INTRODUCTION

The Sumatriptan succinate (SUM) (Fig.1) is the most frequently prescribed anti-migraine drug of triptan class. It is chemically known as 3-[2-(Dimethylamino)ethyl]-N-methyl-1H-indole-5-methane sulphonamide succinate (1:1) base. SUM is a specific and selective 5-hydroxytryptamine receptor (5-HT1D) agonist with no effect on the other 5-HT receptors (5HT2-5HT7) subtypes. It is used widely for prophylaxis and acute relief of migraine attack with or without aura. SUM undergoes an extensive biotransformation mainly through Mono amino oxidase-A. The drug is official in EP 2 and USP 3 suggests chromatographic methods for determination of SUM in bulk and tablet formulations. Several analytical techniques like HPLC 4-9, HPLC-MS-MS 10-13, HPLC-ECD 14-15, HPLC-coulometry 16, capillary LC-MS-MS 17, HPTLC 18, spectrophotometric with HPTLC 19, RP-HPLC and colorimetric 20, UV21, voltammetry 22, capillary electrophoresis 23, and densitometry with spectrophotometric detection 24 have been reported in the literature. The main purpose of the present study was to establish a relatively simple, sensitive, validated and inexpensive visible spectrophotometric method for the determination of SUM in pure form and in pharmaceutical dosage forms, since most of the previous methods involve sophisticated equipments which are costly and pose problems of maintenance. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and acidic dye Tropaeolin ooo in the presence of 0.1M HCl. The method can be extended for the routine quality control analysis of pharmaceutical products containing SUM.

As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the acid dye technique was therefore, utilized in the present work for the estimation of SUM. The present paper describes simple and sensitive extraction spectrophotometric method for the determination of SUM, based on its tendency to form chloroform extractable ion-association complex with acidic dye belonging to Azo (monoazo) category dye TP000 (CI No. 15510) under experimental conditions by exploiting the basic nature of the drug molecule.

MATERIALS AND METHODS

A Systronics UV/Visible spectrophotometer model -2203 with 10mm matched quartz cells was used for all spectral measurements. A pure drug sample of SUM was provided as a gift sample by Orchid healthcare Ltd., India. All the chemicals used were of analytical grade. Tropaeolin 000 (Phka, 0.2%, 5.7x10^-3M prepared by dissolving 200mg of Tropaeolin 000 in 100ml distilled water and subsequently washed with chloroform to remove chloroform soluble impurities), 0.1M HCl (prepared by diluting 8.7ml of Con. hydrochloric acid to 1000ml with distilled water and standardized) were prepared.

Standard solution: The standard stock solution (1mg/ml) of SUM was prepared by dissolving 100mg of SUM in 10ml of 0.1M sodium hydroxide and followed by dilution to 100 ml with distilled water. The working standard solution of SUM (100µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Sample solution: About 20 tablets were pulverized and the powder equivalent to 100mg of SUM was weighed, dispersed in 25ml of IPA, sonicated for 30 minutes and filtered through Whatman filter paper No 41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Assay: Aliquots of the standard SUM solution (0.5ml-2.5ml, 100µg/ml) were placed in a series of 125ml separating funnels. A volume of 6.0ml of 0.1M HCl and 2.0ml of TP000 were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. Then 10.0ml of chloroform was added to each funnel and the contents were shaken for 2 minutes. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 482.5nm (Fig. 2 showing absorption spectra) against a reagent blank within the stability period (5 minutes to 1hour). The amount of drug was computed from its calibration graph (Fig.3 showing Beer’s Law plot).
RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of TPOOO reagent and acid solution and solvent for final dilution of the colored species were studied. The water immiscible solvents tested for the extraction of colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetra chloride, benzene, n-butanol or chloroform. Chloroform was preferred for its selective extraction of colored drug-dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the drug to dye was determined by the slope ratio method and was found to be 1:1. The optical characteristics such as Beer’s law limit, Sandell’s sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4th of the amount of the upper Beer’s law limits). Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-1.
Table 1: Optical and regression characteristics, precision and accuracy of the proposed method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>482.5nm</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>2 - 10</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²/0.001 abs. unit)</td>
<td>0.019851117</td>
</tr>
<tr>
<td>Molar absorptivity (Litre/mole/cm)</td>
<td>20830.0625</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.998</td>
</tr>
<tr>
<td>Regression equation (Y)*</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.011</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.051</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.9524</td>
</tr>
<tr>
<td>% Range of errors (95% Confidence limits)</td>
<td></td>
</tr>
<tr>
<td>0.05 significance level</td>
<td>0.9996</td>
</tr>
<tr>
<td>0.01 significance level</td>
<td>1.5677</td>
</tr>
</tbody>
</table>

$Y = a + bx$, where Y is the absorbance and x is the concentration of sumatriptan in µg/ml

Commercial formulations containing SUM were successfully analyzed by the proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t- and f-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the preanalyzed formulations at three different concentration levels (50%, 75% and 100%). These results are summarized in Table-2.

Table 2: Results of analysis of tablet formulations containing SUM

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulations</th>
<th>Labeled amount (mg)</th>
<th>Found by proposed methods</th>
<th>Found by reference method ± SD</th>
<th>#% Recovery by proposed method ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUM-TPOOO</td>
<td>Tablet-1</td>
<td>50</td>
<td>49.654 ± 0.1568</td>
<td>49.647 ± 0.125</td>
<td>99.307 ± 0.314</td>
</tr>
<tr>
<td></td>
<td>Tablet-2</td>
<td>50</td>
<td>49.709 ± 0.190</td>
<td>49.702 ± 0.169</td>
<td>99.418 ± 0.381</td>
</tr>
</tbody>
</table>

* Different batches (Table-1 & 2) from two different companies (Sun Pharmaceuticals, Dabur Pharmaceuticals)

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t =2.57 and f = 5.05.

# Recovery of 10mg added to the pre analyzed sample (average of three determinations).

Reference method (reported UV method) using double distilled water ($\lambda_{\text{max}}$ =220nm).

CONCLUSION

The reagents utilized in the proposed method are normal cost, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed extractive colorimetric method is validated as per ICH guidelines and possess reasonable precision, accuracy, simple, sensitive and can be used as alternative method to the reported ones for the routine determination of SUM depending on the need and situation.

Chemistry of colored species

The positively charged tertiary nitrogen of SUM molecule in acid medium is expected to attract the negatively charged part of the acidic dye TPOOO and form an ion pair held together through electrostatic attraction. Based on the analogy, the structure of ion association complex in this method is shown in the scheme (Fig.4).

![Scheme of the reaction](image)

Fig. 4: Scheme of the reaction
ACKNOWLEDGEMENT

One of the authors (B.Kalyana Ramu) is grateful to University Grants Commission, New Delhi, for providing financial assistance under the Teacher Fellowship. The authors are very much thankful to the m/s chemistry, Andhra University, for his advice and suggestions given in this work.

REFERENCES