ISSN : 0974-7419 Volume 12 Issue 4

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(4) 2013 [127-132]

A validated stability – Indicating method for the determination of sumatriptan and kinetic study of the degradation

Hayam M.Lotfy[1], Mamdouh R.Rezk[1], Adel M.Michael[2]*, Ayman O.S.El-Kadi[3], Mostafa A.Shehata[1]

[1]Faculty of Pharmacy, Cairo University, (EGYPT)

[2]Faculty of Pharmacy, Ahram Canadian University, (EGYPT)

[3]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, (CANADA)

E-mail: adel_magdy_m@yahoo.com

Received: 27th July, 2012 ; Accepted: 22nd September, 2012

ABSTRACT

A simple, specific, accurate and stability-indicating liquid chromatographic method has been developed for determination of sumatriptan in the presence of its alkaline degradation product and in pharmaceutical formulation. The analysis was carried out on Grace C18 (2.1 x 250 mm with 5 µm particle size) column with a mobile phase consisting of water (contains 0.1 % triethylamine, adjust pH to 6.5 by phosphoric acid): acetonitrile in the ratio (6 : 4, v/v). The detection was accomplished ûuorometrically setting the excitation wavelength at 225 nm and emission wavelength at 350 nm. The retention time was 4.1 min. and 5.2 min. for sumatriptan and its alkaline degradation, respectively, at flow rate 0.2 mL min^{-1}. The developed and validated method was successfully applied to the analysis of pharmaceutical formulation. The calibration curve was linear over the range 50-800 ng mL^{-1}. The LOD and LOQ values were found to be 16.6 and 50 ng mL^{-1}, respectively. Statistical analysis of the results has been carried out revealing high accuracy and good precision. A kinetic study of the degradation reaction was done and proved to follow pseudo-first order kinetics.

KEYWORDS

Sumatriptan;
Stability indicating;
HPLC;
Kinetic study.

INTRODUCTION

Sumatriptan (SUM) is 3-[2-(dimethylamino)ethyl]-N-methyl-indole-5-methanesulfonamide.It'sa serotonin agonist acting at the receptors 5-HT1D and 5-HT1B[1,2]. SUM reduces the vascular inflammation associated with migraine. It decreases the activity of the trigeminal nerve, which, it is presumed, accounts for sumatriptan efficacy in treating cluster headaches[3,4].

The literature review revealed several analytical methods for quantitative estimation of SUM in body fluids and in pharmaceutical formulations. These methods include spectrophotometry[5-8], liquid chromatography[9-12] and capillary electrophoresis[13]. No stability indicating method showed high sensitivity like the developed work in addition to the kinetic study of the degradation.

In modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis. The present work aimed to develop an instrumental method for the quantification of SUM in the presence of its alkaline degradation product which was found to

be simple and rapid (both compounds can be analyzed in less than 6 minutes).

EXPERIMENTAL

Instruments

The HPLC chromatograph consisted of a Waters model 600 (Waters Corp., Canada) and a Kontron model 360 autosampler (Kontron Instruments SPA, Canada) connected in series with a Waters model 470 scanning fluorescence detector.

Materials and reagents

- Pure SUM standard (sumatriptan succinate powder) was kindly supplied by Sigma pharmaceuticals – Egypt. Its potency was found to be 992 μg mg^{-1}.
- Sumagraine® tablets (Sigma pharmaceuticals - Egypt) were purchased from the Egyptian local market. Each tablet is claimed to contain 25 mg sumatriptan succinate.
- Acetonitrile, triethylamine, phosphoric acid and dichloromethane: Caledon, Canada, HPLC grade.
- Sodium hydroxide and 2-Naphthylamine (NA): Sigma Aldrish, Canada.

Standard solutions

Stock solution of sumatriptan succinate

Stock solution of sumatriptan succinate was prepared by dissolving 50 mg of sumatriptan suucinate powder in 100 mL distilled water (500 μg mL^{-1}). Working solutions (5, 50 μg mL^{-1}) were prepared by serial dilution.

Stock solution of 2-naphthylamine

Stock solution of 2-naphthylamine was freshly prepared as 10 μg mL^{-1} solution in methanol and used as internal standard (IS).

Stock solution of the alkaline degradation product

Stock solution of the alkaline degradation product was prepared by heating 50 mg of SUM with 25 mL 2 M NaOH for 4 hours at 85° C then neutralized by 2 M HCl and the volume was completed to 100 mL by distilled water (500 μg mL^{-1}) . Working solutions (5, 50 μg mL^{-1}) were prepared by serial dilution.

Procedures

Degradation of sumatriptan

Accelerated alkaline- degradation was performed as mentioned and the degradation product solution was exposed to evaporation till dryness and the residue was dissolved in dichloromethane for IR analysis for subsequent identification.

Linearity

Aliquot portions of sumatriptan working solutions were accurately transferred into a series of 10-mL volumetric flasks to provide final concentration of 50-800 ng mL^{-1}. The internal standard solution (2-naphthylamine) was added to each flask to provide final concentration of 200 ng mL^{-1} and the volume was completed with the mobile phase. Triplicate 100 μL solutions for each concentration were injected using the following chromatographic conditions: stationary phase; Grace C18 (2.1 x 250 mm with 5 μm particle size) column and the mobile phase composition was water (containing 0.1 % triethylamine, adjust pH to 6.5 by phosphoric acid): acetonitrile = 6:4, v/v. The flow rate was 0.2 ml min^{-1}. The mobile phase was sonicated and filtered through a 0.45 μm millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use. The detection was done at excitation wavelength 225 nm and measuring the emission at 350 nm. The calibration curve was constructed by plotting the relative peak area (peak area of SUM to that of the internal standard [200 ng mL^{-1}]) using versus the corresponding concentration of SUM (ng mL^{-1}) and the regression equation was computed.

Analysis of laboratory prepared mixtures containing different ratios of SUM and its degradation product (specificity)

Mix aliquots of intact drug and the degraded drug to prepare different mixtures containing 7–93 % (w/w) of the degradation product, and proceed similarly as under linearity. The concentrations of SUM were calculated from the corresponding regression equation.

Assay of the pharmaceutical formulation

Ten sumagraine® tablets were weighed and finely powdered to determine the average tablet weight. A portion of the powder equivalent to one tablet (containing 25 mg sumatriptan succinate) was dissolved in 80 mL of water in 100-mL volumetric flask. The solution was stirred with magnetic stirrer for 10 minutes, filtered through filter paper and the volume was then

completed to 100 mL with water. Working solutions were prepared by serial dilutions then the procedures were followed as under linearity. The concentrations of SUM were calculated from the corresponding regression equation.

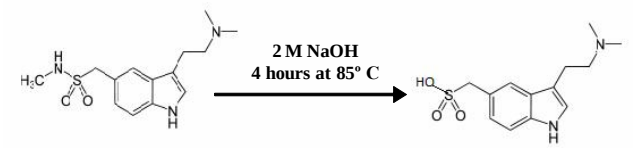
Kinetic study of the degradation

In 8 cell culture tubes, 1mL of the stock solution of SUM (500 μg mL^{-1}) was added and heated with 2 mL of 2 M NaOH for 4 hours at 85º C. Every half hour one tube was removed from the water bath, cooled and transferred quantitatively into 10-mL volumetric flask. The solution was neutralized with 2 M HCl and volume was completed to 10 mL with water then working solution (5 μg mL^{-1}) was prepared. Further dilution from the working solution was taken to provide final concentration of 600 ng mL^{-1} and NA was added to provide final concentration of 200 ng mL^{-1}. The volume was completed with the mobile phase and injection of the solution under the same chromatographic conditions described under linearity. The remaining concentration of SUM was calculated from the corresponding regression equation.

RESULTS AND DISCUSSION

In this work, systematic studies focused on sumatriptan degradation have been performed using different concentration of sodium hydroxide at different temperature for different time interval. It was found that heating with 2 M NaOH in water bath at 85º C for 4 hours was sufficient for complete degradation.

The suggested degradation pathway is shown in the following scheme:



Sumatriptan $C_{14}H_{21}N_3O_2S$ Alkaline degradation product of sumatriptan
Mol. mass 295.402 g/mol

Scheme 1 : The suggested degradation pathway of SUM.

Only one degradation product was observed under the conditions used in the preparation of the degradation product as only one peak appeared in the HPLC chromatogram.

IR spectra for both the intact and the degradation product showed that the function groups in both are typical as the organic chemistry literature[14]. The structure elucidation showed the disappearance of the NH_2 peak of the sulphonamide group of the intact drug at 3379 cm^{-1} and the appearance of the peak OH of the sulphonic acid at 3445 cm^{-1}.

The International Conference of Harmonization (ICH) guideline entitled "stability testing of new drugs substances and products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance[15]. An ideal stability indicating method is the one that quantifies the standard drug alone and also resolves its degradation products.

Method development

Different types of stationary phase C_8 and C_{18} columns with different dimensions and particle sizes were tested to find the best stationary-mobile phases match. It was found that Grace C18 (2.1 x 250 mm) column gave the most suitable resolution for the separation of the drug from its degradation product.

The chromatographic conditions were adjusted in order to provide a good performance of the assay. Various mobile phase systems were tested for optimizing the HPLC-separation. The mobile phase composition was water (containing 0.1 % triethylamine, adjust pH to 6.5 by phosphoric acid): ACN = 6 : 4, v/v. The flow rate of 0.2 ml min^{-1} was found to be quite satisfactory for the good resolution and determination of the studied drug substance in presence of its degradation product. System suitability parameters were tested and displayed in TABLE 1.

The chromatographic system described in this work allows complete base line separation of SUM from its degradation product (Figure 2). Peak purity was confirmed for the HPLC peaks of both intact SUM and its degradation product by a pilot run using a photodiode array detector. Spiking of both intact drug and its degradation product assured the presence of only one degradation product during preparation of the degradation product. Also by changing the mobile phase ratios, just one peak appeared corresponding to the drug at about 4.1 min and another one for the degradation product at 5.2 min. with the peak of IS at about 10.9 min.

Kinetic study for the degradation showed that

Full Paper

TABLE 1 : System suitability data for the developed HPLC method

Parameter	Sumatriptan	alkaline degradation product	Reference value
Capacity factor	2.39	3.33	1- 10 accebtable
T (Tailing factor)	0.98	0.93	T = 1 for a typical symmetric peak
Resolution factor	1.83	6.58	0.8
N	736	903	Increases with the efficiency of separation
HETP	0.034	0.027	The smaller the value, the higher column efficiency

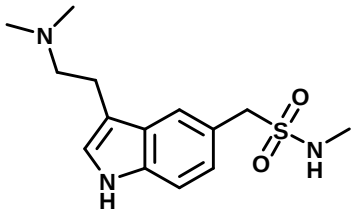


Figure 1 : Structural formula of sumatriptan.

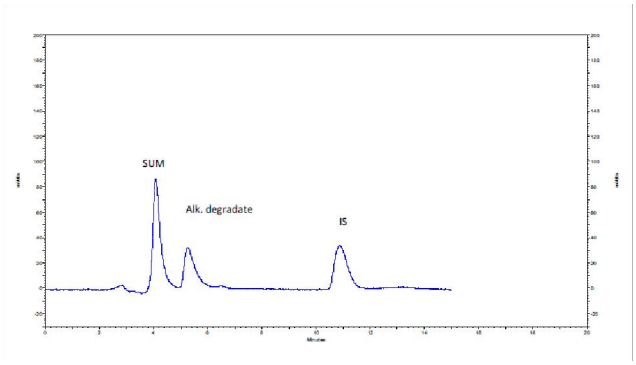


Figure 2 : HPLC chromatogram for the developed method under the specified conditions showing sumatriptan (400 ng mL^{-1}), alkaline degradation (400 ng mL^{-1}) and IS (Rt: 4.07, 5.26 and 10.86 min. respectively).

it follows pseudo-first order kinetics as shown in Figure 3.

Method validation

Linearity and calibration curve

Under the specified optimum conditions, calibration curve was constructed by plotting the relative peak area versus the concentrations of SUM in ng mL^{-1} as shown in Figure 4.

Accuracy and precision

The methods were tested for accuracy and precision for SUM in bulk form and in the dosage form by the analysis of six replicates of three different concentrations. Validation results are represented in TABLE 2. Statistical comparison showed no significant difference between the developed methods and the official method[9] as shown in TABLE 3 which revealed that

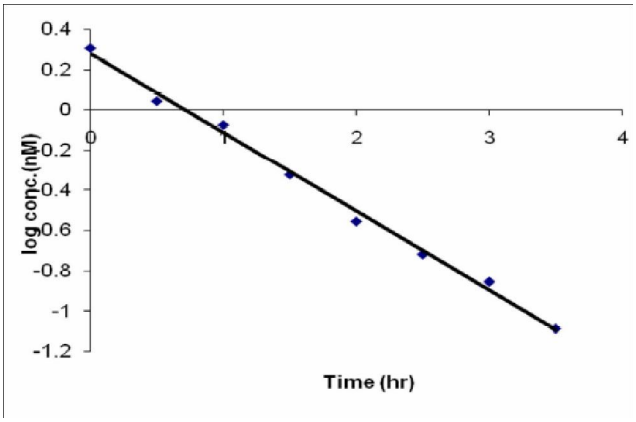


Figure 3 : Kinetic study of the degradation showing pseudo-first order kinetics.

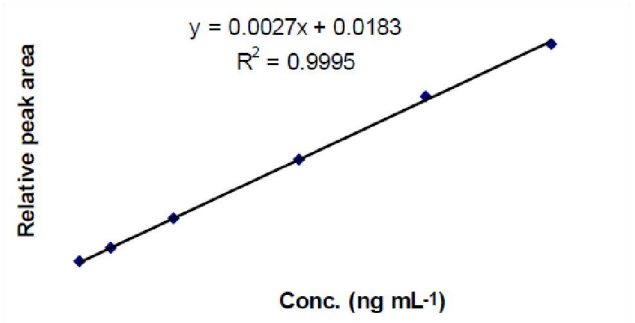


Figure 4 : Linearity of sumatriptan conc. (ng mL^{-1}) and relative peak area in the proposed method.

the calculated t-test and F-value are less than the tabulated ones.

The robustness of the HPLC method was examined by the analysis of samples under a variety of experimental conditions such as small changes in the pH (± 0.2 units), small changes in proportions of mobile phase, by up to ± 0.5 % mainly of the organic part of the mobile phase. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.

TABLE 2 : Validation sheet for the developed HPLC method

Parameters	The developed HPLC method
Linearity range (ng mL^{-1})	50-800
Regression equation	
Slope	0.0027
Intercept	0.0183
Correlation coefficient	0.9995
Accuracy	
Mean ± SD	100.07 ± 1.25
Precision	
Intra-day (Mean ± RSD)	99.80 ± 0.723 %
Inter-day (Mean ± RSD)	100.20 ± 0.844 %

TABLE 3 : Statistical comparison between the results obtained by the proposed method and the official method.

Parameter	The developed method	The official method[9]
Mean	100.07	99.30
SD	1.25	1.10
RSD	1.249 %	1.107 %
Variance	1.56	1.21
N	6	6
Student's t-test	0.31 (2.26)[a]	
F-value	1.29 (5.19)[a]	

[a] **The values in the parenthesis are the corresponding thoretical t- and F-values at P = 0.05.**

Specificity (Analysis of laboratory prepared mixtures of sumatriptan and its alkaline degradation product)

Each laboratory prepared mixture was analyzed as described under linearity then the concentrations of the intact SUM in the prepared mixtures were determined from its corresponding regression equation as shown in TABLE 4.

TABLE 4 : Determination of sumatriptan in laboratory prepared mixtures.

Intact (ng mL^{-1})	Added degradation (ng mL^{-1})	Recovery % of the intact
50	750	103.44
200	600	101.39
400	400	100.91
600	200	102.25
750	50	100.00
	Mean	101.59
	SD	1.31

Analysis of pharmaceutical dosage form

The suggested methods are valid and applicable for the analysis of SUM in sumagraine® tablets. The results in TABLE 5 showed that no interference from the additives in the tablets.

TABLE 5 : Determination of sumatriptan in pharmaceutical dosage form by the proposed HPLC method.

Taken (ng mL^{-1})	Found	Recovery %
	296.79	98.93
300.00	298.94	99.65
	298.94	99.65
Mean		99.41
S.D		0.41

CONCLUSION

The suggested method is found to be accurate and selective with no significant difference of the precision compared with the official HPLC method of analysis[9] with the advantage of being more sensitive. Application of the proposed method to the analysis of sumatriptan in its pharmaceutical formulation shows that neither the excipient nor the degradation product interferes with the determination. The run time is relatively short, which enable rapid determination of many samples in routine and quality control analysis of tablet formulation. The high sensitivity of the method may permit its application for the determination of sumatriptan in plasma with high accuracy.

REFERENCES

[1] J.G.Hardman, L.E.Limbird; Goodman and Gilman's, The Pharmacological basis of therapeutics, 10th Edition, McGraw-Hill, New York, **(2001)**.

[2] P.N.Bennet, M.J.Brown; Clinical pharmacology, 9th Edition, Longman, **(2003)**.

[3] A.C.Moffat, M.D.Osseltons, B.Widdop; Clarke's Analysis of drugs, 3rd Edition, The Pharmaceutical Press, London, U.K., **(2004)**.

[4] Budavari; The Merck Index, 14th Edition, Whitehouse Station, USA, **(2007)**.

[5] K.V.Satyanarayana, P.N.Rao; E.-J.Chem., **8**, 269-275 **(2011)**.

[6] K.Raghubabu, B.K.Ramu; Int.J.Pharm., Biomed. Sci., **1**, 49-52 **(2010)**.

Full Paper

[7] R.P.Gondalia, A.P.Dharamsi; Int.J.Pharm., Biomed Sci. **1**, 24-26 **(2010)**.
[8] M.Trinath, K.Banerjee, C.G.Bonde; Der Pharmacia Sinica **1**, 36-41 **(2010)**.
[9] The United States Pharmacopeia, 30th Edition, 184 **(2007)**.
[10] M.Dunne, P.Andrew; J.Pharm.Biomed.Anal., **6**, 721-726 **(1996)**.
[11] C.R.Shah, B.N.Suhagia, N.J.Shah, R.R.Shah; Indian J.Pharm.Sci., **70**, 831-834 **(2008)**.
[12] L.I.Bebawy, A.A.Moustafa, N.F.Abo-Talib; J.Pharm.Biomed.Anal. **32**, 1123-33 **(2003)**.
[13] K.D.Altria, S.D.Filbey; Anal.Proc., **30**, 363-365 **(1993)**.
[14] L.G.Wade; 'Organic chemistry', 5th Edition, Pearson Education, Inc. New Jersy, 948 **(2003)**.
[15] ICH, Stability testing of new drug substances and products international conference on harmonization, Geneva, **(1993)**.