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# Development, Validation and Application of a Spectrofluorimetric Method for the Quantification of Nevirapine in Pharmaceutical Formulations Tablets and Suspensions

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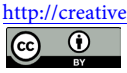



## Abstract

The development of the spectrofluorimetric method can be considered a promising alternative that is relatively less expensive and sufficiently reliable. In the current literature, no method for the analysis of nevirapine by spectrofluorimetric has been reported. The proposed method is based on the transformation of naturally non-fluorescent nevirapine into a fluorescent derivative after chemical synthesis. Maximum excitation and emission wavelengths are 290 nm and 357 nm respectively. The analytical performance of the method demonstrates linearity in the concentration range $1.5 \times 10^{-2}$ and $13.5 \times 10^{-2}$ µg/mL with a correlation coefficient (r) greater than 0.999. The detection (LOD) and quantification (LOQ) limits found are $1.97 \times 10^{-3}$ µg/mL and $5.48 \times 10^{-3}$ µg/mL respectively. Recovery is achieved with 99.9% and 100.3% trueness, intra-day precision with a coefficient of variation of repeatability (CVr) of 0.99% and inter-day precision with a coefficient of variation of precision (CVR) of 1.7%. The method has been successfully applied in the analysis of 10 batches of nevirapine tablets and suspensions.

## Keywords

Spectrofluorimetric Method, Validation Nevirapine, Sodium Hypochlorite

## 1. Introduction

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which,

by binding directly to HIV-1 reverse transcriptase, slows viral Deoxyribonucleic acid (DNA) synthesis and thereby inhibits viral replication. In recent years, the use of nevirapine (alone or in combination) has generated considerable interest because, as shown in clinical trials, it is effective in reducing mother-to-child transmission, inexpensive and convenient to use in PMTCT programs [1]-[6].

The nevirapine drug substance is a white to off-white crystalline powder. Nevirapine is highly lipophilic. It is only slightly soluble in water (0.1 mg/ml), forming a clear colorless solution, and is relatively insoluble in non-polar media. The nevirapine used in the formation of the tablets is anhydrous with a molecular weight of 266.3 g/mol. It is a low molecular weight compound that is lipophilic (partition coefficient = 83) and has a weak base (pKa = 2.8) [7].

At pH values below the pKa, nevirapine is very soluble in an aqueous buffer. At higher pH values, the water solubility of nevirapine decreases asymptotically to about 0.1 mg/ml.

Nevirapine is a weak base because of the two pyridine nitrogens. The ionization constants measured are pKa1 = 2.8; pKa2 = −0.4. The first and second ionization constants were determined by spectrophotometry (UV) and by NMR, respectively [8]. Nevirapine exhibits solubility in chloroform; sparingly soluble in methanol [9].

Analytical methods are reported in the literature for the determination of NVP in tablets and suspensions. They employ techniques such as capillary electrophoresis [10] [11], electroanalysis [12]-[23], spectrophotometry [24]-[33], high performance liquid chromatography (HPLC) [24] [28] [34]-[63], liquid chromatography coupled with mass spectrometry LC/MS/MS [64]-[77], gas chromatography coupled with mass spectrometry (CG-MS) [78] and high performance thin layer chromatography (HPTLC) [58] [79]. The International Pharmacopoeia, USP, British and European Pharmacopoeia have adopted the HPLC method for the quantitative analysis of nevirapine in formulations [80] [81] [82] [83]. The high-performance liquid chromatography method is the most documented analysis technique for nevirapine in the literature due to its sensitivity, repeatability and specificity.

Very often, the proposed mobile phases consist of compounds such as ammonium acetate, potassium hydrogen phosphate, phosphoric acid, etc., respectively, which could shorten the lifetime of a column. Furthermore, the preparation of the mobile phase requires tedious procedures and most of the reported liquid chromatography methods use an internal standard [24].

In the current literature, no method for the analysis of nevirapine by spectrofluorimetric has been reported. The spectrofluorimetric method can be considered a promising simple, faster, direct, sensitive, selective and relatively cheaper; alternative for the determination of nevirapine content in pharmaceutical formulations with sufficient reliability.

The objective of this study is to develop and validate a method for assaying nevirapine in pharmaceutical form (tablet or oral suspension).

## 2. Method

### 2.1. Instruments

Spectrofluorimeter Perkin-Elmer UK model LS 45, equipped with a 150 W Xenon arc lamp, grating excitation and emission monochromator coupled to a Fuitsusiemens computer with Winlab software version 4.00.02/2001. Starsonic C200 ultrasonic shaker was used for solution degassing, pH meter Metrohm 913.

Statistical analysis was performed using minutab 18 and origin 2019b software. The later origin was also used to draw the figures.

### 2.2. Materials and Reagents

Nevirapine reference substance USP 99.5%, Zidovudine reference substance USP 99.4%, Lamivudine reference substance USP 99.7%, Methanol 99.9% and sodium hydroxide 98.5% supplied by Sigma-Aldrich. Sodium hypochlorite of the brand Maadar manufactured and marketed in Senegal was used. The galenic forms were found in the health structures of the Eastern Democratic Republic of the Congo and Dakar in Senegal. The distilled water of analytical quality was used.

### 2.3. Preparation of Solutions

#### 2.3.1. Synthesis of Fluorescent Product

The nevirapine stock solution was prepared by dissolving 10 mg of nevirapine in 10 mL of methanol. After complete dissolution 1 mL of this solution was diluted to 10 mL in distilled water; then approximately 300 µL of this was mixed with 10 mL of 0.1N NaOH and 300 µL of sodium hypochlorite. This was incubated at 60˚C for 10 minutes. After cooling 150 µL were diluted to 10 mL with distilled water.

#### 2.3.2. Preparation of the Standard Validation Solution

The stock solution of nevirapine was prepared by dissolving 10 mg of nevirapine in 10 ml of methanol. After complete dissolution 1 mL of this solution was diluted to 10 mL in distilled water; following this, the respective volumes of 260, 300 and 340 µL were mixed with 10 ml of 0.1N NaOH and 300 µL of sodium hypochlorite. This was incubated at 60˚C for 10 minutes. After cooling 150 µL were diluted to 10 mL with distilled water.

#### 2.3.3. Preparation of sample validation solutions

1) *Constitution of the matrix*

The matrix is made up of 4.00 g of starch, 4.00 g of sodium saccharin and 4.00 g of mannitol after trituration in the mortar with a pestle; the matrix was kept for the preparation of the validation solution.

2) *Preparation of the sample*

The validation sample consists of 6 g of matrix and 2 g of nevirapine by trituration in a mortar with a pestle.

The validation solutions were established from the validation sample by making a test portion containing 8.00 mg, 10.00 mg and 12.00 mg of nevirapine in

10mL of methanol corresponding to 80%, 100% and 120% solutions respectively. After sonication for 30 min and filtration, 1 mL of the filtrate was diluted to 10 mL in distilled water; subsequently about 300 µL of the latter were mixed with 10 mL of 0.1N NaOH and 300 µL of sodium hypochlorite. This was incubated at 60˚C for 10 minutes. After cooling 150 µL were diluted to 10 mL with distilled water.

## 2.4. Optimisation of Experimental Parameters

The reaction of synthesis of the fluorescent derivative is carried out between pH 1 to 12 by a mixture of nevirapine and sodium hypochlorite, which are constituted in the presence of 10ml of the respective buffer solutions of pH 1 to 12. The effect of the concentration for it is evaluated by the variation in the general method of the concentration of sodium hypochlorite while maintaining constant that of nevirapine; Then the variation of the concentration of nevirapine while maintaining that of sodium hypochlorite. To optimize the temperature, the reaction is carried out for 90 min at temperatures of 30˚C, 40˚C, 50˚C, 60˚C and 70˚C.

## 2.5. Method Validation

The method validation study was conducted for linearity, trueness, precision, sensitivity, selectivity, and recovery, in accordance with the guidelines of the ICH International Conference on Harmonization [84].

### 2.5.1. Linearity

The stock solution of 1 mg/mL of nevirapine was prepared in methanol. After complete dissolution, 1 ml of this solution was diluted to 10 ml in distilled water; then 0.1, 0.3, 0.5, 0.7 and 0.9 ml were mixed respectively with 10 ml of 0.1 N NaOH and 300 µL of sodium hypochlorite. This was incubated at 60˚C for 10 minutes. After cooling, 150 µL were diluted with 10 mL of distilled water. Thus the solutions obtained have the following respective concentrations: $1.5 \times 10^{-2}$; $4.5 \times 10^{-2}$, $7.5 \times 10^{-2}$, $10.5 \times 10^{-2}$ and $13.5 \times 10^{-2}$ µg/mL.

Subsequently, the validity of the proposed linearity concentration was confirmed by plotting the ratio graph of response and concentration [34].

### 2.5.2. Trueness and Precision

The trueness, intra-day and inter-day precision of the method were determined by the mean percentage recovery (n = 9). The samples were subjected to analysis, emission intensities were observed, and percentage accuracy or percentage RSD values were calculated. The trueness of the method was also confirmed based on the criteria that the deviation from the nominal concentration must be ± 5% for the three concentration levels and the three repetitions.

### 2.5.3. Sensitivity

Sensitivity was established in terms of limit of detection (LOD) and limit of quantification (LOQ).

#### 2.5.4. Selectivity

Pharmaceutical formulations contain inert ingredients (excipients) such as talc, magnesium stearate, mannitol, starch, lactose, sugar and sometimes even associated active ingredients, which can sometimes interfere with the analysis.

The study of the specificity and selectivity of the nevirapine assay was carried out in the presence of two molecules often associated with nevirapine in the galenic forms currently available, zidovudine (AZT) and lamivudine (3TC), a hand, and also in the presence of the three major excipients of the solid and liquid dosage forms (starch, mannitol and sugar) on the other hand. Active ingredient-excipient ratios of 1:1, 1:3 and 1:6 were considered.

### 2.6. Application to the Quantification of Pharmaceutical Forms

Ten tablets containing nevirapine were weighed, finely powdered and thoroughly mixed; then an accurately weighed amount of the powder equivalent to 10.0 mg NVP was transferred to 25 mL volumetric flasks and extracted with 10 mL methanol. The flasks were sonicated for 30 min and then filtered. 1 mL of the filtrate was diluted to 10 mL in distilled water; then approximately 300 µL of this were mixed with 10 mL of 0.1N NaOH and 300 µL of sodium hypochlorite. This was incubated at 60˚C for 10 minutes. After cooling, 150 µL were diluted to 10 mL with distilled water.

## 3. Results and Discussion

### 3.1. Determination of Spectra after Synthesis Reaction

The excitation and emission spectra of nevirapine-sodium hypochlorite are well resolved and characterized by bands (peaks) located at wavelengths of 290 nm maximum excitation and 357 nm maximum emission (Figure 1). The emission

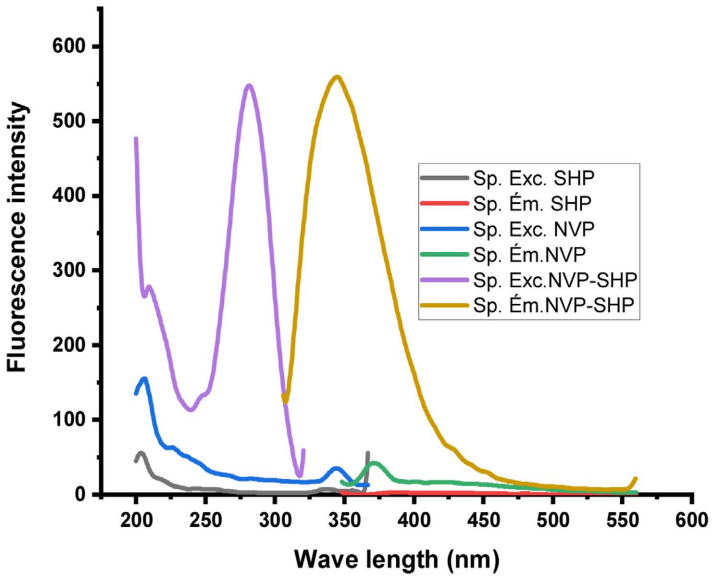


Figure 1. Excitation and emission spectrum of NVP-SHP with SHP = NaOCl.

of a fluorescent signal from the nevirapine-sodium hypochlorite mixture is an indicator of a reaction that has occurred between the two molecules. These two compounds are naturally non-fluorescent, hence the regeneration of a highly fluorescent compound resulting from chemical synthesis.

Thus, nevirapine undergoes a reaction in the presence of sodium hypochlorite in a strongly basic aqueous medium to give a synthetic nevirapine derivative that is highly fluorescent with excitation between 275 nm - 375 nm (maximum at 290 nm) and emission between 350 nm - 475 nm maximum at 357 nm).

It is established in the literature that secondary amides ($R_1$-CO-$NHR_2$) are hydrolyzed in the presence of sodium hypochlorite in strongly basic media.

It has been established by several authors that secondary amides ($R_1$-CO-$NHR_{2)}$ are hydrolyzed in the presence of sodium hypochlorite plus sodium hydroxide under exceptionally mild conditions (room temperature, absence of light, good agitation) to give the corresponding carboxylic acids [85] [86]. This hydrolysis gives the amide function of NVP the opportunity to undergo the Hoffman reaction (the Hoffmann rearrangement), also known as the Hofmann degradation [87] [88] which is a reaction of an amide with a halogen (chlorine or bromine) in a strongly basic aqueous medium (sodium or potassium hydroxide) to convert the amide into a primary amine. This proposed reaction mechanism between nevirapine and sodium hypochlorite in a basic medium is presented in **Figure 2**,

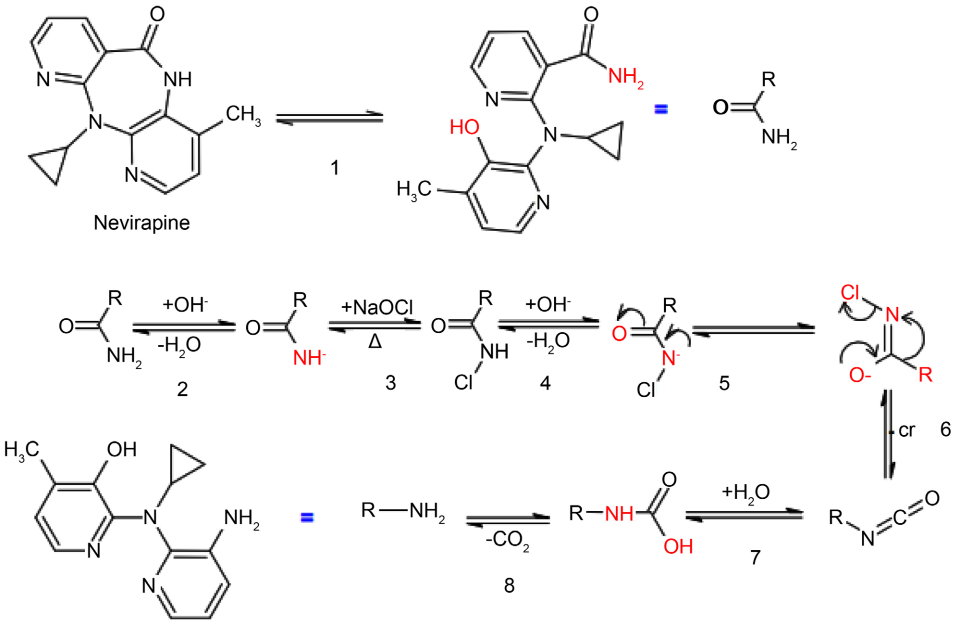


**Figure 2.** Synthesis reaction mechanism (Hoffman reaction). 1. Hydrolysis of Nevirapine; 2. The base extracts an acidic NH proton, giving an anion; 3. The anion reacts with sodium hypochlorite ($Cl^-$) in a substitution reaction to give an N-Chloro-2-; 4. The base deprotonates NH acid, giving an anion; 5. Electronicrearrangement; 6. The R group attached to the carbonyl carbon migrates to the nitrogen as the chloride ion leaves, giving an isocyanate; 7. The isocyanate adds water in a nucleophilic addition step to give a carbamic acid; 8. The carbamic acid spontaneously loses $CO_2$, giving the product amine attached to the radical R.

would justify the enhancement of the fluorescence intensity, thus reflecting the formation of a fluorescent derivative.

## 3.2. Optimisation of Experimental Parameters

### 3.2.1. Effect of pH

The fluorescence signal intensity of many fluorophores is sensitive to changes in the pH of the medium [89]. The pH is an important factor in chemical reactions, so solutions of phosphoric acid (0.1 M) and NaOH (0.1 M) were used to get the pH variations between 2 and 13.

However, in an alkaline environment the fluorescence signal increases progressively with increasing pH values, reaching a maximum at pH 12.5 (**Figure 3**). This is justified by the fact that the Hofmann rearrangement involving the amide and sodium hypochlorite only takes place in a basic medium.

### 3.2.2. Optimal Concentration of Sodium Hypochlorite

Nevirapine prepared at a concentration of 0.1 mg/mL was mixed with sodium hypochlorite ranging from 0.008˚ to 2.00˚ in chlorine.

There is an increase in fluorescence intensities (FI) with increasing sodium hypochlorite up to a certain level of chlorine followed by a plateau which is subsequently accompanied by a decrease in FI with increasing sodium hypochlorite (**Figure 4**). The plateau zone is where the response is maximal, whereas a subsequent increase in sodium hypochlorite causes a fluorescence inhibiting effect. Under these working conditions, the use of sodium hypochlorite between 0.16˚ - 0.64˚ in $Cl^-$ facilitates the reaction of fluorescent nevirapine derivative formation.

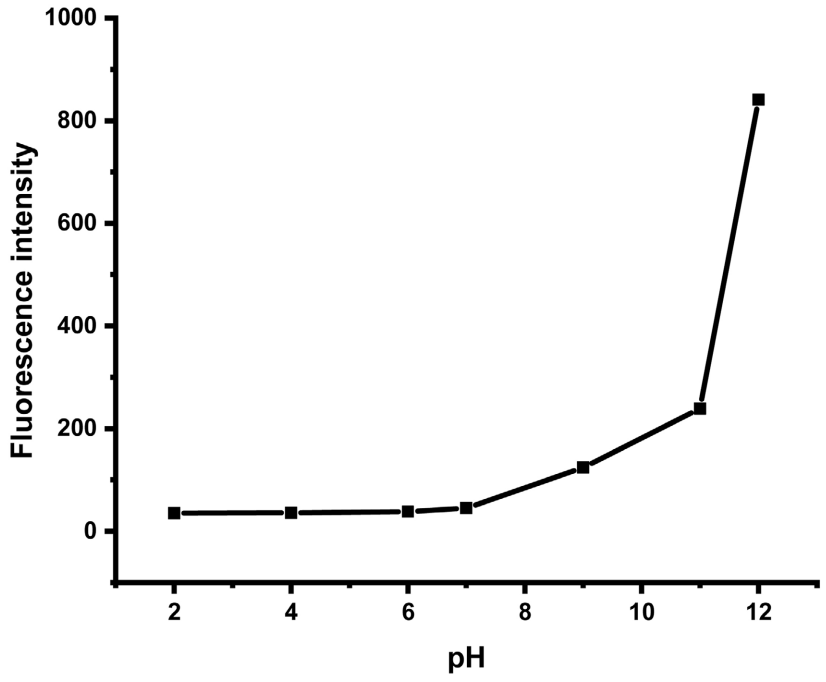


**Figure 3.** Impact of pH.

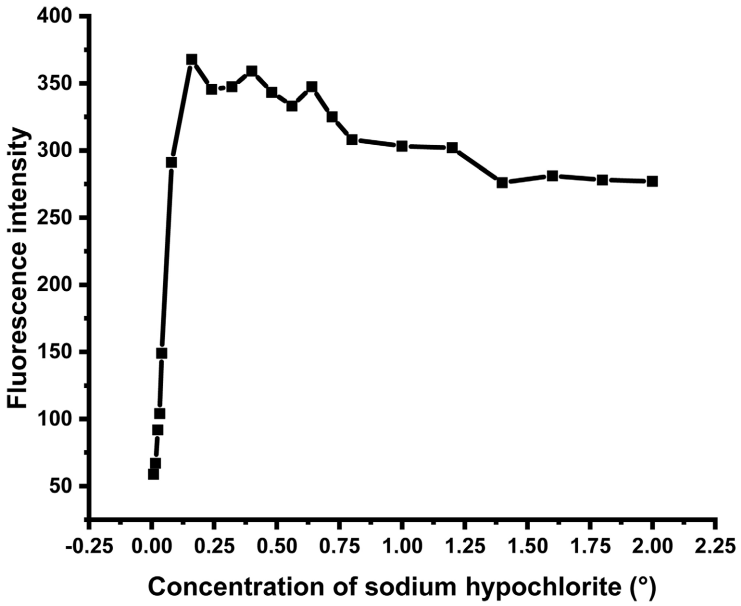


**Figure 4.** Effect of sodium hypochlorite concentration on FI.

### 3.2.3. Effect of Nevirapine Concentration

To test the effect of nevirapine concentration, the concentration of sodium hypochlorite was kept constant while varying the concentration of NVP (nevirapine). No band shifts were observed and no changes in the shape of the fluorescence spectra in the basic medium were observed with the change in concentration. However, there was an increase in fluorescence intensity with increasing concentration (**Figure 5**).

### 3.2.4. Effect of Temperature

Forced degradation generally takes place at more than 10˚C above the accelerated stability study temperature of 50˚C or 60˚C [90].

To evaluate the effect of heat on the kinetics of fluorescent derivative formation in the presence of sodium hypochlorite, the heating time and temperature of the heat treatment were determined. The plotted curve shows maximum and stable fluorescence intensities between 50˚C and 70˚C with a stabilisation at 60˚C (**Figure 6**). Thus, this increase would be due to the favourable reaction at these temperatures.

Treatment at 60˚C for 10 - 15 minutes resulted in a higher amount of fluorescent transformed species, which decreased after 20 minutes of treatment at 60˚C (**Figure 7(a)** and **Figure 7(b)**).

## 3.3. Stability Kinetics

The stability kinetics of the product formed was carried out under optimized experimental analytical conditions and performed immediately after the reaction between the different components.

The stable signal for 1200 seconds (20 minutes) was recorded. The recorded FIs did not show any variations during the time of the stability study, which is

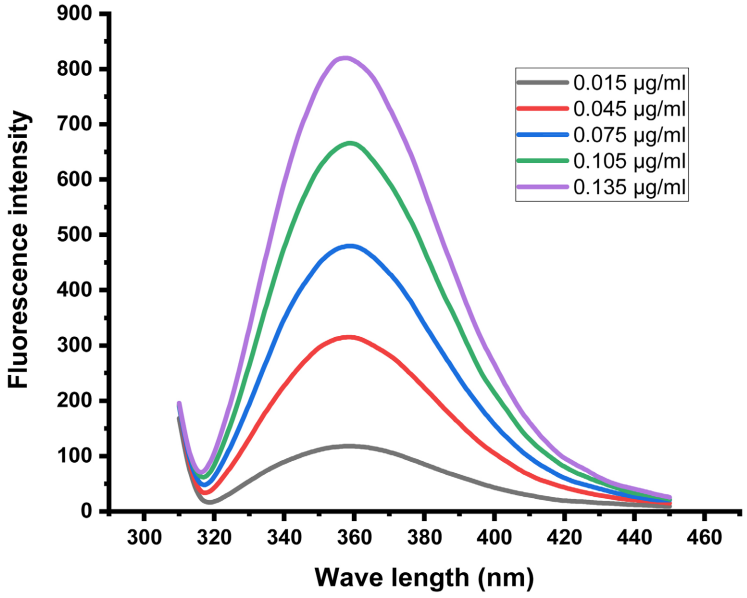


**Figure 5.** Effect of nevirapine concentration.

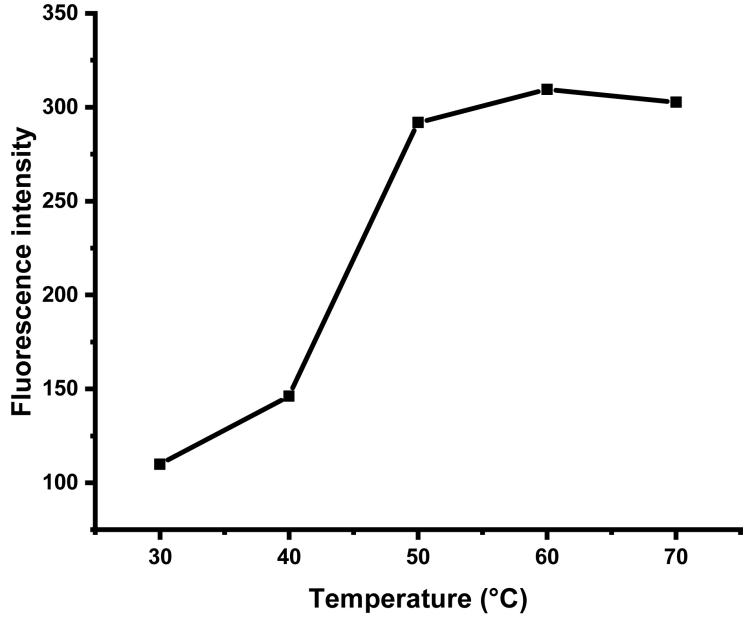


**Figure 6.** Effect of temperature on fluorescence intensity.

reflected by the straight line found parallel to the X-axis (**Figure 8**) and also by the analysis of the relative standard deviation, which is less than 0.5% (RSD = 0.33%) for n = 121.

## 3.4. Method Validation

### 3.4.1. Selectivity

Pharmaceutical formulations contain inert ingredients (excipients) such as talc, magnesium stearate, mannitol, starch, lactose, sugar and sometimes even associated active ingredients that can sometimes interfere with the analysis.

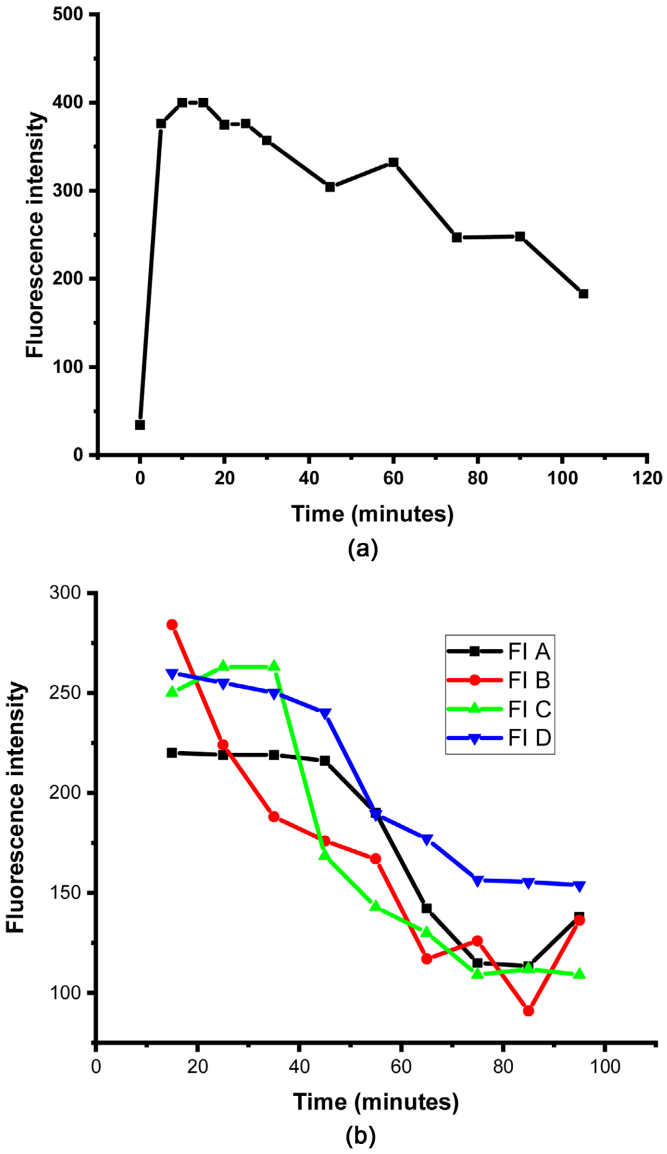


**Figure 7.** (a) effect of heating time on IF. (b) effect of heating time on IF.

The study of the specificity and selectivity of the nevirapine assay was carried out in the presence of two molecules that are often in combination with nevirapine in the currently available dosage forms, zidovudine (AZT) and lamivudine (3TC), on the one hand, and also in the presence of the three major excipients of the solid and liquid dosage forms (starch, mannitol and sugar) on the other hand. The active ingredient-excipient ratio 1.1, 1.3 and 1.6 was considered. No interference was observed in nevirapine dosing in the presence of these common excipients and active ingredients with recovery between 95.8% and 104.3% in **Table 1**.

### 3.4.2. The Calibration Curve

Linearity was established by the least-squares regression of the calibration curve.

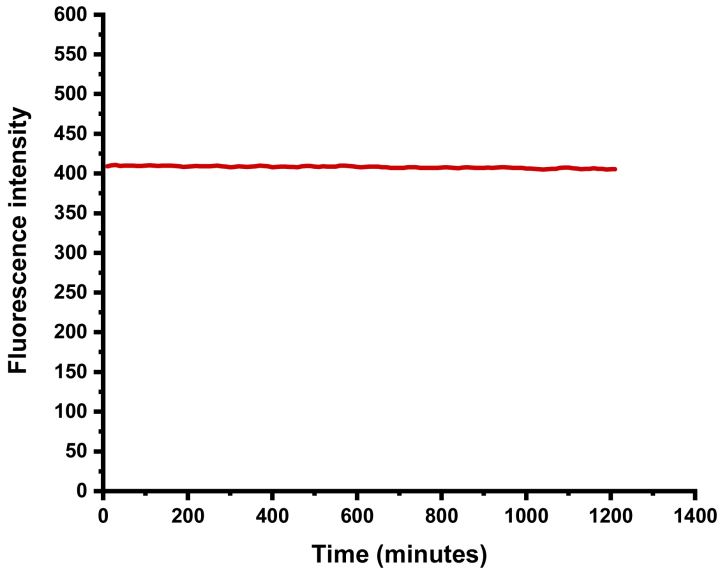


**Figure 8.** Signal stabilitykinetics.

**Table 1.** Percentage recovery of nevirapine (3 µg/mL) in the presence of excipients and other molecules in combination.

| Excipients | API ratio: Excipients | Added excipient (µg) | Recovery %. and RSD | |
|---|---|---|---|---|
| Mannitol | 1:1 | 3 | 96.4 | ±3.2 |
| | 1:3 | 9 | 96.9 | ±5.2 |
| | 1:6 | 18 | 101.8 | ±4.4 |
| Starch | 1:1 | 3 | 99.3 | ±3.9 |
| | 1:3 | 9 | 97.1 | ±3.7 |
| | 1.6 | 18 | 101.9 | ±1.7 |
| Saccharin | 1:1 | 3 | 99.3 | ±0.8 |
| | 1:3 | 9 | 95.8 | ±1.6 |
| | 1:6 | 18 | 102.5 | ±4.2 |
| 3TC | 1:1 | 3 | 103,0 | ±5.4 |
| | 1:3 | 9 | 103.9 | ±5.4 |
| | 1:6 | 18 | 98.3 | ±0.2 |
| AZT | 1:1 | 3 | 104.3 | ±0.2 |
| | 1:3 | 9 | 96.5 | ±4.8 |
| | 1:6 | 18 | 100.3 | ±1.0 |

This calibration curve was obtained over the concentration range of $1.5 \times 10^{-2}$ to $13.5 \times 10^{-2}$ µg/mL. The correlation coefficient (r) is greater than 0.999. The results showed an excellent correlation between fluorescence intensities and nevirapine concentrations. The regression equation is as follows: $y = 6017x + 33.6$ where the intercept and slope are 33.6 and 6017 respectively.

### 3.4.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limit is the lowest amount of an analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy [91]. They were determined as specified in the ICH (International Conference on Harmonisation) protocol.

$\mathrm{LOD} = 3.3\sigma/S$ $\mathrm{LOQ} = 10\sigma/S$ [84] where $\sigma$ = the standard deviation of the response and $S$ = the slope of the calibration. Hence the LOD and LOQ found are $1.97 \times 10^{-3}$ µg/mL and $5.48 \times 10^{-3}$ µg/mL respectively.

### 3.4.4. Sensitivity

The fluorometric method suggests very low detection limit ($1.9 \times 10^{-3}$ µg/mL) and LOQ quantification limit ($5.0 \times 10^{-3}$ µg/mL) values. This indicates a high sensitivity of the method developed for the dosage of nevirapine in pharmaceutical form. These extremely low values of the detection and quantification limits indicate much higher sensitivity of the method compared to methods found in previously published literature (**Table 2**).

### 3.4.5. Accuracy Profiles

Calibration standards were made for three concentration levels 80%, 100% and 120%, with two replicates per level and for three runs. The linear response function was found for each series, as well as the equation of the line, and the correlation coefficient.

The validation samples were established at 3 concentration levels, with 3 repetitions per level and for 3 series (different days), treated as real samples (in the matrix used in the routine). The results obtained (**Table 3** and **Table 4**) were calculated in concentration by the inverse relation of the response function (calibration standards).

The accuracy is measured by the bias (or recovery rate) between the assumed true value and the average of the results. The method is accurate if the average of the results is close to the true value. The trueness found is between 99.9% and

**Table 2.** Comparison of major characteristics of various methods for the determination of NVP.

| Range µg/mL | Regression coefficient | LOD µg/mL | LOQ µg/mL | Sample | Instrumental method | Réf |
|---|---|---|---|---|---|---|
| $1.5 \times 10^{-2}$ - $13.5 \times 10^{-2}$ | 0.999 | $1.9 \times 10^{-3}$ | $5.0 \times 10^{-3}$ | Pharmaceuticals | spectrofluorimetric | Presentwork |
| 4 - 30 | 0.9997 | $2.5 \times 10^{-1}$ | $7.6 \times 10^{-1}$ | Pharmaceuticals | Spectrophotometry | [24] |
| 5 - 40 | 0.997 | $1.37 \times 10^{-1}$ | $4.16 \times 10^{-1}$ | Pharmaceuticals | Spectrophotometry | [26] |
| 1 - 10 | 0.9998 | $1.2 \times 10^{-1}$ | $3.7 \times 10^{-1}$ | Pharmaceuticals | HPLC | [24] |
| 1.223 - 53.2 | 0.9996 | $1.94 \times 10^{-1}$ | $6.46 \times 10^{-1}$ | -------- | Fluorescence nano-probe | [23] |
| 0.1 - 4 | 0.995 | $1.13 \times 10^{-2}$ | $1 \times 10^{-1}$ | Human plasma | HPLC/UV | [34] |
| 10 - 40 | 0.996 | 3.6 | 10.8 | Pharmaceuticals | LC–MS/MS | [66] |

**Table 3.** Recovery.

| Recovery | Serie 1 | Serie 2 | Serie 3 |
|---|---|---|---|
| 80 | 97.3% | 101.1% | 99.8% |
| | 98.7% | 101.3% | 97.9% |
| | 98.2% | 99.9% | 97.5% |
| 100 | 98.9% | 102.9% | 99.6% |
| | 99.3% | 97.3% | 99.8% |
| | 97.8% | 100.0% | 100.4% |
| 120 | 99.5% | 99.8% | 97.2% |
| | 100.3% | 99.3% | 97.1% |
| | 100.0% | 100.2% | 100.9% |

**Table 4.** Summary of validation data.

| Concentration | Trueness | intra-day precision | inter-day precision | uncertainty | Lowertolerancelimit | Uppertolerancelimit | lowerlimit | Upperlimit |
|---|---|---|---|---|---|---|---|---|
| 80% | 100.1% | 0.9% | 1.1% | 2.7% | 97.4% | 102.9% | 95% | 105% |
| 100% | 100.3% | 1.7% | 1.7% | 4.1% | 96.2% | 104.3% | 95% | 105% |
| 120% | 99.9% | 1.4% | 1.4% | 3.4% | 96.5% | 103.2% | 95% | 105% |

100.3%, relative to the true value (100%).

The repeatability variances or intra-day precision (CVr) found for the method ranged from 0.9% to 1.7%. The intermediate fidelity variances or inter-day precision (CVR) were found to range from 1.1% to 1.7%. They are considered to be low because they are less than 2%. This implies that there is no significant difference between the different replicates of the input and inter-day analyses.

At a confidence level of 95%, for a degree of freedom of 6, the calculated t student is 2.45. The uncertainty was estimated between 2.7% to 4.1%.

Looking at the profile (**Figure 9**), one can see that the lower and upper tolerance limits found vary from 96.2% to 104.3% inside the acceptance limits. So this method is valid in this concentration range.

### 3.5. Application of the Method in the Analysis of Nevirapine in Pharmaceutical Forms

The content of the samples was determined for the 10 batches by the validated spectrofluorimetric method. **Table 5** represents the results obtained during the dosage of the pharmaceutical forms. These samples consist of an oral suspension of nevirapine, uncombined tablets and tablets combined with other antiretrovirals.

The recovery rates obtained vary from 91% to 105% for nevirapine. The margin is 100 plus at least 10% as acceptable limits of the United States Pharmacopoea [80]. The method has been applied successfully.

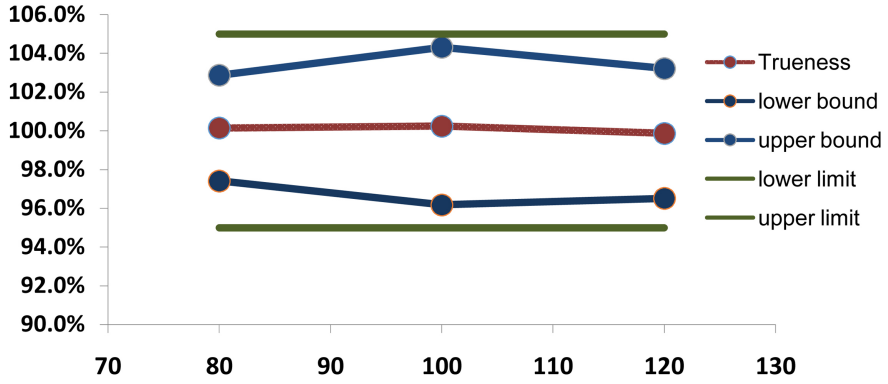


**Figure 9.** Diagram of the accuracy profile of proposed method.

**Table 5.** Sampleassay results.

| Number | Batch number | Mean | | Concentration % | RSD % |
|---|---|---|---|---|---|
| | | Sample | Standard | | |
| 1 | 7228211 | 362.60 | 342.30 | 105.9 | 3.5 |
| 2 | IA80534 | 329.42 | 325.96 | 101.1 | 2.8 |
| 3 | 3048961 | 334.24 | 325.96 | 102.5 | 3.9 |
| 4 | LZV18033 | 303,73 | 325.96 | 93.2 | 2.4 |
| 5 | LZV17032 | 298.57 | 325.96 | 91.6 | 2.6 |
| 6 | 8031425 | 300.99 | 325.96 | 92.3 | 1.0 |
| 7 | 3035942 | 289 | 326 | 88.6 | 2.1 |
| 8 | 3065693 | 321.1 | 329.3 | 97.5 | 0.7 |
| 9 | LZV18032 | 299.8 | 329.3 | 91.1 | 2.5 |
| 10 | LZV17070 | 325.5 | 329.3 | 98.9 | 4.6 |

## 4. Conclusion

The current study concerns the development and validation of a simple, rapid and selective method for the quantification of nevirapine in medicinal products. The accuracy profile is the analytical approach applied for method validation. The analysis of nevirapine goes through the chemical synthesis of a fluorescent derivative in this work. Method validation studies confirmed the excellent linearity, trueness, precision, high sensitivity and selectivity of the developed method. In addition, an application study of the method has been successfully carried out. The advantages of this method were its simplicity, speed and the fact that it did not require various elaborate treatments, which makes the developed method suitable in quality control laboratories for routine analysis.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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