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Determination of Saxagliptin Monohydrate by Derivatization UV-Vis Spectroscopy

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Abstract: *Specific, accurate, precise and simple UV spectrophotometric method was developed for determination of SAXA in pharmaceutical dosage form and in laboratory prepared mixtures. In this method the amplitudes of zero order spectra of the dosage form containing SAXA was measured at 578 nm respectively. The method is based on derivatization technique for making SAXA UV detectable, employing reaction of ninhydrin with primary amino group of SAXA by heating at 65°C for 30min. A purple colour product forms which is used for quantitative determination of SAXA by measuring the absorbance at 578 nm. The linearity ranges for SAXA was found to be 5 – 25 µgmL^{-1} respectively. The proposed method is validated for linearity, recovery, precision, and specificity by statistical evaluation as per ICH guidelines. The formulation excipients and additives did not interfere in their determinations. Commercial tablet formulations and laboratory prepared mixture was successfully analyzed using the developed methods.*

Keywords: UV Visible Spectrophotometry, Saxagliptin, Ninhydrin, Saxa, Onglyza

1. Experimental

Apparatus and Software

Shimadzu UV 1601 double beam spectrophotometer connected to a computer loaded with Shimadzu UVPC software was used for all the spectrophotometric measurements. The spectral bandwidth was 1 nm and the wavelength scanning speed was 2800 nm min-1. The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 400-800 nm.

Reagents and Pharmaceutical Preparations:

The standard drug was used without further purification. All the solvents used in spectrophotometric analysis were of analytical reagent grade. ONGLYZA tablets claimed to contain 5 mg of SAXA. were used in the analysis.

Preparation of standard SAXA solution:

Accurately weighed 25 mg of pure drug was taken in clean, dry 25 ml volumetric flask and dissolved in small volume of methanol and the volume was made up with methanol. This gave 1000 µg ml^{-1} of drug concentration.5 ml of above solution was pipetted out into 100 ml volumetric flask and volume was made up to 100 ml with methanol. This solution gave 50µg ml-1 concentration.

Preparation of ninhydrin reagent: 4g of ninhydrin in 95 ml of n-butanol and 5ml of [2M acetic acid gives 4 % (w/v) ninhydrin.

UV Spectrophotometry

A calibration set of five dilutions of SAXA (5-25 µg mL-1) in Methanol were prepared, 1 ml of 4% of ninhydrin reagent was added and heated in water bath at 65ºC for 30mins and UV-Vis spectra were recorded in the wavelength range 400-800 nm versus solvent blank. The zero order spectra of standard solution of SAXA is shown in the Fig.1 respectively.

Optimization and selection of method parameters

All the optimized method parameters are summarized in Table 1 Methanol was selected as solvent, 578 nm was selected for the determination of SAXA respectively.

Method parameters	Optimized Values
Solvent	Methanol
Scanning range	400 nm to 800 nm
Slit width	1 nm
Scan speed	Fast (2800 nm min^{-1})
Wavelength for determination of SAXA	578nm

Validation of Analytical Method

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Linearity

The linearity of the method was demonstrated over the concentration range of 5-25 µgml^{-1}for SAXA. Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml of 50 µgml^{-1}of SAXA solution was pipetted into 10 ml volumetric flasks. A Calibrations curve is produced by analyzing different concentrations of the pure drug with absorbance. Fig.2 shows the calibration curve of SAXA. Calibration curve parameters of SAXA were obtained as shown in table 2.

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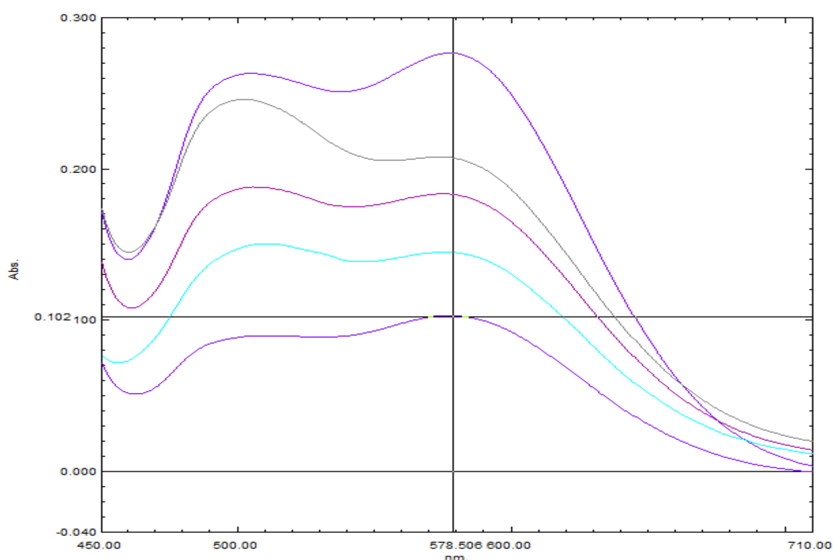

Figure 1: Zero order spectra of SAXA

Table 2: Calibration curve parameter, LOD and LOQ

Parameters	UV
	SAXA
Wavelength (nm)	578
Linearity and range (μgml^{-1})	5-25
Correlation coefficient (r^2)	0.9987
Intercept	0.07
Slope	0.0073
LOD (μgml^{-1})	1.2
LOQ (μgml^{-1})	4.3

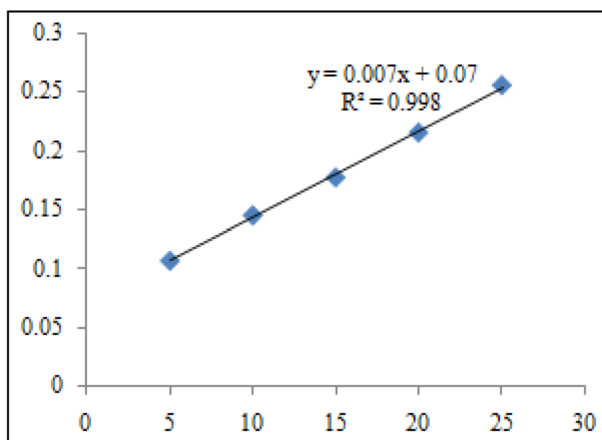

Figure 2: Linearity curve of SAXA

Precision

Inter and intra-day precision:

3Different concentrations of standard solution of SAXA were prepared, these samples are in linearity range. Absorbance was measured at 578nm for the determination of SAXA respectively the values were calculated. The experiment was repeated three times in a day and also on three different days, the average % RSD values of the results were calculated as shown in Table 3

Table 3: Intraday and Inter-day precision

SAXA		
Conc. ($\mu g\ mL^{-1}$)	Intraday (%RSD)	Inter – day (%RSD)
5	1.0094	1.0222
15	0.9925	0.9765
25	0.9896	1.0091

Average of three readings

Accuracy

The accuracy of a method is affected by systematic (bias) error components, the term accuracy will be used in the sense of bias. "Bias is the difference between the expectation of the test results and an accepted reference value". Accuracywas found out by recovery study from prepared mixture at three levels of standard addition, from 50%, 100% and 150% of the label claim.

To keep an additional check on the accuracy of the developed assay methods and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of standard SAXA solution to pre-analysed samples of commercial dosage form and the mixtures were are analysed by the proposed method. (table 4)

Table 4: Accuracy

Average of three readings

Analyte	Drug added to analyte (%)	Final conc.	%recovery± % RSD
SAXA	50%	5	99.17±0.9079
	100%	10	100.11±0.8538
	150%	15	100.58±0.3078

Results of analysis of commercial formulation

Applicability of the method was tested by analyzing the commercially available tablet formulation containing SAXA. The values of % recovery from formulation as shown in the table 5 were found to be very close to each other as well as to the label claim of commercial pharmaceutical formulation, which showed that the method is applicable for determination SAXA from the formulation.

Table 5: Analysis of commercial formulation (ONGLYZA)

Label Claim (mg/tablet) ONGLYZA	Conc. found mg	%recovery
5	5	100

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2. Conclusion

The proposed method enables the quantification of SAXA in the presence of different excipients, with good accuracy and precision, either in laboratory prepared samples or in spiked pharmaceutical dosage form. Finally it was concluded that the short analysis time and low costs were the main advantages of this derivative spectrophotometric method for the determination of SAXA in routine analysis. High percentage recovery showed that the method is free from interferences from the excipients and additives commonly used in the formulations of the drugs. Although derivative spectroscopy has been used for a long time in pharmaceutical analysis this procedure is simple and practical in application in routine laboratories.

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