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Development and validation of stability indicating assay method for naratriptan by ultra performance liquid chromatography

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ABSTRACT

A novel stability-indicating ultra-performance liquid chromatographic assay method was developed and validated for Naratriptan and its degradant products. An isocratic UPLC method was developed to separate the drug from the degradation products, using an Acquity UPLC BEH C18 (50mm x 2.1 mm). Mixture of water : acetonitrile (pH3.4) (60:40) was used as mobile phase. The flow rate was kept 0.3 mL min-1 and the detection was carried out at 224 nm. The linearity of the proposed method was investigated in the range of 10-50µg mL-1 (r =0.9996) for Naratriptan. The limit of detection was 0.5µg mL-1 and limit of quatitation was 1µg mL-1. The percentage recovery of Naratriptan was ranged from 97.2 to 99.5. The %R.S.D. values for intra-day precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise. The validation studies were carried out fulfilling International Conference on Harmonisation (ICH) requirements. The procedure was found to be specific, linear, precise (including intra and inter day precision), accurate and robust.

Keywords: Degradation; Stress testing; Stability Indicating Assay; Validation.

INTRODUCTION

The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods [1, 2]. It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, and light, oxidizing agents as well as susceptibility over a wide range of pH values [3-

7]. Naratriptan is selective 5- hydroxytryptamine receptor sub type agonist formulated and its salt form is currently prescribed for the treatment of migrate.[8-10]

The objective of the present study was to study degradation of Naratriptan under different ICH recommended stress conditions, and to establish a validated stability indicating UPLC method [11-13]. There is no report yet on the development of stability-indicating assay method for the drug.

MATERIALS AND METHODS

2.1 Materials

Naratriptan was received from Working standard, Glaxo smithline Ltd. Bombay, Sodium hydroxide and hydrogen peroxide were purchased from CDH chemicals Ltd. Hydrochloric acid and acetonitrile were procured from Merck India ltd. All other chemicals were also of analytical grade.

2.2 Instrumentation

Thermal stability study was performed in a hot air oven (Oven universal with thermotech thermostat TIC-4000N, S.M. Industries, New Delhi, India). pH of the mobile phase was checked on microprocessor waterproof pH tester (pH tester 20, eutech instruments, oakton, USA). Lux meter (Lutron LX-102 digital light meter, Marcucci S.P.A, vignate, Milan). The chromatographic separation was carried out on Water Acquity UPLCTM BEH C18 Column (1.7µm, 50mm x 2.1mm) with EMPOWER software.

2.3 Degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on Naratriptan powder. The analysis was carried out by UPLC with a PDA detector. 2µl of each of forced degradation samples were injected at regular intervals.

2.3.1. Acid degradation:

50 mg of naratriptan sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M hydrochloric acid solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn at appropriate time interval and subjected to UPLC analysis after suitable dilution.

2.3.2. Base degradation:

50 mg of naratriptan sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M sodium hydroxide solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn at appropriate time interval and subjected to UPLC analysis after suitable dilution.

2.3.3. Hydrolytic degradation:

50 mg of naratriptan sample was taken into a 50 ml round bottom flask, 50 ml of Milli Q water was added and the contents were mixed well and kept for constant stirring for 48 h at 80ºC. Samples were withdrawn at appropriate time intervals and subjected to UPLC analysis after suitable dilution.

2.3.4. Oxidative degradation:
50 mg of naratriptan sample was taken into a 50 ml round bottom flask, 50 ml of 30% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 h at room temperature. Samples were withdrawn at appropriate time intervals and subjected to UPLC analysis after suitable dilution.

2.3.5. Thermal degradation:
50 mg of naratriptan sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks.

2.3.6. **Photolytic degradation:**
The naratriptan was exposed to sunlight during the daytime (70,000–80,000 lux) for 2 days.

2.4 Separation studies
UPLC studies were carried out first on all reaction solutions individually, and then on a mixture of those solutions in which decomposition was observed. Separations were achieved by isocratic elution using acetonitrile -buffer (a pH 3.4) as the mobile phase. The mobile phase was filtered through 0.22µm nylon membrane and degassed before use. The injection volume was 2 µL and the mobile phase flow rate was kept constant at 0.6 mL min-1. The analysis was carried out at 224 nm.

2.5 Validation of the method
2.5.1 Linearity and range
A stock solution of the drug was prepared at strength of 1mg mL-1. It was diluted to prepare solutions containing 10-50µg mL-1 of the drug. The solutions were injected six times into the UPLC column, keeping the injection volume constant (2µL).

2.5.2 Precision
Six injections, of five different concentrations (10-50µg mL-1), were given on the same day and the values of relative standard deviation (%R.S.D.) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

2.5.3 Accuracy
Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 1,6 excellent recoveries were made at each added concentration, despite the fact that the drug was fortified to a mixture that contained drug as well as the degradation products, formed under various reaction conditions.

2.5.4 Specificity and selectivity
The specificity of the method was established through study of USP resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks.

2.5.5 Limit of detection and Limit of quantitation
The LOD was determined by using the signal to noise ratio method [14, 15]. The concentration that resulted in a signal to noise of 3:1 was found to be 0.5µg mL-1 and was determined to be the LOD. The signal to noise ratio of 10:1 was used to determine the LOQ and was the concentration

that elicited a response that could be accurately and reliably measure. This concentration was found to be 1µg mL-1.

Table 1. Recovery study

Spiked concentration (µgmL-1)	Measured concentration R.S.D. (%)	Recovery(%)
10	9.92 ± 0.07234, 0.729	99.2
20	19.43± 0.0665, 0.342	97.2
30	29.58± 0.2389, 0.8	98.6
40	39.8 ± 0.2055, 0.51	99.5
50	49.01± 0.6325,1.29	98.0

S.D. = Standard deviation.
R.S.D. =Relative standard deviation.

Table 2 Reproducibility and precision data evaluated through intra-day and inter-day Studies

Actual concentration(µgmL-1)	Intra-day measured concentration ± S.D.,R.S.D. (%)	Inter-day measured concentration ± S.D.,R.S.D. (%)
10	9.85 ± 0.0153, 0.155	9.79 ± 0.0709, 0.72
20	19 . 6 8 ± 0.1183, 0.601	19.75 ± 0.1724, 0.872
30	29.91 ± 0.2874, 0.96	29.81 ± 0.289, 0.96
40	39.98 ± 0.3654, 0.91	38.96 ± 0.257, 0.65
50	49.32 ± 0.2425, 0.491	50.43 ± 0.6589, 1.30

S.D. = Standard deviation.
R.S.D. =Relative standard deviation

Table 3 Purity Data

S.NO.	Peak	Purity angle	Purity threshold	USP resolution
1	naratriptan	9.32	41.2	1.89
2	Degradant-1	2.14	20.58	3.6
3	naratriptan	0.34	3.67	3.6
4	Degradant-2	38.81	90	7.4
5	Degradant-3	6.56	32.29	4.65

RESULTS AND DISCUSSION

3.1 Degradation behavior

UPLC studies on naratriptan under different stress conditions suggested the following degradation behaviours:

3.1.1. Acidic condition

The drug gradually decreased with time on heating at 80 ºC in 0.1M HCl, forming degradation products at RT 0.42 min, 1.12 min, 1.51min. The drug naratriptan was found to be labile to acid hydrolysis.

3.1.2. Alkaline Hydrolysis:

The drug naratriptan was found to be labile to alkaline hydrolysis. Drug was degraded at 8 hrs in 0.1 M NaOH, one degradant was obtained at 0.44 min and drug peak was obtained at 0.631min.

3.1.3. Neutral hydrolysis:
The drug naratriptan found to be very stable to neutral hydrolysis. After refluxing the drug for 2 days, one degradant was obtained at 0.428 min and drug peak was obtained at 0.623 min.

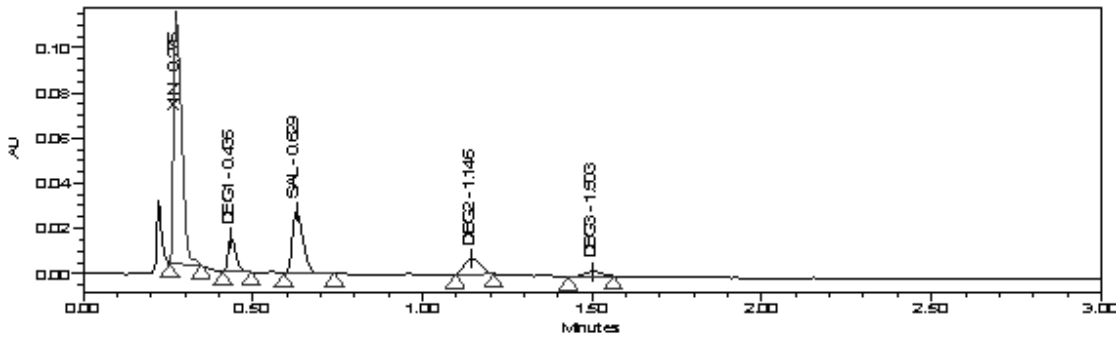
3.1.4. Oxidative degradation:
The drug showed no degradation in extreme condition as per protocol i.e. in 30% $H2O_2$ for 24hrs.

3.1.5. Solid-state study
naratriptan showed no degradation after exposure it to dry heat at 50 ºC in hot air oven for 4weeks.

3.1.6. Photolytic degradation:
naratriptan remain stable after exposure to sunlight at 70,000 to 80,000 lux for 2 days.

3.2. Development and optimization of the stability-indicating method
The method was optimized to separate major degradation products formed under various conditions. Resolution was also checked on mixture of the degradation solutions to confirm the separation behavior. The resulting chromatogram is shown in Fig. 1 It indicates that the isocratic method was successful in separation of drug and all degradation products.

3.3. Validation of developed stability-indicating method
The response for the drug was strictly linear in the concentration range between 10 and 50µg mL-1. The mean (± %R.S.D.) values of slope, intercept and correlation coefficient were 1808.5, 626.26 and 0.9996 respectively.



The data obtained from precision experiments are given in Table 1 for intra and inter day precision studies. The %R.S.D. values for intra-day precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise. Excellent recoveries were made at each added concentration. Fig. 2 shows that the method was sufficiently specific to the naratraption. The USP resolution factor for the drug peak was >3 from the nearest resolving peak. Good separations were always achieved, indicating that the method remained selective for all components under the tested conditions.

CONCLUSION

The study shows that naratriptan is a labile molecule in acid, alkali and water. It is stable to oxidation, dry heat and photolytic condition. A stability-indicating method was developed, which separates all the degradation products formed under variety of conditions. The method proved to be simple, accurate, precise, specific and selective. Hence it is recommended for analysis of the drug and degradation products in stability samples by the industry.

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