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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND SUMATRIPTAN IN NASAL *IN-SITU* GEL FORMULATION

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ABSTRACT

Simple, precise and accurate UV-Spectrophotometric simultaneous equation methods for estimation of Naproxen (NAX) and Sumatriptan (SMP) were developed and validated as per ICH guidelines. This method involves solving of simultaneous equations based on measurement of absorbance at two wavelengths *i.e.* 276 nm and 228 nm (λ_{max} of NAX and SMP) in simulated nasal fluid (SNF) at pH 7.4. Both the drugs obey the Beer's law in the concentration ranges 2-10 and 10-50µg/ml for SMP and NAX respectively. Percent recovery for both the drugs was in the range of 99.059±0.358% indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercial formulation.

Keywords: Naproxen, Sumatriptan, Spectrophotometric analysis, Simultaneous equation method.

1. INTRODUCTION

Triptans are a group of tryptamine-based drugs used in the acute treatment of migraine headaches. Sumatriptan (SMP, Fig.1 A) is one among them and is structurally related to the neurotransmitter serotonin. Sumatriptan (SMP) is a 5-hydroxytryptamine (5-HT) receptor subtype (a member of the 5-HT 1D family) having only a weak affinity for 5-HT1A, 5-HT5A, and 5-HT7 receptors and chemically designated as [3-[2-(Dimethylamino) ethyl]-1H-indol-5-yl]-N-methyl-methanesulfonamide hydrogen butanedioate [1]. SMP acts by selectively binding to serotonin type-1D receptors (serotonin agonist) and rapidly terminates a migraine attack while eliminating associated symptoms such as nausea, vomiting, and light and sound sensitivity [2]. SMP has official monographs in BP [1] and EP [3], which describe liquid chromatographic methods for the assay of SMP, and also in USP [4]. Naproxen (NAX, Fig.1 B) is chemically (2S)-2-(6- methoxynaphthalen-2-YL) propionate. NAX is a propionic acid derivative that belongs to the aryl acetic group of non-steroidal anti-inflammatory agents [5, 6]. They act by inhibiting the prostaglandin synthesis thereby producing several side effects such as ulceration and gastrointestinal bleeding [7, 8]. A tablet formulation containing 85 mg of Sumatriptan succinate and 500 mg of Naproxen sodium has recently been approved for the acute treatment of migraine. The combination product was proved to have superior efficacy compared to its individual components for the acute treatment of migraine. Sumatriptan, work early in the migraine process at the trigeminovascular unit as agonists of the serotonin receptors (5-HT receptors) 1B and 1D. They block vasoconstriction and block transmission of signals to the trigeminal nucleus and thus prevent peripheral sensitization. The analgesic effect of Naproxen sodium helps relieve the headache, while the anti-inflammatory effect decreases the neurogenic inflammation in the trigeminal ganglion, thus preventing the development of central sensitization [9]. So far, several liquid chromatography procedures have been described for the determination of SMP and NAX [10-28]. But, these procedures were developed to estimate either SMP or NAX individually and in combination with other drugs from formulation, plasma, urine, intestinal perfusion samples and in bulk drugs. For simultaneous determination of SMP and NAX in formulation, there are two spectrometric methods [29, 30] and an HPTLC [31] method was reported. However, no UV-Spectrophotometric simultaneous equation method is available for simultaneous determination of the SMP or NAX in combined pharmaceutical dosage form. In the present study, an

attempt was made to develop a simple, precise and accurate method for the simultaneous estimation of these drugs in combined pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines [32].

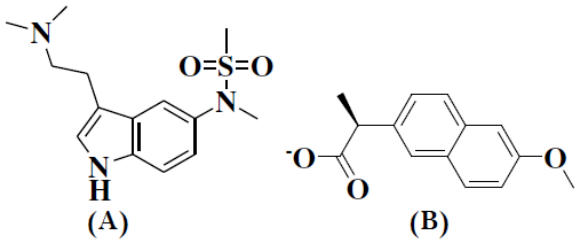

Fig. 1: Chemical structure of (A) Sumatriptan and (B) Naproxen

2. MATERIAL AND METHOD

2.1. Reagents and chemicals

SMP and NAX standard were obtained from Sun Pharmaceuticals Ltd. Vadodara, Gujarat. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Ammonium acetate AR, sodium dihydrogen phosphate AR sodium chloride, calcium chloride and potassium chloride AR grade were procured from Central Drug House (P) Limited, New Delhi, India. The 0.45- mm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). HPLC grade water was used throughout the study. Other chemicals used were of analytical or HPLC grade.

2.2. Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

2.3. Method development

2.3.1. Standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml simulated nasal fluid (SNF), pH 7.4 in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilizing the drug and the volume was made up to the mark 100ml with simulated nasal fluid (SNF), pH 7.4 to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

2.3.2. Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of SMP and NAX and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with simulated nasal fluid (SNF), pH 7.4 that gave concentration of 100µg/ml (Stock-B).

2.3.3. Determination of λ_{max}

10 µg/ml standard solutions of both SMP and NAX were prepared from respective sub-stock solutions. Both the solutions were scanned in the wavelength region of 200-400 nm and the λmax was found to be 228 nm and 276 nm for SMP and NAX respectively. They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Figs. 2-4).

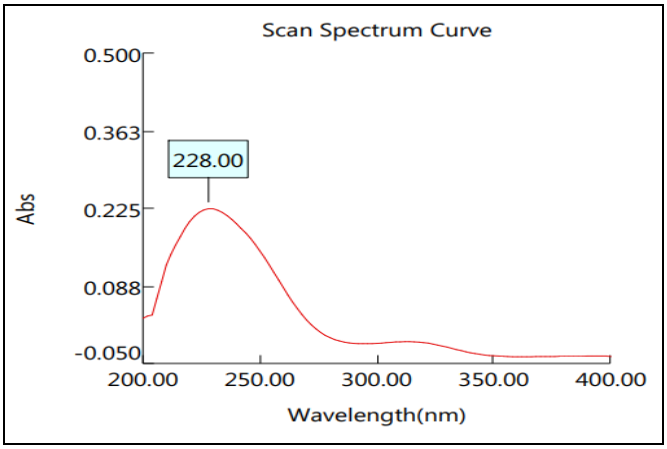

Fig. 2: Determination of λ_{max} of SMP in simulated nasal fluid (pH 7.4)

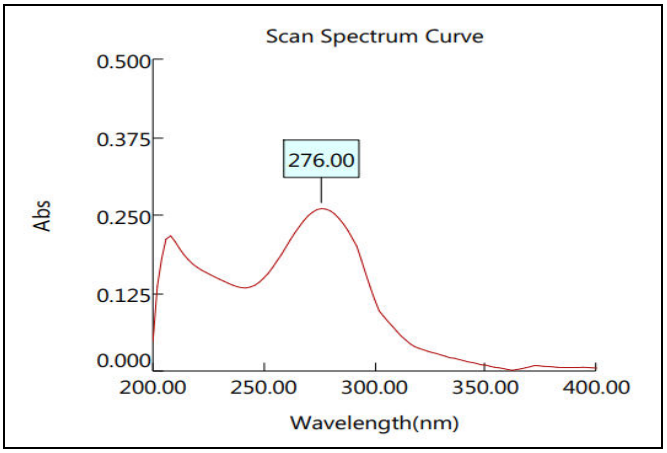

Fig. 3: Determination of λ_{max} of NAX in simulated nasal fluid (pH 7.4)

2.3.4. Preparation of calibration curve

From the standard stock solution of each drug, appropriate aliquots were pipetted out into a series of 10 ml volumetric flasks. The volume was made up to

the mark with in simulated nasal fluid (pH 7.4) to get a set of solutions having a concentration range of 2-10 and 10-50µg/ml for SMP and NAX respectively. Triplicate dilutions of each drug solutions were prepared separately. The prepared working solutions of SMP and NAX were scanned 228 nm and 276 nm, respectively. The absorbance's were recorded and were plotted against the concentrations to obtain their respective calibration curves.

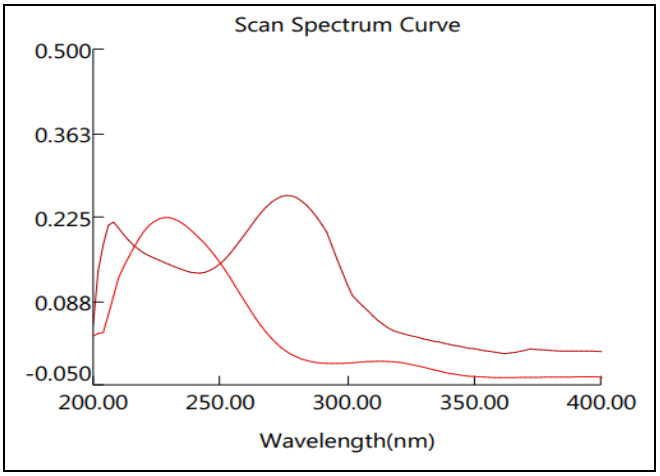

Fig. 4: overlay spectra of SMP and NAX

2.3.5. Simultaneous equation method (Vierordt's)

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of SMP and 50µg/ml of NAX were scanned in the spectrum mode over the range of 200-400 nm against simulated nasal fluid (pH 7.4) as blank and the overlain spectra of the two were recorded. SMP showed an absorbance peak at 228.0 nm, whereas NAX at 276.0 nm. The overlain spectra also showed isoabsorptive points at 250.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method. Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 228.0 nm and 276.0 nm that are λ_{max} of SMP and NAX respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations

$$C_{SMP} = A_1ay_2 - A_2ay_2 / aX_1ay_2 - aX_2ay_1 \quad \text{Eq. (1)}$$

$$C_{NAX} = A_1aX_2 - A_2aX_1 / aX_1ay_2 - aX_2ay_1 \quad \text{Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 228.0 nm and 276.0 nm respectively, ax_1 and ax_2 are absorptivities of SMP at λ_1 (228.0 *i.e.* λ_{max} of SMP) and λ_2 (276.0 *i.e.* λ_{max} of NAX) respectively and ay_1 and ay_2 are absorptivities of NAX at λ_1 and λ_2 respectively. C_{NAX} and C_{SMP} are concentrations of NAX and SMP respectively. Fig. 4 represent the overlain spectra of both the drugs in 10:50 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the SMP and NAX [33].

2.4. Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [32].

2.4.1. Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in Table-1.

2.4.2. Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in Table 2.

2.4.3. Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in Table 3.

2.5. Analysis of synthetic mixture

Equivalent to 10mg of naproxen were weighed and taken in 50 ml volumetric flask. This was dissolved in

25 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times (Table 4).

3. RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis [34]. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials.

Table 1: Results of Linearity of Sumatriptan and Naproxen

Parameter	Sumatriptan	Naproxen
Concentration (µg/ml)	2-10	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.059	0.022
Intercept (c)*	0.002	0.004

**value of three replicate*

Table 2: Results of Recovery Study

% Level	% Mean±SD*	
	Sumatriptan	Naproxen
80%	98.736±1.076	98.549±1.875
100%	98.870±0.951	99.059±0.358
120%	98.449±0.211	99.000±0.465

** Value of three replicate and five concentrations.*

Table 3: Results of Precision

Parameter	% Mean±SD*	
	Sumatriptan	Naproxen
Repeatability	98.719±0.166	98.887±0.143
Intermediate precision		
Day to day	99.075±0.146	99.183±0.098
Analyst-to-Analyst	98.847±0.143	99.556±0.093
Reproducibility	99.666± 0.039	98.311±0.145

** Value of five replicate and five concentrations*

To develop suitable methods of analysis, various solvents were studied. Based on sensitivity of the method and non-toxic behaviour simulated nasal fluid (SNF), pH 7.4 was selected as a solvent for the methods. Overlain spectra (Fig. 4) shows that at λ_{max} of SMP (228 nm) interference of NAX and at λ_{max} of NAX (276nm) interference of SMP occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with 99.666± 0.039 (SMP), 98.311±0.145 (NAX) and 98.870±0.951 (SMP), 99.059±0.358 (NAX) respectively. The standard deviation, coefficient of variance and standard error were obtained for SMP and NAX were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine SMP and NAX simultaneously.

Table 4: Assay of formulation

Conc. Present (µg/ml)		% Conc. Found	
Sumatriptan	Naproxen	Sumatriptan	Naproxen
2	10	99.00	97.40
4	20	98.50	99.80
6	30	97.93	97.07
8	40	99.75	98.25
10	50	83.28	99.84

**Average of three replicate and five concentrations*

4. CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of SMP and NAX in synthetic mixture. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of SMP and NAX in commercial formulation.

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