Method development and validation of UV spectrophotometric method for Estimation of Nevirapine in human plasma

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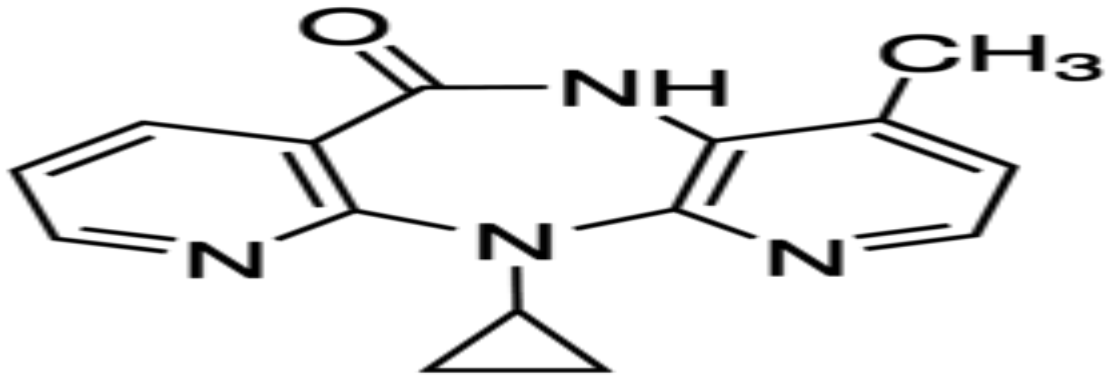
Astract: *A rapid and simple method of spectrophotometric estimation in UV-region has been developed for the estimation of nevirapine in spiked human plasma .this is accomplished by extracting nevirapine from spiked human plasma using ethyl acetate and after extraction it was scanned between 200-400nm by using UV detector and its absorption maxima (λ_{max}) was found to be 282.5nm.The calibration curve was linear in the range of 6-20 µg/ml.Recovery and assay studies of nevirapine were within 98 to 99% indicating that the prposed method can be adaptable for quality control analysis of nevirapine.*

KEYWORDS:*Nevirapine,liquid-liquid extraction,human plasma,UV-method.*

1.Introduction

Nevirapineis chemically 2-cyclopropyl-7-methyl-2,4,9,15-tetrazatricyclopentadeca-1(11),3,5,7,12,14-hexaen-10-one.

1.1.Structure of nevirapine



Nevirapine is a non-nucleoside reverse transcriptase inhibitor (nNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase

activities by causing a disruption of the enzyme's catalytic site. The activity of nevirapine does not compete with template or nucleoside triphosphates.

2. Introduction to UV spectroscopy

UV spectroscopy is a absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n,π or σ or electrons.These bonding(σ and π) and non-bonding(n) electrons absorb the radiation and undergoes transition from ground state to excited state . By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated easily.

UV spectroscopy obeys the Beer-Lambert law,

Beer law:This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances,the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

$$I=I_0*e^{-k1*c} \quad \text{——————} \quad 1$$

Where, I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute
K_1=constant

Lambert's law:This law can be stated as follows "Whwn a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly propotional to the intensity of the light".

$$I=I_0*e^{-k2*l} \quad \text{——————} \quad 2$$

Where ,I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
L = length of sample cell (cm.)
K_2=constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$$A = \log (I_0/I) = \varepsilon cl \quad \text{——————} \quad 3$$

Where, A = absorbance
I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute

L = length of sample cell (cm.)
Ɛ = molar absorptivity

A literature search has shown that there are less quantitative analytical methods for estimation of rivastigmine [1,2]; further, very few methods were available that shows the quantification of rivastigmine in biologicalfluids [3,4]; these methods include LC-MS and GC-MS, which needs high end instrumentation which are costly and not available in regular bioanalytical laboratory. Thus,it was clear that there was a necessity to develop a rapid, simple and economical method. Our method was based on liquid–liquid extraction (LLE) for sample preparation and UV detection for the quantification of rivastigmine from spiked human plasma.

3. Bioanalytical Method Validation And Sample Preparation

3.1.Bioanalytical Method Validation

A bioanalytical method involves the collection, processing, storage, and analysis of a biological matrix for a chemical compound. Bioanlysis involves the quantitative estimation of Xenobiotics of drugs such as their metabolites etc, Bioanalytical method validation includes all of the procedures that shows us that the method developed and used for quantitative estimation of analytes in the given biological matrix is reliable and reproducible [5]. Validation of a bioanalytical method is the process by which it is established that the .The fundamental bioanalytical method validation parameters like precision and accuracy, sensitivity,robustness and ruggedness requirements are met.

3.2.Sample Preparation

The sample preparation portion of the analysis is often the most vital part there is time involed in extracting the analyte and also this part is the difficult one. Each matrix has its own unique properties. For example, in urine we find high salt content, plasma consist of lot of phospholipids, whole blood consist of red blood cells that must be lysed [6] .The different analyte has different characteristics and the matrix is the one that decides the type of extraction approach that should be used. Good bioanalysis begins with careful and good sample collection procedures. Thus, the samples integrity must be preserved from the time of collection till the time it is used for analysis, in such a manner that the determined concentration closely reflects the in vivo concentrations.

The most frequently used matrix is plasma; however, depending on the drug’s metabolic behaviour and properties the matrix might change. Sometimes it is appropriate to know the concentration of drug in the urine , this further helps us understand the drug’s behaviour used for analysis, especially if most of the unchanged drug is excreted by urine. The vital point in biological sample collection is to collect them rapidly and store them at the appropriate temperature and it is an important part that the samples are labelled correctly.

The different types of bioanalytical sample preparation are

a. Solid phase extraction

b .liquid-liquid extraction

c. Protein precipitation

B.Liquid-Liquid Extraction

The bioanalytical sample preparation method used in the current experiment is liquid–liquid extraction.It is also known as partitioning.It is a separation process which involves the transfer of a solute from one solvent to another,the two solvents maybe immiscible or partially miscible with each other [7] . Usually,one of the solvent is water or aqeous mixture and other is a non-polar organic liquid.As in all extraction processes,liquid-liquid extraction consists a step of mixing followed by a step of phase separation.

4. Experimental

4.1.Instrumentation

Double beam UV spectrophotometer; Model : SL 210 ; Make: ELICO.The data was obtained using Spectra Treats 3.11.01Rel 2b

Vortex mixer ; Model:CM 101; Make: REMI

Hot Air Oven, Model:PSI-003;Make:PSI

4.2.Materials

Nevirapine was obtained as a gift from a pharmaceutical company. Nevirapine tablets containing 200 mg was obtained from market .Analytical grade sodium hydroxide, ethyl acetate and methanol was obtained from SD fine chemicals , Mumbai, India .

4.3. Procedure

4.3.1. Selection of wavelength

10mg of nevirapine drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with methanol as diluent .Then from this 0.1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with methanol to give 10ppm solution and this was scanned between 200 to 400 nm and its absorption Maxima was identified as to 282.5nm(Figure-1)

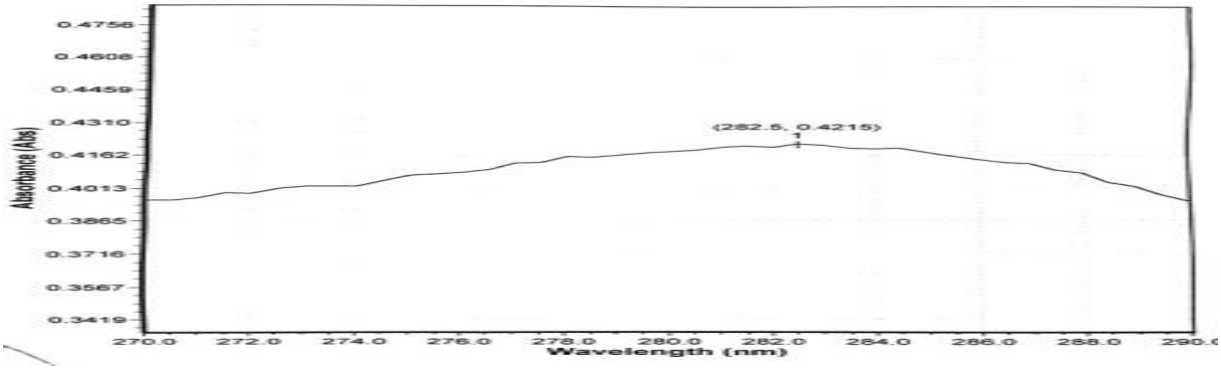


Figure-1: UV spectrum of nevirapine

4.3.2.Assay

4.3.2.1.Standard preparation

10mg of nevirapine drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with methanol to give 1000ppm.From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to give 10ppm solution and its absorbance was measured at 282.5nm.

4.3.2.2.Test preparation

20 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of nevirapine was weighed and taken into 10ml volumetric flask then volume was made upto the mark with methanol .From that 0.1 ml of solution was withdrawn and taken in 10ml volumetric flask. The volume was adjusted with diluent up to 10ml to get 10ppm solution and its absorbance was measured at 282.5nm.

The % Assay is calculated by using the following formula

% Assay=((absorbance of the sample/absorbance of the standard)*(concentration of the standard/concentration of the sample))*100

4.3.3.Preparation of standard stock solution

10mg of nevirapine drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with methanol to give 1000ppm

4.3.4.Preparation of 20ppm standard solution

From the standard stock solution 0.02 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to give 20ppm solution and its absorbance was measured at 282.5nm

4.3.5. Spiking procedure

2.5ml of human plasma was taken in a centrifuge tube and 2.5ml of 20ppm nevirapine solution was added.

4.3.6. Extraction procedure

From the drug spiked plasma 1ml was withdrawn and vortexed for 1 minute. After which 0.5ml of 0.5 N sodium hydroxide was added and vortexed for 1 minute. The drug was then extracted using 5ml of Ethyl Acetate by vortexing for 10 minutes .Then it is allowed to stand for 20 minutes .after this the organic layer get separated and this Organics layer is taken in a china dish and evaporated in a hot air oven for 10 minutes .Then 0.1ml was taken from the china dish after evaporation and the volume was made up to 10ml with methanol and then it is measured at 282.5 nm and its absorbance was noted.

5. Method validation parameters

5.1. Linearity

From the stock solution 0.06 ml , 0.09 ml, 0.12 ml, 0.15 ml and 0.18 ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml with methanol to give 6ppm, 9ppm, 12ppm. 15 ppm and 18 ppm solutions respectively and absorbance was measured at 282.5 nm using methanol as blank and the calibration curve is plotted.

5.2. Precision

10ppm standard solution of nevirapine pure drug is selected for Precision study. From the standard stock solution 0.1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using methanol to give 10ppm solution .This procedure is repeated 6 time and observances of all were measured at 282.5 nm using methanol as blank and its %RSD was calculated by using the formula

%RSD = (standard deviation of the measurement / mean value of measurement) *100

5.3. Accuracy

Accuracy of the method was ascertained by standard addition method at three levels. Standard quantity equal into 50% , 100% and 150 % is to be added in sample.

5.3.1. Preparation of sample solution: 6ppm of sample solution is prepared after appropriate solutions

5.3.2. Preparation of standard solution: 3ppm, 6ppm, 9ppm standard solutions were prepared after appropriate dilutions from the standard stock solution.

5.3.3.Standard addition method: Three 10 ml volumetric flasks are taken ,into the first 2 ml of 3ppm standard solution is added and to the second one 2 ml of 6ppm standard solution is added and into the third 2 ml of 9ppm standard solution is added and then to each volumetric flask 2 ml of 6 ppm. sample solution is added and they are thoroughly mixed and this is repeated three times and their absorbance is measured at 282.5 nm and the %recovery is calculated by using the formula :

% Recovery = (amount found / amount added) *100

5.4. Limit of Detection

The detection limit (DL) may be expressed as:

$DL = 3.3*\sigma/ S$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

5.5. Limit of Quantification

The quantitation limit (QL) may be expressed as:

$QL = 10*\sigma / S$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

5.6.Robustness

6 aliquots of 12ppm standard solutions were prepared and was scanned at wavelength +/-1 nm of absorption maxima the absorbance values was noted down.

6. Results and discussion

The %assay was found to be 99%.

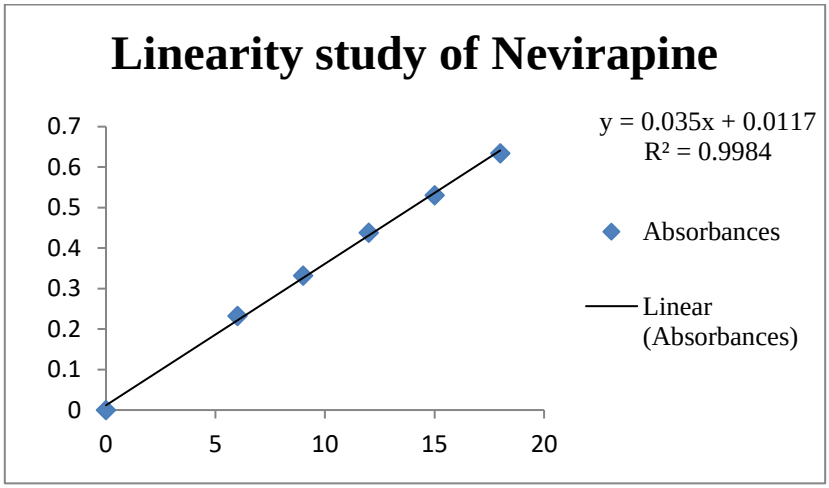


Figure-2: Linearity curve of nevirapine

Concentration(ppm)	Absorbance(nm)
0	0
6	0.2325
9	0.3326
12	0.4383
15	0.5308
18	0.6345

Table-1 : Conc. Vs Abs. table for Linearity Study

Sample no.	%RSD
1	0.4383
2	0.4383
3	0.4484
4	0.4484
5	0.4382
6	0.4482
Mean	0.4434
SD	0.0055143
%RSD	1.2

Table-2: Evaluation data of precision study.

% Recovery level	%Recovery	Mean % recovery
50%	99.65	99.66
	99.75	
	99.60	
100%	99.78	99.81
	99.89	
	99.78	
150%	99.76	99.76
	99.65	
	99.87	

Table-3: Evaluation data of accuracy study.

The limit of detection was found to be 0.51 ppm and limit of quantificationis found to be 1.57ppm.

Sample no.	281.5nm	282.5nm	283.5nm
1	0.4383	0.4381	0.4382
2	0.4383	0.4382	0.4383
3	0.4382	0.4383	0.4380
4	0.4383	0.4383	0.4384
5	0.4380	0.4383	0.4382
6	0.4381	0.4383	0.4383
Mean	0.4383	0.43825	0.43823
SD	0.000136	0.000083	0.00013
%RSD	0.031	0.0189	0.029

Table-4: Evaluation data of robustness study.

7. Acknowledgment

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