Journal of Current Chemical and Pharmaceutical Sciences

Research | Vol 12 Iss 8

Novel Fluorimetric Procedure for Estimation of Sumatriptan in its Tablet Formulation and in Human Plasma; Implementation to Content Symmetry Testing

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Received: 30-December-2020, Manuscript No. JCCPS-22-001-Pre QC 22; **Editor assigned:** 04-August-2022, PreQC No. JCCPS-22-001-Pre QC 22 (PQ); **Reviewed:** 18-August-2022, QC No. JCCPS-22-001-Pre QC 22; **Revised:** 25-August-2022, Manuscript No. JCCPS-22-001-Pre QC 22 (R); **Published:** 29-August-2022; **DOI:** 10.4172/JCCPS 2277-2871.001.

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Abstract

A rapid, simple and specific fluorimetric procedure is described for estimation of Sumatriptan succinate (STS) in its pharmaceutical preparation and in human plasma. The suggested procedure is depended on the measurement of the fluorescence intensity (FI) of the studied drug in sulphuric acid / methanol at 350 nm (λ_{ex} 286 nm). Straight correlation was observed between the fluorescence intensity and concentrations studied over the concentration range 0.1 -2.0 µg/ml with correlation coefficient of 0.9990. The detection and quantitation limits were 0.019 and 0.057 µg/ml, respectively. The suggested procedure was validated according to ICH and USP guidelines. It was applied successfully implemented to the estimation of STS in its tablet formulation and content uniformity testing. The suggested method was sensitive enough to allow the estimation of STS in human plasma.

Keywords: *Sumatriptan; fluorescence behavior; Dosage form; content symmetry testing; Spiked human Plasma*

Introduction

Migraine is a disease characterized by frequent attacks of headache that may continue for 72 hours. Serotonin (5-HT1) agonists are the drugs of choice for treatment of migraine attack. STS (Figure 1) is a selective serotonin (5-HT1) agonist and commonly utilized for the remediation of severe headache period of migraine symptoms [1]. Different procedures were reported for the estimation of STS in different matrices such as spectrofluorimetry [2], spectrophotometry [3-8], HPLC [9,10]

and thin-layer chromatography. The current work pursued to promote and validate a straightforward, fast, sensitive, and specific spectrofluorimetric method for the estimation of STS, using its inherent fluorescence of its methanolic solution at acidic pH. The described method is specific, simple and rapid compared to the reported spectrofluorimetric method. Up to date, solely one spectrofluorimetric procedure was reported regarding spectrofluorimetric estimation of STS. The reported method lacks simplicity and specificity which are the main merits of our proposed method. Therefore, up to date, the suggested spectrofluorimetric procedure is considered more suitable for estimation of the STS for its simplicity, time saving, and specificity. The described method was entirely validated according to ICH guidelines and perfectly implemented for assay of the cited drug in its tablets formulation. Owing to its good sensitivity, the suggested procedure was utilized to estimate STS in human plasma using a suitable extraction method. Additionally, it was used for content symmetry test of the cited drug in its tablet preparation according to USP guidelines.

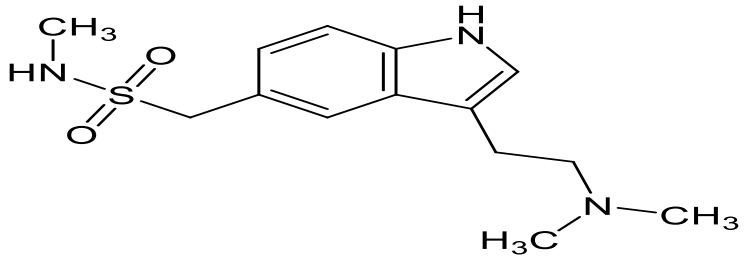

FIG. 1. **Chemical structure of Sumatriptan.**

Experimental

Apparatus

Spectroflurometer FS-2 (Scinco, Korea) linked to Dell PC, with 1 cm quartz cell, Xenon arc lamp, grating excitation and emission monochromators with slit widths set at 5 nm and PMT voltage 400 V were used for all fluorescence measurents. A UV-1601 PC UV–visible spectrophotometer (Shimadzu, Tokyo, Japan) with 1 cm quartz cell, laboratory centrifuge (Bremsen ECCO, Germany) and Jenwey pH meter model 350 (E.U) were also used in the study.

Chemicals and reagents

Sumatriptan succinate (STS, 99.1%) was generously offered by sigma for pharmaceutical industries company, Cairo, Egypt. IMIGRAN® tablets (GlaxoSmithKline NZ Ltd. for pharmaceutical industries company, Cairo, Egypt) claimed to include 50 mg STS per tablet. Sodium dodecyl sulfate (SDS), Tween-80 and Carboxymethylcellulose (CMC) were purchased from El Nasr chemical Co. (Abu Zaabal, Cairo, Egypt). 0.5%w/v aqueous solution of either SDS or CMC and 2.0% v/v aqueous solution of Tween-80 were prepared. Cetyl trimethyl ammonium bromide, 0.1% Aqueous solution, (CTAB, Danochemo a subsidiary of Ferrosan, manufacturing chemists, Copenhagen, Denmark). Methanol, ethanol, butanol, isopropanol, sulphuric acid, nitric acid, orthophosphoric acid 85%, acetone, hydrochloric acid and acetonitrile were bought from El Nasr chemical

(Co.(Abu Zaabal, Cairo, Egypt). Pooled blank plasma was supplied by Hospital of Assiut University (Assiut, Egypt) and preserved at -30 ºC till use after gentle thawing.

Standard drug solution

Ten mg of STS was exactly weighed and precisely transferred into a 100-ml calibrated flask. Then 20 ml distilled water was added with continuous shaking. The resultant solution was then diluted to mark with distilled water to get a stock solution of 100 µg/ml of STS. This solution was further diluted quantitatively with same solvent to get working standard solution of STS of 10 µg/ml.

General analytical procedure

Different aliquots were precisely pipetted from the working standard solutions of STS into a series of 10-ml calibrated flasks to get final concentrations in 0.1-2.0 µg/ml range. To each flask, 1.5 ml of 0.05 N H2SO4 was added followed by of methanol to the mark. The FI was measured immediately at 350 nm (λex 286 nm) at room temperature (25 ± 5 oC). Blank experiments were carried out in parallel, omitting drug addiction. The FI observed in correspondence to drug concentrations was used to construct calibration graphs. GraphPad InStat version 3.05 program was used to derive regression equations for this correlation.

Application of the suggested method

Application to tablet dosage form: A number of 20 tablets of STS was accurately weighed, finely pulverized in a mortar to powder form and blended thoroughly. Then an amount of the powder tablets equivalent to 10 mg of the studied drug was carefully weighed and transferred into a 100-ml flask followed by addition of about 60 ml of distilled water. The content of the flask was shaken for about 5 min, and then completed to the volume with distilled water. The content of the flask was filtered through Whatman filter paper No. 4 and the first part of the filtrate was rejected. Different aliquots of the filtrate were quantitatively measured and pipetted into a series of 10-mL volumetric flasks to get several sample solutions containing an equivalent amount of drug in the range of 0.1-2.0 µg/ml. Then general analytical procedure described under 2.4 was applied starting from 1.5 ml of 0.05 N H_2SO_4 to the end of the procedure.

Application to spiked human plasma: Spiked human plasma samples with different known concentrations of STS were used to construct another standard calibration curve. Human plasma was prepared as follows: into a heparinized tube, 5ml of human blood sample free from drug was withdrawn from healthful volunteers, centrifuged at 18,659 g-forces for 10 min. After centrifugation, 1.0 mL of the resultant supernatant was carefully measured and poured into 10-ml stoppered calibrated tube and then spiked with 1.0 mL of STS working standard solution. 2 ml of methanol was added to precipitate protein and the resultant mixture was completed to volume ml with methanol and then centrifuged at 18,659 g-forces for about 10 min. 1.0 ml of the resultant supernatant was accurately measured and poured into 10 ml volumetric flask and diluted to volume with distilled water. Then the general procedure, described under section 2.4 was followed using the newly prepared working solution. A blank experiment was carried out in parallel to spiked human plasma with different known concentrations by treatment the drug-free blood sample by the same manner. Calibration graph was plotted using the observed FI in

correspondence to the final concentration of the drug studied the regression equation for this correlation was obtained as explained under section 2.4.

Results and Discussion

STS contains indole ring produces a low inherent FI in aquatic solution, which can be detected at 350 (λex 286 nm). The FI of STS was greatly increased using aqueous methanol at acidic pH. This may be attributed to the formation of protonated species of the cited drug in acidic medium which have enhanced fluorescence more than neutral species. Its native fluorescence intensity, in aqueous methanol, was dramatically increased by about 20-folds in the presence of 0.05 N H_2SO_4 as shown in Figure 2. Therefore, the emission band of STS at 350nm was used to develop a novel procedure intended for its analysis in tablets formulation and human plasma.

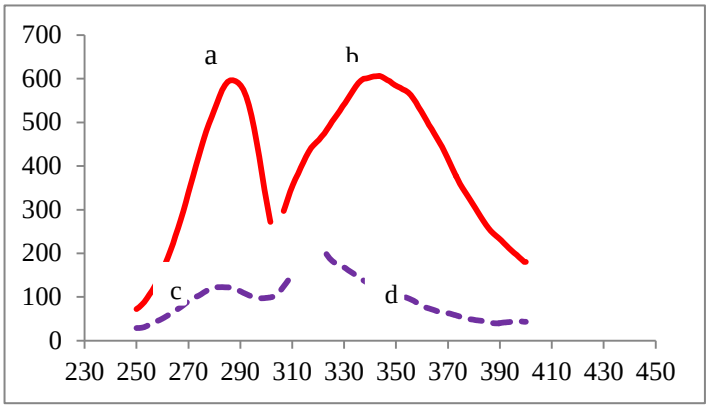

FIG. 2. **Fluorescence spectra of Sumatriptan succinate (STS) in methanolic acidic solution; Where; (c, d`) blank; (a, b) STS (1.2 µg/ml).**

Optimization of experimental conditions

The experimental parameters influencing the FI of STS were studied and optimized. The general procedure was applied where the parameter under study was changed one at a time but the others were kept the same.

Various acids such as HCl, HNO_3, H_2SO_4, and H_3PO_4 were tried to predict their effect on FI of drug under study. Highest FI with lowest blank reading were observed upon using sulphuric acid (Figure 3). Therefore, H_2SO_4 was chosen as the appropriate acid for the drug under study.

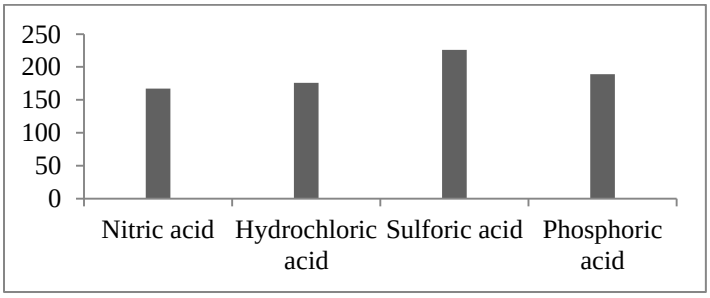

FIG. 3. **Effect of different acids (0.05N of each solution) on RFI of Sumatriptan (0.5µg/ml).**

The influence of the volume of 0.05 N H_2SO_4 on the FI was investigated using various volumes of reagent solution varied from 0.5 to 3.0 ml. It was observed that a steady increase in FI occurred upon using volumes of acid reached to 1 ml, after that no more raise in FI was achieved. Consequently, 1.5 ml of 0.05 N H_2SO_4 was selected as the suitable volume for estimation of cited drug.

The effect of several diluting solvents such as distilled water, ethanol, methanol, acetonitrile, acetone, and ethanol on the FI of the studied drugs was investigated. According to this study, highest FI and the lowest blank were observed upon using methanol. Thus, it was used as diluting solvent for in the general procedure. The FI of STS in several organized media was examined. Examples of these media used throughout this study are anionic surfactant (SDS), cationic surfactant (CTAB), non-ionic surfactant (tween-80) and macromolecules (CMC). 1.5 ml from each media was used (0.05 N). It was observed that, FI of the studied drug in all cases was decreased compared to treated control sample of drug freed from the organized media used. This finding can be explained based on the fact that the micelle formation was hindered by addition of methanol which in turn leads to partial or full damage of the micelle-enhancement effect of these surfactants on the FI of cited drug . Under the described experimental condition provided under 2.4., the FI was developed instantly after dilution with methanol and remained stable for about 1hr, time selected for study. Consequently, FI was measured directly after dilution of all the studied concentrations. FI was checked against variation of temperature after dilution with methanol, over 25–55oC range, utilizing temperature controlled water bath. The observed FI was decreased upon increasing the temperature. This finding can be explained based on the higher internal conversion path at higher temperature, thus leads to suppression of the excited singlet state by other way. Thus, all FI measurements were done at ambient temperature.

Validation of the suggested method

To confirm that the performance features of the proposed procedure fulfill the demands for the designed analytic implementations, validation of the proposed method should be approved through ICH guidelines. The entire groups of validation steps were accomplished within the particular range of the suggested procedure to guarantee its validation.

Linearity and range

A sequence of 6 concentrations of studied drug were analyzed and the results obtained were utilized to construct standard calibration graphs where the FI reading were plotted against concentrations within the calibration range. The test results were analyzed by least squares method and the regression equation which correlate FI to its corresponding concentration was computed. The analytical parameters such as effective range, intercept (Sa), slope (Sb), correlation coefficient (r) and determination coefficient (r2) are illustrated in Table 1. In addition detection Limit (LOD) and quantification limits (LOQ) were also calculated as reported.

TABLE 1. **Analytical parameters for the analysis of Sumatriptan by proposed fluorimetric method.**

Item	**Sumatriptan (STS)**
λ_{ex} (nm)	286
λ_{em} (nm)	350
linear range (µg /ml)	(0.1-2.0)
LOD (µg /ml)	0.019
LOQ (µg /ml)	0.057
Correlation coefficient (r)	0.999
Determination coefficient (r^2)	0.999
Slop(b)	0.437
Intercept(a)	27.230
SD of the intercept (Sa)	2.495
SD of slope (Sb)	0.002
SD of residual (Sy.x)	4.771
LOD: Limit of detection (µg /ml). LOQ: Limit of quantitation (µg/ml)	

Accuracy and precision

The accuracy of the suggested procedure was checked out by analysis for six various concentrations of authentic sample of drug by the suggested procedure. As illustrated in Table 2, it was clear that there is a good coincidence between the found and real values, which reflect the perfect accuracy of the suggested procedure.

Precision at both Intraday and interday were also evaluated using 3 concentrations (high, medium and low) where each concentration were analyzed six times. Data of such study (Table 3) reflect perfect repeatability and reliability of the suggested procedure as proved by values of relative standard deviation values where the values obtained with all concentrations studied were below 2%.

TABLE 2. **Evaluation of the accuracy of the proposed fluorimetric method**

	Sumatriptan		
Sample no.	**Added (µg/ml)**	**found* (µg /ml)**	**% Recovery***
1	0.3	0.29	97.50
2	0.5	0.49	98.00
3	0.9	0.89	98.50
4	1.0	0.99	99.00
5	1.5	1.47	98.30
6	2.0	1.97	98.70
Mean			**98.30**
SD			**0.53**
RSD			**0.54**
*Average of three replicate measurements SD: Standard deviation. RSD: Relative standard deviation.			

TABLE 3. **Evaluation of Interday and Intraday precision of the proposed fluorimetric method**

Parameter		**Sumatriptan (% found)***		
		0.3 µg/ml	0.6 µg/ml	1.0 µg/ml
Intraday assay	**1**	97.40	98.90	98.20
	2	98.70	98.60	97.50
	3	98.00	99.30	99.00
	Mean	98.00	98.90	98.20
	SD	0.65	0.35	0.75
	RSD	0.66	0.35	0.76
Interday Assay	**1**	98.10	98.60	99.00
	2	98.70	98.00	98.50
	3	98.40	99.30	98.00
	Mean	98.40	98.60	98.50
	SD	0.30	0.65	0.50
	RSD	0.31	0.66	0.51

*Average of six determinations.

Robustness of the suggested procedure

The robustness of the suggested procedure was checked versus little intentional variations in the experimental conditions such as variation in the volume of 0.05N H_2SO_4 (1.5 ± 0.5 ml), the excitation wavelengths (286 ± 5 nm) and emission wavelengths (350 ± 5 nm). The good percentage recoveries and standard deviations observed proved that the method is robust since small variations in volume of 0.05 N H_2SO_4, excitation or emission wavelengths have no considerable effect on the results of the proposed procedure. Consequently, the current procedure is reliable and appropriate for the analysis of the cited drug in either pure form or in different matrices.

Implementation to pharmaceutical dosage form

STS tablet dosage form available in the local market was subjected to analysis by the suggested and reference [23] methods in parallel to each other. The results obtained from both methods were statistically analyzed by student's t-test and the variance ratio F-test at 95% confidence level. According to the data provided it was obvious that there is no considerable variation between the results of both methods. Thus, the proposed procedure is accurate and precise enough and is appropriate for analysis of cited drug in its available pharmaceutical formulations.

Content uniformity test

Because of the higher precision and accuracy of the current procedure and its capability to find the concentration of the drug in a single tablet extract within short period, with sufficient accuracy, the suggested procedure was utilized for content uniformity testing. The method described for analysis of tablet was implemented for analysis of 10 different tablets separately and the uniformity of their contents was evaluated according to the USP guidelines (Chapter 905: Uniformity of Dosage Units). The acceptance value (AV) was computed for each tablet and the results obtained proved excellent STS uniformity where all values were within the maximum allowed acceptance value (L1=15).

Implementation to spiked human plasma

Owing to the good sensitivity of the current procedure, it was decided to apply it for estimation of STS concentrations in its/or their sample(s) spiked with human plasma. The data of such investigation, which reflects the appropriateness of the present procedure for analyses of STS in human plasma.

Conclusion

The current work describes for the first time how to enhance FI of STS through measurement of its native fluorescence in methanolic H2SO4 acid solution without using any buffer or critical pH control or other additives. The suggested procedure is time saving, low cost and simple since no need for tedious and long treatment upon comparison with the existed fluorimetric procedures. Consequently, the suggested procedure can be considered the method of choice for studying the cited drug during its content uniformity testing and in human plasma.

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