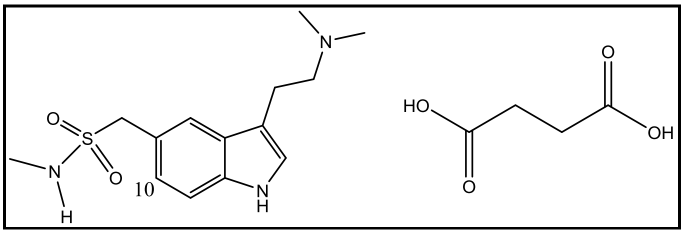

Fig.1:Chemical Structures of Sumatriptan succinate

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Nuclear magnetic resonance spectroscopy itselfwas a quantitative spectroscopic tool due to the intensity of resonance peak is directly proportional to the number of corresponding resonance nuclei.This fact facilitates to determination of the amount of substance with accurate and precisely.Quantitative NMR spectroscopy[7-11] was first described by Jungnickel,forbes and hollis in 1963.The most attractive advantage with quantitative NMR than other analytical techniques that can achieve precise quantification without analyte reference standards(CRS). Analytical methods based on HPLC [12-13], spectrophotometry[14-16] ,UPLC-MS method[17] ,HPTLC method[18] have been reported for the determination of sumatriptan succinate .The aim of the study was to develop specific and accurate quantitative NMR method to determine the assay of sumatriptan succinate in bulk as well as in pharmaceutical tablet dosage form and validated as per International Conference on Hormonisation(ICH)[19] guidelines.

Materials and Method

Chemicals and Reagents

Samples of Sumatriptan succinate standard sample obtained from local pharmaceutical company.High purity analytical grade Maleic acid (99.90%)purchased from Sigma-Aldrich and deuterium oxide-D2O(99.99%),deuterated dimethyl sulfoxide-DMSO-*d*6(99.99%) purchased from Merck.Tablets containg 50 mg of sumatriptan succinate were purchased from local market, India.

EQUIPMENT

All NMR spectral data obtained from BRUKER 400 Avance-III NMR with field strength of 9.4 Tesla and operating at frequency 400.12MHz for protons.The spectrometer equipped with 5mm BBO(Broad Band Observe) probe with multi nuclear probehead. Semimicro balance was Sartorious with accuracy 0.001mg.

Preparation of Stock Solution

Maleic acid (IS) Stock Solution Preparation

Accurately weighed about 83.33 mg of Maleic acid and transferred to 10mL volumetric flask (5mg/0.6mL),shake it to completely dissloved and made up to the mark with deuterium oxide(D2O).

Suamtriptan succinate Standard preparation

Accurately weighed about 10mg of sumatriptan sucinate standard in a stoppered glass vial to that added 0.6 mL stock solution of maleic acid(IS),shake it to completely dissolved.

Suamtriptan succinate Sample(Tablets) preparation

Ten tablets of sumatriptan succinate were weighed, crushed with mortar and pestle ground in to fine powder,take portion equivalent to 10mg of sumatriptan succinate was weighed accurately ,transferred to stoppered glass vial to that added 0.6 mL stock solution of maleic acid(IS),the solution was thourouly mixed it to completely dissolved and supernatant solution was taken for analysis.

Results and Discussions

Determination of Relaxation Time

Toaccurate quantification, the key parameter is relaxation delay(t) which depend on the longitudinal relaxation time(T_1) of all signals of interest. Longitudinal relaxation time(T_1)is calculated with following foumula(1).

$M_z=M_0[1-e^{-(t/T1)]}$

Here, M_z is the magnetization along z-axis after time t

M_0 is the magnetization along z-axis at thermal equilibrium

In this study relaxation time was determined experimentally by inversion recovery experiment for all the protons in sumatriptan succinate and maleic acid internal standard in D2O.The longest relaxation time was found for the maleic acid as 7.89 sec and for the monitong analyte proton was found to 0.5sec. The T_1 results were summarized in the table1. Relaxation delay (t) should not be less than five times the T_1 to ensure a reliable experimental data, so that 40sec relaxation time was kept for all experiments.

Table.1 Summarised results for T_1 values of all corresponding protons

S.No	Chemical shift	Relaxation delay(T1)sec
1	2.54	1.86
2	2.61	1.29
3	2.78	0.96
4	3.09	0.57
5	3.10	0.54
6	3.31	0.59
7	4.43	0.50
8	6.19	7.89
9	7.14	1.70
10	7.21	5.38
11	7.39	2.60
12	7.41	2.49
13	7.54	1.51

Optimised Data Acquisition Parameters

The optimised experimental quantitative proton NMR(qNMR) parameters were number of scans (ns) 64 collected for each experiment into 32K datapoints using 30° pulse length.Aquisition time 3.94 sec ,Delay time of 40sec as five times of the calculated from the T_1 value.Preacquision delay 6.5 sec and spectral width (25.0ppm).For all proton NMR spectra data, manual phase correction and base line correction were used for the signal integration.

Sumatriptan succinate structure confirmation by various NMR experiments

Sumatriptan succinate standard was dissolved in deuterated dimethyl sulfoxide (DMOS-*d*6) and performed 1H-NMR,13C-NMR, DEPT,COSY,HSQC,HMBC and NOESY experiments.The assaignment of 1H-NMR and 13C-NMR chemical shifts were assigned and summarized in the table2.The chemical shifts were referenced to the residual DMSO-d6 peak at 2.50 ppm and 39.7 ppm for ^{1}H-NMR and ^{13}C-NMR respectively.

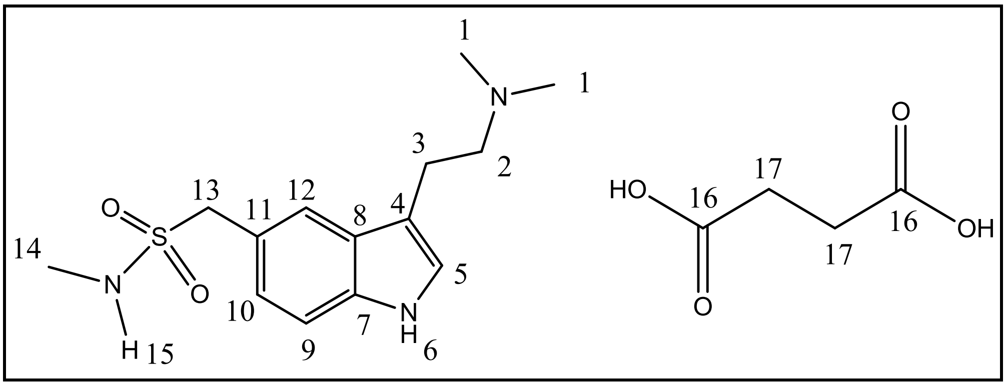


Fig.2: Structure of sumatriptan succinate

Table.2 Summarised results for assaignment of ^{1}H-NMR &^{13}C-NMR of sumatriptane succinate

S.No	^{1}H- NMR			^{13}C- NMR		Assaignment
	Chemical shift(ppm)	**Multiplicity & Coupling constant (*J*=Hz)**	**No.of Protons**	**Chemical shift(ppm)**	**No.of Carbon**	
1	2.36	s	4	30.7	-2**C**H_2-	17
2	2.41	s	6	43.6	-2**C**H_3	1
3	2.53-2.54	d(*J*=3.9)	3	29.2	-**C**H_3	14
4	2.75-2.79	t(*J*=8.0)	2	58.4	-**C**H_2-	3
5	2.87-2.90	t(*J*=7.0)	2	21.6	-**C**H_2-	2
6	4.34	s	2	56.8	-**C**H_2-	13
7	6.77-6.81	q(*J*=5.1)	1	-	-	15
8	7.07-7.09	dd(*J*=8.2,1.2)	1	124.1	-**C**H-	10
9	7.193-7.198	d(*J*=2.0)	1	123.7	-**C**H-	12
10	7.314-7.334	d(*J*=8.3)	1	111.5	-**C**H-	9
11	7.52	s	1	120.9	-**C**H-	5
12	10.89	Broad singlet	1	-	-	15
13				111.2	quary-**C-**	11
14				120.1	quary-**C-**	7
15				127.2	quary-**C-**	8
16				136.1	quary-**C-**	4
17				174.9	**-C=O**	16

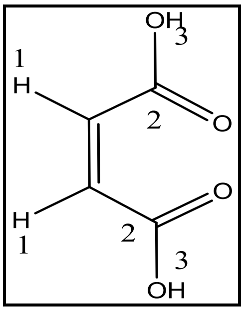


Fig.3: Structure of Maleic acid internal standard

Table.3 Summarised results for assaignment of ^{1}H-NMR &^{13}C-NMR of Maleic acid(IS)

S.No	^{1}H-NMR			^{13}C -NMR		Assaignment
	Chemical shift(ppm)	**Multiplicity & Coupling constant (*J*=Hz)**	**No.of Protons**	**Chemical shift(ppm)**	**No.of Carbon**	
1	6.25	s	2	130.43	-2**CH-**	1
2	12.67	s	2		-2C=O-**OH**	2
3			-	167.09	-2**C**=O-	3

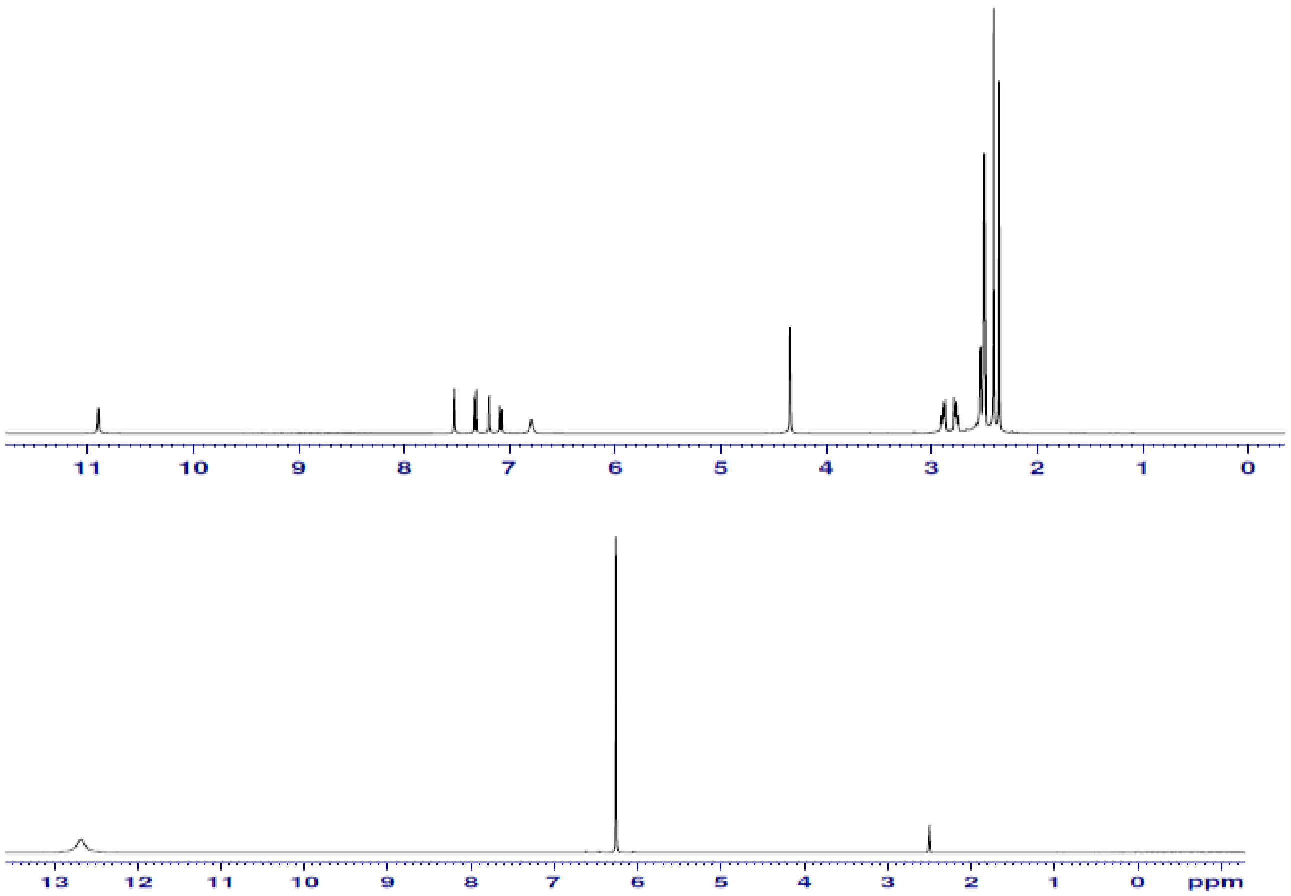


Fig.4 (a) ^{1}H-NMR spectrum of Sumatriptan succinate standard in DMSO-*d*6 (b)^{1}H -NMR spectrum of Maleic acid (IS) in DMSO-*d*6.

From the above ^{1}H-NMR spectra sharp singlet peak at 4.43 ppm choosen as quantitative monitoring peak of analyte and the singlet peak at 6.20 ppm , corresponding to Maleic acid internal standard.The assay and amount of sumatriptan succinate was calculated by using below formula

$$W_{Spl} = \frac{I_{Spl}}{I_{Std}} \times \frac{M_{Spl}}{M_{Std}} \times \frac{N_{Std}}{N_{Spl}} \times P_{Std}$$

$$P_{Spl} = \frac{I_{Spl}}{I_{Std}} \times \frac{M_{Spl}}{M_{Std}} \times \frac{N_{Std}}{N_{Spl}} \times \frac{W_{Std}}{W_{Spl}} \times P_{Std}$$

Here, I_{Spl}=Integral area of analyte proton signal obtained at 4.32 ppm
I_{Std}=Integral area of Maleic acid internal standard proton signal obtained at 6.23 ppm
M_{Spl}=Molar mass of sumatriptane succinate 432.15g/mol
M_{Spl}=Molar mass of Maleic acid internal standard 116.07g/mol
N_{Spl}=No.of protons for sumatriptane succinate(2.0)
N_{Std}=No.of protons for Maleic acid internal standard(20.)
W_{Spl}=Weight of analyte,
W_{Spl}=Weight of internal standard(Maleic acid)
PStd=Potency of Maleic acid internal standard (99.90%)

Method Validation

The Optimised final method was validated as per ICH guidelines Q2(B) interms of Specificity, Limit of detection (LOD), Limit of Quantitation (LOQ), Precision (Method precision and Intermediate precision), Linearity, Accuracy, Robustness, Solution stability and mobile phase stability.

System Suitability

System suitability was evaluated by perfoming six replicates were analyzing 1H-NMR spectra recorded. The acceptance criterion for system suitabilty ,the percentage relative standard deviation of the integral value of analyte signal should not be more than 2.0,the average Signal to noise raio(S/N) of the analyte signal should be more than 150 and the difference of the chemical shift value should not be more than 0.2ppm.The results were summarized in the table 4.

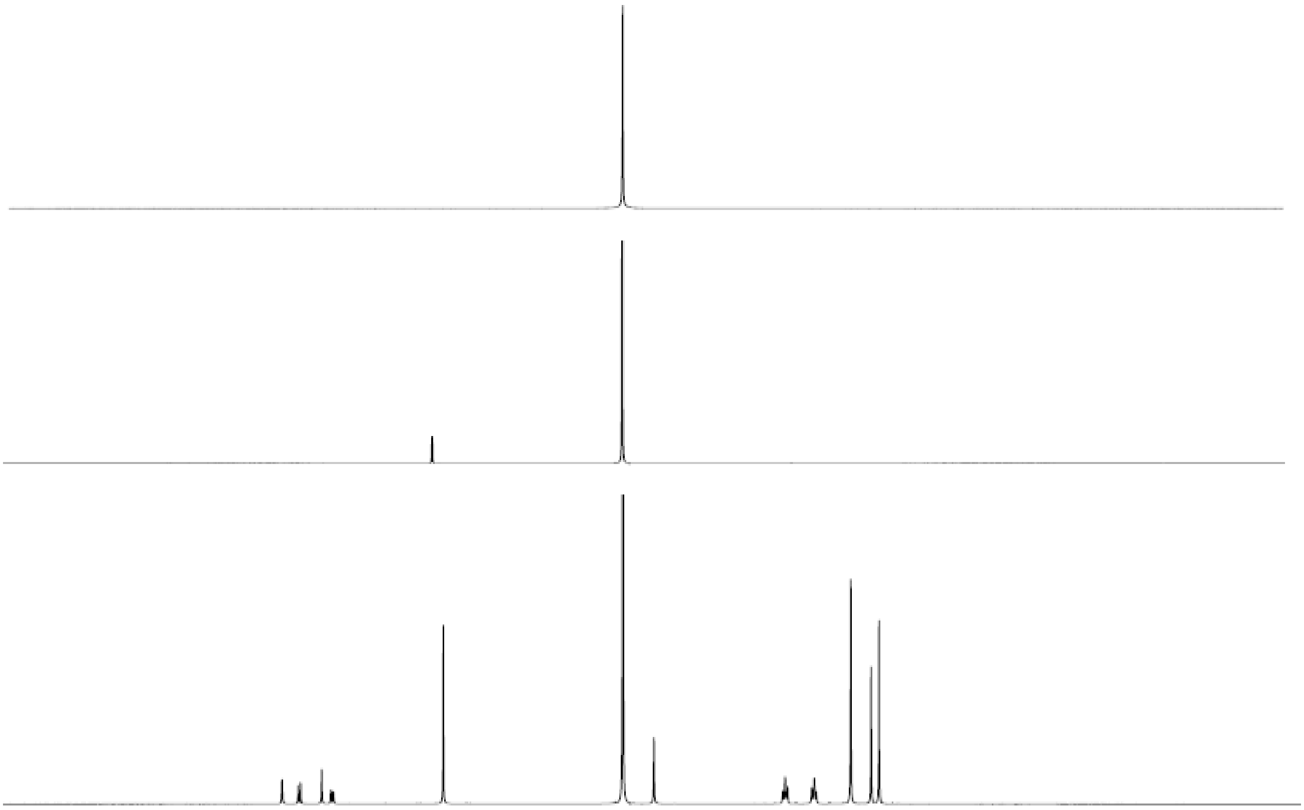
Table 4 : Summarised results of system suitability

Sample Name	**Integral value**	**Signal to noise**	**Chemical shift (ppm)**	**Difference in chemical shift**
Standard solution-1	1.0427	5317.54	4.439	-
Standard solution-2	1 .0428	5378.80	4.438	0.001
Standard solution-3	1.0425	5333.63	4.438	0.001
Standard solution-4	1.0425	5338.61	4.437	0.002
Standard solution-5	1.0428	5300.34	4.437	0.002
Standard solution-6	1.0426	5369.33	4.437	0.002

The percentage relative standard deviation of the integral value of analyte signal was found to 0.01% ,the average signal to noise ratio was found to be 5339:1 and the difference of the chemical shift value was found to 0.002ppm.

Specificity

Recorded the 1H-NMR spectra individually of Sumatriptan succinate standard, sample, Maleic acid internal standard and diluents D2O. It was observed that there was no interference peaks found at the analyte monitor peak and the maleic acid internal standard peak.



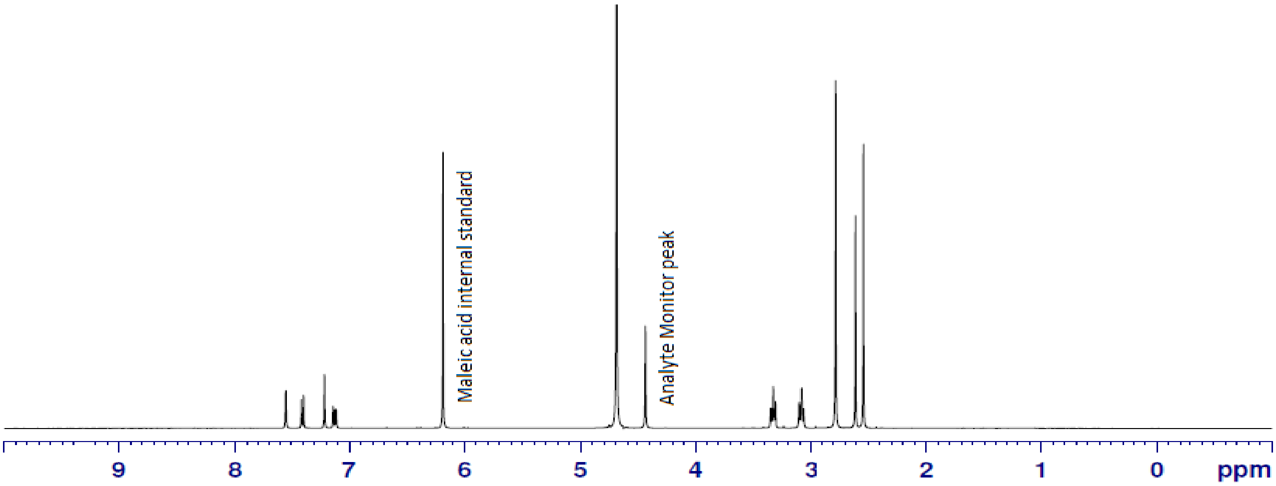


Fig5: 1H-NMR spectra of (a) Diluent D2O (b) Maleic acid internal standard (c)Sumatriptan succinate sample (d) Sumatriptane succinate standard

Precision

Method Precision/reproducibility was performed by six replicate preparations (n=6) of sumatriptane succinate sample were prepared and recorded the ^{1}H-NMR spectra. The acceptance criterion for method precision was the percentage relative standard deviation(%RSD) for assay results of sumatriptan succinate was not more than 2%.It was found to be 0.01%.Intermediate precision/repeatability was performed by different analyst and on different day by preparing six replicate injections(n=6)of sumatriptan succinate sample were prepared and recorded the ^{1}H-NMR spectra. The acceptance criterion for method precision was the percentage relative standard deviation(%RSD) for assay results of sumatriptane succinate was not more than 2% and the percent relative standard deviation for twelve preparations(Method precision and intermediate preparation) was not more than 5%.It was found to be 0.01% and 0.01% respectively,the results were summarized in table5.

Table5 : Summarised results of Precison

Sample name	**Precision**			**Intermediate precision**		
	Taken drug wt (mg)	**Found drug (mg)**	**%Assay (as such)**	**Taken drug wt (mg)**	**Found drug (mg)**	**%Assay (as such)**
Preparation-1	10.44	10.54	99.97	10.18	10.28	99.94
Preparation-2	10.27	10.37	99.95	10.20	10.3	99.93
Preparation-3	10.37	10.47	99.94	10.33	10.43	99.96
Preparation-4	10.25	10.35	99.98	10.24	10.33	99.93
Preparation-5	10.34	10.44	99.97	10.55	10.65	99.93
Preparation-6	10.21	10.31	99.95	10.20	10.29	99.94

Linearity

Linearity was established by preparing seven calibration points from 3mg to 20mg(30% to 200%) of sumatriptan succinate drug weight taken.Linearity calibration curve was obtained from integral peak area againt the amount of drug found(mg) by using least squares method.The regression coefficient,slope and intercept was calculated. The acceptance criterion for linearity was the regression coefficient should not be less than 0.995.The equation for curve was $y = 0.1069x + 0.001$ and the regression coefficient found to 1.000, indicates that good linearity.The results were summarized in the table6.

Table 6 : Summarised results of Linearity

Sample name	Taken drug (mg)	Found drug (mg)	Integral value
Linearity-1	3.29	3.31	0.3549
Linearity-2	5.30	5.34	0.5721
Linearity-3	8.23	8.3	0.888
Linearity-4	10.25	10.34	1.1064
Linearity-5	12.21	12.32	1.3177
Linearity-6	15.45	15.54	1.6622
Linearity-7	20.15	20.26	2.167

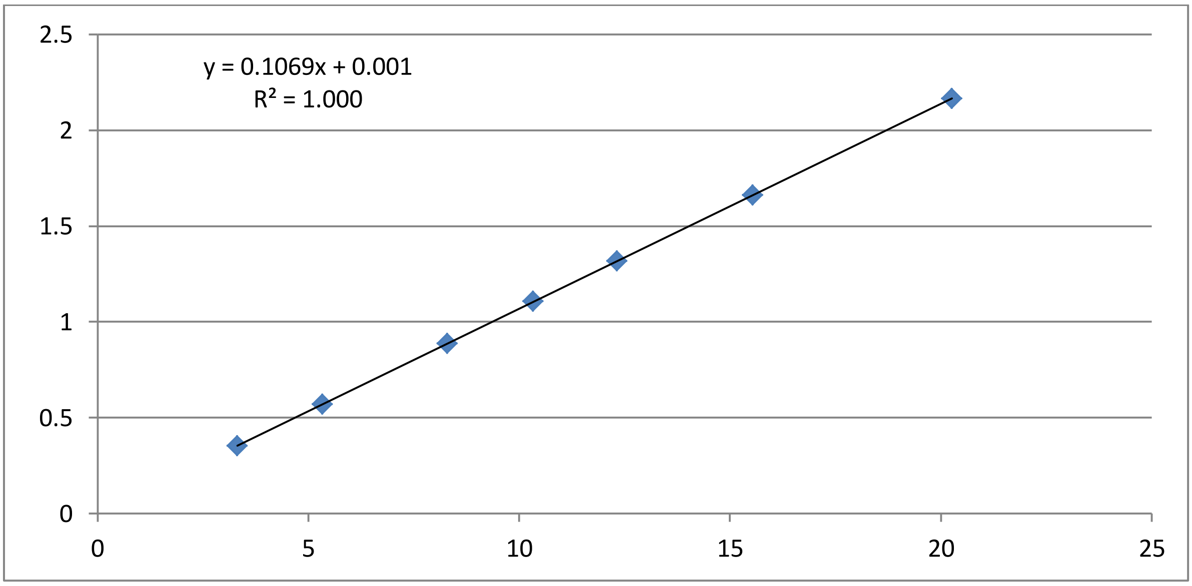


Fig6: Linearity curve of integral value vs found drug amount in mg

Limit Of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection (LOD) and limit of quantitation (LOQ) can be calucalted by the standard deviation of the response and slope S of a calibration curve obtained.The LOD and LOQ were calculated by using below formula

LOD = 3.3 σ / S

LOQ = 10 σ / S

where σ = the standard deviation of the response

S = the slope of the calibration curve

From the linearity calibration data, calculated the standard deviation of the response was found to 0.00021 and the slope was 0.10191.LOD and LOQ were found to 0.007mg/0.6mL and 0.020 mg/0.6mL.

Accuracy

The accuracy/Recovery experiment carried out by preparing in triplicate preparations at 50%,100% and 150% levels with respect to the amount of sample preparation. The acceptance criterion for the percentage recovery at 50%,100% and 150% levels should be with in the range of 90-110%.It was found to 100.13 to 101.15,the results were shown in table 7.

Table 7 : Summarised results of accuracy

Level	Preparation	Taken drug in mg	Found drug in mg	%Recovery
50%	1	5.20	5.26	101.15
	2	5.29	5.34	100.95
	3	5.24	5.29	100.95
100%	1	10.3	10.39	100.87
	2	10.29	10.39	100.97
	3	10.41	10.43	100.19
150%	1	15.2	15.34	100.92
	2	15.35	15.49	100.91
	3	15.10	15.24	100.93

Robustness of the Optimized Method

To perform the robustness of the method, experimental parameters(No.of scans, Acquisition time, relaxation delay and different analyte) were altered. For that No.of scans was altered from 48 to80 i.e 64±16 scans. Acquisition time changed from 3 to 5 i.e 4±1 sec.Relaxation delay changed from 30 to 50sec i.e 40±10 sec and the different analyte peak integral value taken at 7.22 ppm instead of 4.43 ppm.The acceptance criterion for robustness was the absolute difference between the results obtained in Repeatability of Sample preparation-1 and those obtained by carrying out modifications in the method was not more than 10.0%.

Table 8 : Summary of robustness results

Parameter	Changes	Taken drug in mg	Found drug in mg	%Assay	Absolute difference w.r.t unaltered condition
No.of scans	48	10.28	10.38	99.97	-0.01
	64	10.34	10.44	99.96	NA
	80	10.09	10.19	99.94	0.02
Acquisition time	3	10.33	10.43	99.97	-0.01
	4	10.31	10.41	99.96	NA
	5	10.25	10.35	99.97	-0.01
Dealy time(t)	30	10.32	10.42	99.98	-0.02
	40	10.25	10.35	99.96	NA
	50	10.20	10.3	99.97	-0.01
Different analyte peak	7.22ppm	10.16	10.26	99.97	-0.01
	4.43 ppm	10.28	10.44	99.96	-

Solution Stability

The solution stability of sumatriptan succinate in this method was performed on standard and sample preparation at initial,after 6Hrs, 12Hrs, 18Hrs, 24Hrs and 48Hrs intervals,recorded the ^{1}H-NMR spectra and calculated the assay results of the sample and solution preparations, it was found that there was no remarkable change in the assay results shown in table9.

Table 9 : Results of solution stability

For Sample solution					**For Standard solution**			
Time interval	**Taken mg**	**Found mg**	**%Assay**	**%Difference**	**Taken mg**	**Found mg**	**%Assay**	**%Difference**
Initial	10.24	10.34	99.97	-	10.24	10.33	99.92	-
after 6Hrs	10.24	10.33	99.90	0.07	10.1	10.19	99.93	0.01
After 12Hrs	10.24	10.34	99.96	0.01	10.35	10.15	99.92	0.00
After 18Hrs	10.24	10.34	99.96	0.01	10.22	10.32	99.89	0.03
After 24Hrs	10.24	10.33	99.91	0.06	10.4	10.5	99.93	0.01
After 48Hrs	10.24	10.34	99.97	0.00	10.23	10.33	99.93	0.01

Conclusion:

In conclusion, A new, simple and accurate quantitative proton nuclear magnetic resonance(qNMR) spectroscopic method was developed to determine the sumatriptan succinate in pharmaceutical tablet formulationand validated as per ICH guidelines.The proposed method meets all acceptance criterion and can be used to determine the Sumatriptan succinate content in bulk andas well as in tablet dosageform.

Acknowledgements

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