

# Sensitive and rapid determination of Naratriptan, a second generation Antimigraine drug in human plasma by LC-ESI-MS/MS: Application to a bioequivalence study

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## Introduction

Naratriptan is a novel second generation triptan antimigraine drug used to treat moderate to acute migraine cases. It is a selective 5-HT receptor agonist, with high affinity at the 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub> and 5-HT<sub>1F</sub> receptor subtypes [1,2]. The probable sites of therapeutic action of naratriptan include cranial vasculature; the peripheral terminations of trigeminovascular sensory nerves; the first order synapses of the trigeminovascular sensory system; the descending pain control system; and the nuclei of the thalamus [3]. It has very high oral bioavailability (63-74%) and higher lipophilicity compared to other triptan analogs and exhibits a distinct clinical therapeutic profile. The clinically recommended dose of naratriptan is 2.5mg and has a plasma half life of 6h [3-5].

Few methods are presented for the determination of naratriptan in biological matrices. Dulery et al. [6] have developed a liquid chromatographic-electrospray-mass spectrometric assay for the determination of naratriptan, sumatriptan and MDL 74,721 in rabbit plasma (100 or 300µL). The primary objective was to compare their pharmacokinetics after intravenous and oral administration of these three antimigraine compounds in rabbits. Vishwanathan and co-workers [7] have reported a rapid, sensitive and selective method for the determination of antimigraine drugs rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by LC-ESI-MS/MS. The drugs were extracted by solid phase extraction on Oasis HLB cartridges employing 1.0mL serum sample. The curves were linear from 1-100ng/mL and the chromatographic analysis required 5min to separate all four compounds. The limit of detection was 100pg/mL for naratriptan based on the signal to noise ratio of 3. So far there are no reports in the literature for the estimation of naratriptan in human plasma.

In the present study, a simple, sensitive, selective and rapid high performance liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and validated for the quantification of naratriptan, using sumatriptan as internal standard (IS). The method concerned liquid-liquid extraction of naratriptan and IS in methyl-*tert*-butyl ether and dichloromethane mixture from 100µL human plasma. The chromatographic separation was achieved on ACE C-18 (50mm x 2.1mm, 5µm) analytical column under isocratic conditions, using 0.1% acetic acid and acetonitrile (15:85, v/v) at a flow-rate of 0.4mL/min.

The parent → product ion transitions for naratriptan (m/z 336.10→98.06) and IS (m/z 296.09→251.06) were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) and positive ion mode. The linearity of the method for naratriptan was ascertained in the range of 103-20690pg/mL with the analysis time of 1.5min. The method was fully validated as per USFDA guidelines. The developed method was applied to study the bioequivalence of naratriptan in human plasma.

## Method

### Major Equipment Involved :

Equipment	Make	Model
UPLC	Waters	Acquity
LC/MS/MS	Waters	Quattro Premier XE

### Liquid chromatographic conditions :

Column : Ace C18 (50mm x 2.1mm), 5µm particle size  
 Mobile Phase : 0.1% Acetic Acid : Acetonitrile (85:15 v/v)  
 Flow Rate : 0.4mL/minute  
 Injection Vol. : 5µL  
 Run Time : 1.5 minutes

### Mass spectrometric conditions:

#### MRM File Parameters :

	Naratriptan	Sumatriptan
Ion Mode:	Positive	Positive
Parent (Da):	336.10	296.09
Daughter (Da):	98.06	251.06
Dwell (s):	0.200	0.200
Cone (V):	43.00	28.00
Collision (eV):	24.00	19.00

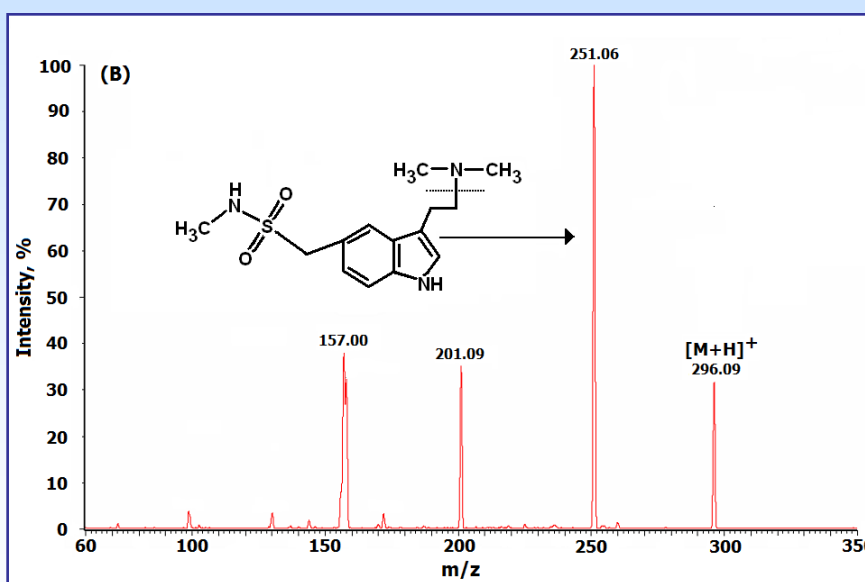
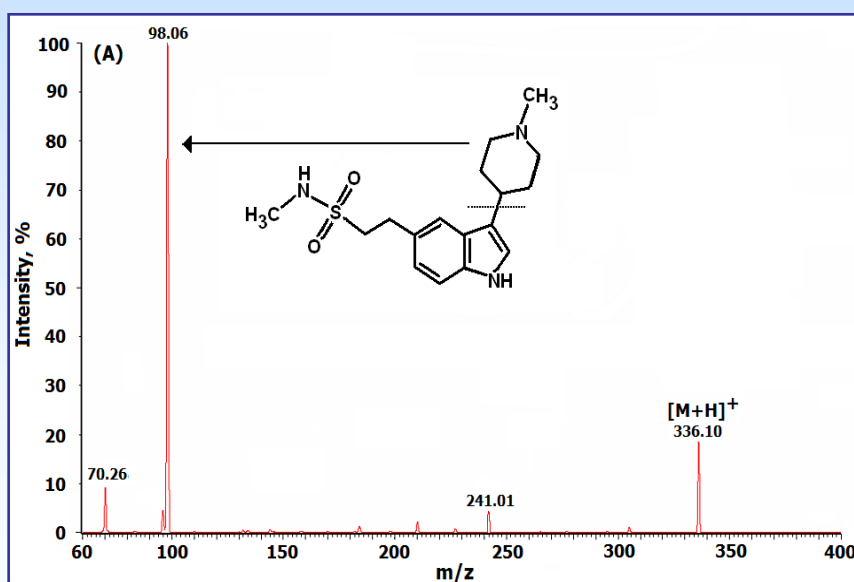


Fig. 1:- Product ion mass spectra of (A) naratriptan (m/z 336.10→98.06, scan range 50-400amu) & (B) sumatriptan, internal standard (m/z 296.09→251.06, scan range 60-350amu) in positive ionization mode.

## Sample Preparation: (LLE)

- 50µL of internal standard was added to 100µL of plasma sample and vortexed for 10s.
- 50µL of 0.1N sodium hydroxide solution was added and vortexed for another 10s.
- Then the samples were extracted with 2.5mL of methyl-*tert*-butyl ether: dichloromethane (80:20, v/v)
- On cyclo-mixer for 10 min at 32 x g and centrifuged at 3204 x g for 5 min at 10°C
- 2.0 mL of the supernatant organic layer was transferred to an evaporation tube and evaporated to dryness in a thermostatically controlled water-bath maintained at 35±5°C under a stream of nitrogen
- After drying, the residue was reconstituted in 100µL of mobile phase and 5µL was injected in the chromatographic system

## Results and Discussion

### Ion Suppression

No ion suppression or enhancement was found at the retention time of analyte and IS in presence of matrix ions through post column infusion of neat solution of analyte and IS.

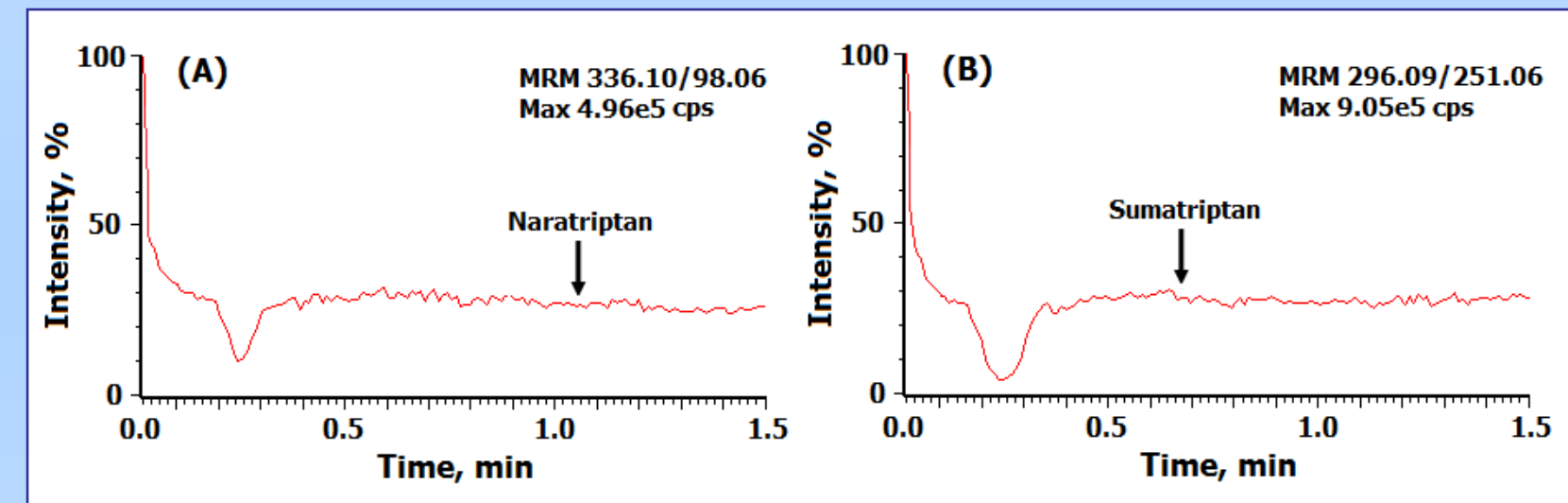


Fig.2:- shows the post column infusion spectra of analyte and ISTD.

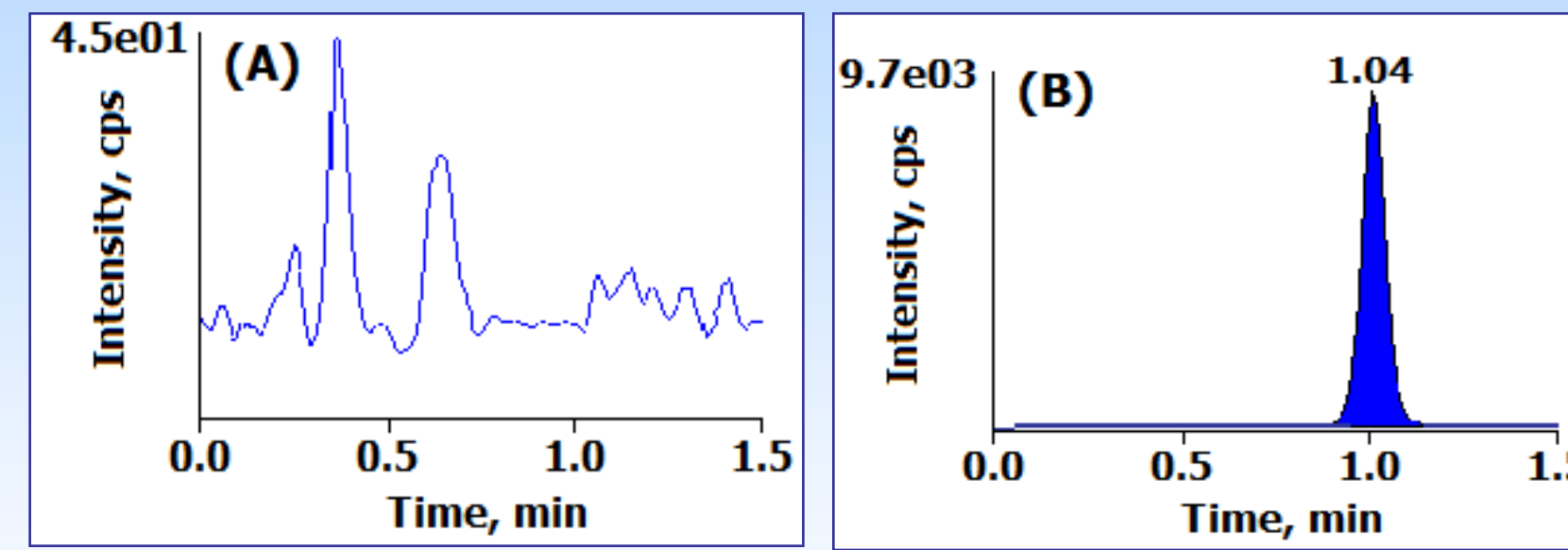


Fig. 3:- Representative chromatograms of double blank (A) and LLOQ (B) in human plasma

### Linearity

The method was validated over the range of 103pg/mL -20690pg/mL with excellent linearity  $r^2 > 0.99$ .

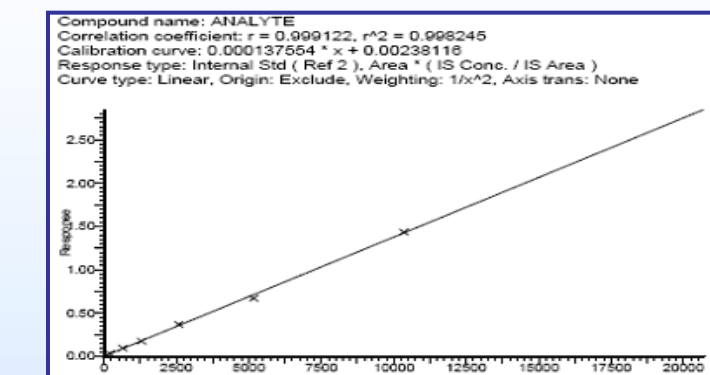


Fig. 4:- Representative linearity curve for Naratriptan

Table 2 - Intra-batch / inter-batch precision & accuracy for naratriptan

>QC ID	>Nominal conc. (pg/mL)		>Mean conc. observed (pg/mL) a		>% CV		>% Accuracy		>Mean conc. observed (pg/mL) b		>% CV		>% Accuracy	
	>n	>5	>n	>5	>n	>5	>n	>5	>n	>5	>n	>5	>n	>5
>HQC	>18636	>5	>17621.7	>5	>0.9	>94.5	>25	>17529.2	>2	>94				
>MQC	>1789.3	>5	>1776.3	>5	>1.8	>99.3	>25	>1750.4	>3.9	>97.8				
>LQC	>304.2	>5	>304.7	>4	>100.2	>25	>289.9	>4.8	>95.3					
>LLOQ	>105.5	>5	>112	>4	>105.2	>25	>110.8	>6.1	>104.2					

CV, coefficient of variance; n, total number of observations.  
 a Mean of 5 replicates at each concentration.  
 b Mean of 5 replicates for five precision and accuracy batches

### Stability, Selectivity & Recovery Parameters

- Bench Top Stability > 6 h
- Wet Extract Stability > 22h
- Dry Extract Stability at -20 C > 24h
- Freeze Thaw Stability > 3 Cycles(-20°C & -70°C)
- Long Term Stability in Human Plasma for 80 Days
- Specificity > No significant interference
- Absolute (ME) > 98 %
- Relative recovery > 97 %
- Process Efficiency > 96 %
- Ruggedness > Analyst, Column & Instrument

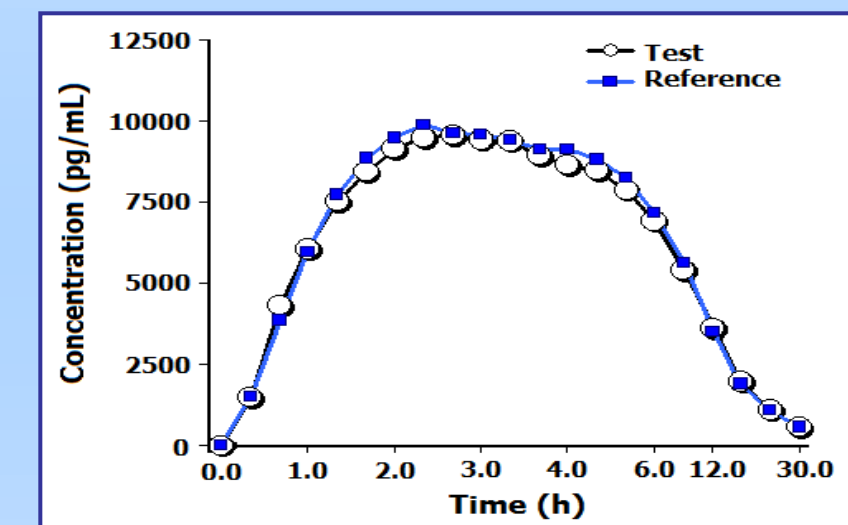


Fig. 5:- shows the plasma concentration Vs time profile of naratriptan in human subjects.

### Application of Method for Bioequivalence Study

The validated method was successfully used to quantify the naratriptan concentration in the human plasma samples after the administration of a single 2.5mg oral dose of naratriptan to 31 healthy volunteers.

## Acknowledgment

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## Conclusion

- To summarize, the LC-ESI-MS/MS method for the quantitation of naratriptan in human plasma was developed and fully validated as per USFDA guidelines with acceptable precision, accuracy and adequate sensitivity for the quantification of naratriptan in human plasma in a clinical study.
- The method offers significant advantages over those previously reported, in terms of lower sample requirements, simplicity of extraction procedure, sensitivity and overall analysis time. The on-column loading of sample at LLOQ level (0.52pg/injection) was much lower compared to other reported procedures
- The efficiency of liquid-liquid extraction and a chromatographic run time of 1.5 min per sample make it an attractive procedure in high-throughput bioanalysis of naratriptan. More than 1500 plasma samples were analyzed during a period of 5 days (more than 300 samples/day) with acceptable precision and accuracy.

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