Development of UV Spectroscopic Method for Nefopam and Escitalopram as INN Drugs in Tablet Dosage Form

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ABSTRACT

Nefopam and Escitalopram are INN drugs and as such it has not been yet included in the BP or USP. The objective of this work is to develop a simple, sensitive, accurate, precise and reproducible UV-Spectrophotometric method for quantitative estimation of Nefopam and Escitalopram in tablet dosage forms. Various solvents were used to find out the medium for maximum solubility of each drug. The λ_{max} of Nefopam and Escitalopram was 266nm and 284nm in water respectively. Both drugs obey Beer- Lambert's law in the range of 50-400µg/ml for Nefopam and 25-200µg/ml for Escitalopram. The correlation coefficients of std. curves were 0.998 and 0.995. The values of SD were 0.131 and 0.081 respectively. %RSD (Relative standard deviation) of interday absorbance of Nefopam was 0.766 and Escitalopram was 0.854. The LOD (Limit of Detection) were 0.393 and 0.243 and LOQ (Limit of Quantification) were 1.310 and 0.810 respectively. The percent potencies were 92.16 and 102.06 for Nefopam and Escitalopram. The potency of these tablets complied with their claimed quantity (±10%). So, based on these data, it may be concluded that these proposed method are simple, sensitive, precise, reproducible and accurate for the analysis of these drugs in tablet dosage form.

Key Words: INN drugs, correlation coefficients, LOD, LOQ, Nefopam and Escitalopram.

INTRODUCTION

International Nonproprietary Names (INN) identifies pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is globally recognized and is public property. The names which are given the status of an INN are managed and selected by the World Health Organization (WHO). The procedure for selecting recommended INN is carried out in accordance with a text adopted by the WHO Executive Board (Drug information, 2005).

Most compounds include chromophores within their molecular structure. Thus these compounds absorb electromagnetic energy in the visible (350–700nm) and/or ultraviolet range (200–350nm). Within specific concentration ranges the amount of energy absorbed is proportional to the concentration of the compound in the sample. UV Spectroscopy method is one of the instrumental analytical methods that are widely used in pharmaceutical industries for the assay of pharmaceutical products, because it is simple, easy, less time consuming and an economical method (Ultraviolet-visible spectroscopy, 2009).

Various analytical methods such as HPLC (High performance liquid chromatography), TLC (Thin-layer chromatography), GLC (Gas-liquid chromatography), HPTLC (High performance thin layer chromatography), RP-HPLC (reversed-phase high performance liquid chromatography), Ion-pair reverse-phase liquid chromatographic method have been reported for the assay of Nefopam and Escitalopram however, no UV-Spectrophotometric study has been found in literature survey in tablets dosage forms for these drugs.

Drug development is required to establish the physicochemical properties of the New Chemical Entities: its chemical makeup, stability, solubility. The method was developed in terms of solubility, linearity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurements as well as repeatability of sample application (Drug Development, 2009).

During patient sample analysis, QCs are incorporated to confirm that the method continues to perform consistently within specifications, thus allowing patient-derived data to be confidently accepted as valid. Performance parameters that are studied during the second stage of validation of bioanalytical methods would normally include: selectivity, sensitivity, calibration response, choice of QC samples, analyte recovery, precision, accuracy and reproducibility (Cummings et al., 2008).

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a liquid solvent to form a homogeneous solution. The extent of the solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase the concentration of the solution (Solubility of things, 2007). The solubility of a compound in water is given in mg/L at 20°C (Water solubility, 2009). Approximate weight of solvent in between 10 and 30 (g) necessary to dissolve 1g of solute, samples are soluble (Aulton, 2002). Nefopam ($C_{17}H_{19}NO$) hydrochloride is a non-narcotic analgesic used parenterally and orally as a racemic mixture for the relief of postoperative pain. However, no information is presently available on the oral kinetics of (+) and (-) nefopam in humans. Also, nefopam is metabolized by N-demethylation but it is not known whether the desmethylnefopam enantiomers are present in plasma for intravenous or oral administration of parent drug (Heel et al., 1980).
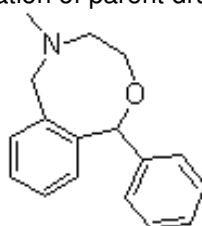
Figure 1: The Chemical Structure of Nefopam hydrochloride.

Escitalopram ($C_2H_2O_4$ or $C_{20}H_{21}FN_2O$) is an oral drug that is used for treating depression and generalized anxiety disorder. Escitalopram oxalate is chemically known as S-(+)-1-[3-(dimethylamino)propyl]-1-(p -fluorophenyl)-5-phthalancarbonitrile oxalate and belongs to the class of compounds known as antidepressants. It is the pure S-enantiomer (single isomer) of the racemic bicyclic phthalane derivative citalopram. Preclinical studies have demonstrated that the therapeutic activity of citalopram resides in escitalopram and the R-enantiomer is approximately 30-fold less potent than escitalopram (Montgomery SA et al., 2001).
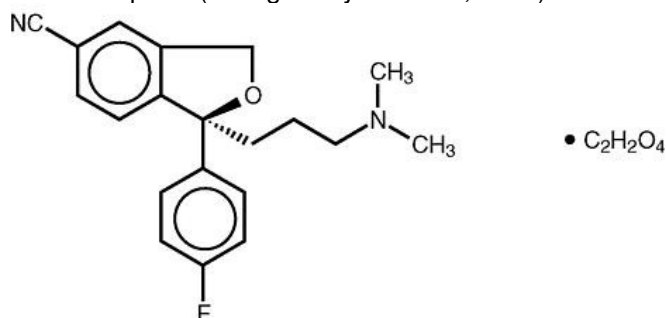
Figure 2: The Chemical Structure of Escitalopram Oxalate.

MATERIALS AND METHODS

Nefopam (potency: 100.47%), Escitalopram (potency: 100.34%) were gift samples from Eskayef Bangladesh Ltd. Tablet of Nefopam, Escitalopram were purchased from the local market.

Preparation of standard solution of Nefopam and Escaitalopram
For the preparation of standard solution 100 mg of nefopam standard was accurately weighed and added water. Then shake to dissolve and made up the volume to 200ml. This was the mother solution. From the mother solution 5, 10, 15, 20, 25, 30, 35, & 40ml had been taken to 8 volumetric flask and added water. Then the final volume was made to 50ml. Thus the concentrations of this standard solution were 50, 100, 150, 200, 250, 300, 350, & 400µg/ml.
For the preparation of standard solution 50 mg of escitalopram standard was accurately weighed and added water. Then shake to dissolve and made up the volume to 100ml. This was the mother

solution. From the mother solution 2.5, 5, 7.5, 10, 12.5, 15, 17.5, & 20ml had been taken to 8 volumetric flask and added water. Then the final volume was made to 50ml. Thus the concentration of this standard solution was 25, 50, 75, 100, 125, 150, 175, & 200µg/ml.

Scanning of wave length for Nefopam and Escitalopram in water
The stock solution of Nefopam and Escitalopram was scanned over a range of 200-400nm, two peaks were observed for Nefopam at 264 and 266nm and four peaks were observed for Escitalopram at 272, 274, 282 and 284nm, using water as a blank, whereas peak at 266nm was the sharpest peak for Nefopam and at 284nm for Escitalopram. Taken the Nefopam solution of concentration of 50, 100, 150, 200, 250, 300, 350, & 400µg/ml had given the absorbance of 0.127, 0.259, 0.389, 0.515, 0.646, 0.768, 0.923 and 1.058 respectively at 266nm. So, λ_{max} is 266nm for Nefopam. And for Escitalopram the concentration of 25, 50, 75, 100, 125, 150, 175, & 200µg/ml had given the absorbance of 0.104, 0.184, 0.267, 0.359, 0.445, 0.520, 0.598 and 0.695 respectively at 284nm. So, λ_{max} is 284nm for Escitalopram.

Preparation of sample solution of Nefopam and Escaitalopram
20 tablets from the supplied sample were taken and their total weight was taken accurately in an analytical balance. Average weight of each tablet was determined. The tablets were powdered by using a mortar and pestle. Approximately 153.4 mg of tablet powder of Nefopam and 118 mg for Escitalopram were weighed three times and taken in three volumetric flasks for each. Water was added and the final volume was made to 100ml.

Analytical Requirements
The absorbance of sample and standard solution were measured by spectrophotometer in 1 cm cell at specific wavelength using water as blank.

Linearity
The linearity was studied by preparing standard solution at different concentration level (Method Validation, 2009). Linearity usually refers to the response of the detector. A detector is linear if the output of a detector is given by the product of a constant and the solute concentration (or, for a mass sensitive detector, the mass of solute passing though it per unit time). If a detector is declared to be linear, the linearity is usually limited to a specific concentration range (or range of mass of solute passing though it per unit time). The linearity range represents the equation of the regression line, correlation coefficient (R^2), relative standard deviation (RSD %) values of the slope and R^2 (Sharma et al., 2003).

Precision
Precision study was performed to find out intra-day and inter-day variations in the estimation of different concentration, with the proposed method (Patel AB et al., 2009). The precision of the method {intraday and interday (5 days) variation of replicate determination} was checked by preparing one concentration of standard solution for 3 times. The precision of the method, expressed as the RSD % of intraday and interday variation.

Reproducibility
Reproducibility was determined analyzing samples under different times.

RESULTS AND DISCUSSION

Specificity
The maximum wavelength of Nefopam at 266nm and Escitalopram at 284nm respectively in water was found. So, from the scanning result, it can be said that this developed analytical method is very specific for quantitative estimation of Nefopam, Escitalopram in tablet dosage form. Results are shown in Table 2.

Table 1: Specificity of Nefopam, Escitalopram in water.

Compound	Media	λ_{max}
Nefopam	Water	266nm
Escitalopram	Water	284nm

So, the maximum wavelengths of Nefopam, Escitalopram were showed that these methods were very specific.

Linearity
Figure 3, 4 and Table 2 represent the equation of the regression line, correlation coefficient (R^2), relative standard deviation (RSD %) values of the slopes and R^2. Excellent linearity was obtained for Nefopam between 50-400µg/ml, Escitalopram between 25-200µg/ml.
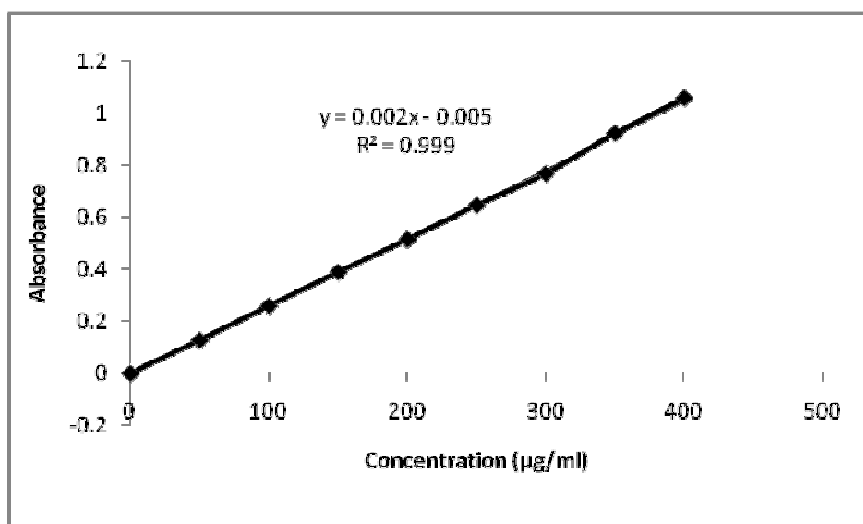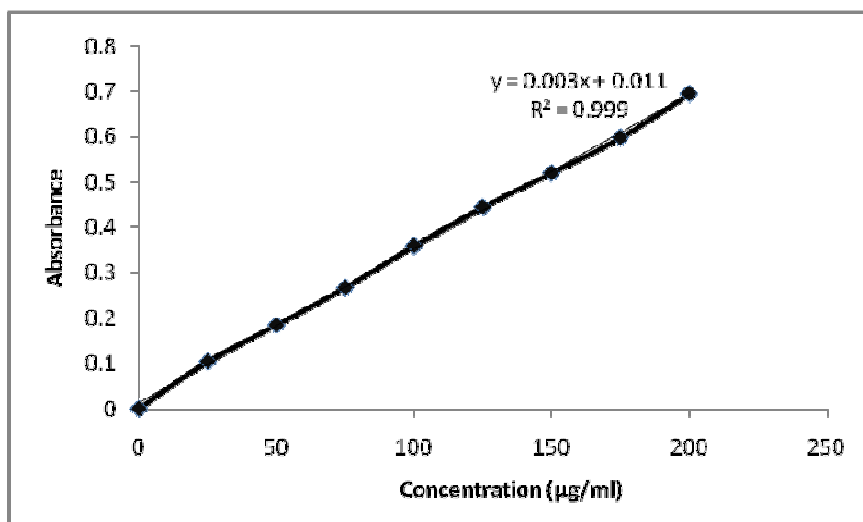

Figure 3: Standard curve of Nefopam in water.

Figure 4: Standard curve of Escitalopram in water.

Table 2: Linearity of Nefopam and Escitalopram.

Compound	Equation	R^2 (mean±SD)	% RSD of R^2	Slope (mean±SD)	% RSD of slope
Nefopam	y = 0.0026x − 0.005 y = 0.0026x − 0.006 y = 0.0025x − 0.034	0.998±0.001	0.115	0.0025±0.000	2.249
Escitalopram	y = 0.0034x + 0.011 y = 0.0034x + 0.017 y = 0.0037x + 0.040	0.995±0.003	0.352	0.0035±0.000	2.857

The plots of peaks area showed that these developed methods were linear. The correlation coefficient of Nefopam was 0.998 and Escitalopram was 0.995. Linear relationships were found for these components.

Precision
The precision of the method (intraday and interday (5 days) variation of replicate determination) was checked by preparing one concentration of standard solution of Nefopam and Escitalopram for 3 times. The precision of the method, expressed as the RSD % of intraday and interday variation is given in Table 3.

Table 3: Intraday and Interday precision of Nefopam and Escitalopram.

Compound	Conc. (µg/ml)	Intraday absorbance (mean±SD)	% RSD	Interday absorbance (mean±SD)	% RSD
Nefopam	100	0.251±0.007	2.788	0.261±0.002	0.766
Escitalopram	50	0.187±0.003	1.601	0.234±0.002	0.854

The precisions of these methods were established by carrying out the analysis of analyte (n=3) using the proposed method. The values of %RSD for Nefopam and Escitalopram showed that the methods were precise.

Reproducibility
Three different standard working solution-containing Nefopam and Escitalopram was prepared. The absorbance of prepared mixture of standard solution was measured 3 times as a test sample. From the respective absorbance counts, the concentration of Nefopam and Escitalopram was calculated (Table 4).

Table 4: Reproducibility of Nefopam and Escitalopram.

Compound	Conc. µg/ml	Absorbance (mean±SD)	Equation	Measured Conc. (µg/ml) (mean±SD)	%RSD	% Deviation
Nefopam	50	0.127±0.002	Y=0.002X-0.003	49.999±0.787	1.574	1.601
	100	0.251±0.007		99.999±2.873	2.873	0.001
	150	0.388±0.001		150.26±0.222	0.147	-0.171
Escitalopram	25	0.109±0.005	Y=0.003X+0.018	24.999±1.146	4.584	0.004
	50	0.187±0.003		50.087±0.939	1.874	-0.173
	75	0.262±0.004		75.095±1.191	1.585	-0.126

%RSD (Relative Standard Deviation) = SD (Standard Deviation) X 100/mean; Y=mX+C, where, Y=absorbance, X=concentration (µg/ml), m=slope and C=intercept.
% Deviation = (Theoretical concentration – measured concentration) X 100/measured conc.

The values of % RSD were 0.147-2.873 for Nefopam and 1.585-4.584 for Escitalopram. So, the values of %RSD and repeatability study showed that the developed methods were reproducible.

Sensitivity
The sensitivity (Sathe et al., 2007) of measurement of Nefopam and Escitalopram was estimated in terms of the limit of quantification (LOQ) and the limit of detection (LOD) (Table 5).

Table 5: Sensitivity of Nefopam and Escitalopram.

Compound	Conc. µg/ml	Absorbance	Equation	SD	LOD	LOQ
Nefopam	50	0.127	Y=0.0026X-0.005	0.131	0.393	1.310
	100	0.259				
	150	0.389				
Escitalopram	25	0.104	Y=0.0034X+0.011	0.081	0.243	0.810
	50	0.184				
	75	0.267				

The detection wavelength of 266 and 284nm were chosen in order to achieve a good sensitivity for quantitative determination of Nefopam and Escitalopram in tablet dosage form. The values of SD, LOD and LOQ were found to be Nefopam, Escitalopram were 0.131 and 0.081, 0.393 and 0.243 and 1.310 and 0.810. These values showed that the developed methods were sensitive.

Determination of Potency
The potency was determined for five different marketed brands of Nefopam and Escitalopram tablets shown in Table 6.

Table 6: Potency of Nefopam and Escitalopram.

Compound Claimed amount	Avg. wt. of each tablet (mg)	Avg. amount of powder (mg)	Dilution factor	Absorbance (mean±SD)	Amount (mg) (mean±SD)	% potency (mean±SD)
Nefopam 30 mg	153.40	153.50	100	0.714±0.001	27.65±0.04	92.16±0.13
Escitalopram 10 mg	118.00	118.40	100	0.356±0.002	10.20±0.07	102.06±0.70

At the selected wavelengths, the percent potencies were found to be 92.16 and 102.06 for Nefopam and Escitalopram respectively. The potencies of the tablets were complying with their claimed quantity (±10%).

CONCLUSION

As INN drugs are not included in the BP or USP, so there are no official methods for the assay of these drugs. Therefore, certain analytical methods must be developed for the assay of such drugs. Various analytical methods have been reported for the assay of Nefopam and Escitalopram, however, no UV-Spectrophotometric study has been found in literature survey in tablets dosage forms. UV Spectroscopy method is one of the instrumental analytical methods that are widely used in pharmaceutical industries for the assay of pharmaceutical products, because it is simple, easy, less time consuming and an economical method.

Various solvents were used to find out the medium in maximum which solubility of each drug may be achieved. The detection wavelengths of Nefopam at 266nm and Escitalopram at 284nm respectively in water were chosen in order to achieve a good sensitivity in tablet dosage forms. The percent potency of all tablet were complied with their claimed quantity (±10%). The study shows that the developed method is simple, sensitive, accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviations which make it versatile and valuable and use for the analysis of these drugs in tablets dosage form.

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