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Spectrofluorimetric determination of benidipine in pharmaceutical preparation and spiked plasma samples using 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole

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ABSTRACT: A new spectrofluorimetric technique was produced for the analysis of benidipine (BDP) in tablets and spiked plasma samples. The developed method was based on coupling between Benidipine, (a secondary amine group), and 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) forming fluorescent dervatived as a NBD-Benidipine. The reaction was occured by using pH 8.5 buffer solution to form fluorescent derivatives that are measured λ_{em}: 550 nm and λ_{ex}: 465 nm. A variable parameters effecting on the derivatization process were studied. The calibration graph was linear in the range of 10–200 ng mL^{-1}. LOD and LOQ values were a 0.445 ng mL^{-1} and 1.348 ng mL^{-1}, respectivelly. The developed method was applied to BDP in commercially available film tablets. An average recovery was found as 99.80% without interference from the available excipients. Besides, the fluorimetric technique was also succesfully applied to spiked human plasma.

KEYWORDS: Benidipine; 7-fluoro-4-nitrobenzofurazan; spectrofluorimetry; pharmaceutical preparations; human plasma.

1. INTRODUCTION

Calcium antagonist group drugs are very frequently used cardiovascular drugs [1]. (±)-(R*)-2,6-Dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (R*)-1-benzyl-3-piperidinyl ester, methyl ester hydrochloride (benidipine hydrochloride) (Figure 1) is often used as an anti anginal and antihypertensive agent. Treatment may be administered daily for hypertension at a 4 or 8 mg single dose or twice a day [2, 3]. In a study on volunteers receiving a 16 mg of oral benidipine tablet, C_{max} value was found as 10.54 ± 6.02 ng mL^{-1} [4].

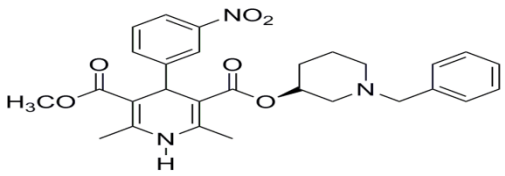

Figure 1. Chemical structure of benidipine.

Literature research has been shown in a couple of methods for determination of BDP such as spectrophotometric [5] voltammetric and HPLC [6] gas chromatographic [7–9] and LC–MS methods [10]. The purpose of the present method is to develop a novel spectrofluorimetric method based on the reaction between benidipine and 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) reagent. Amines react with NBD-F reagent to perform the fluorescent products with excitation at 460–470 nm and emission at 525–530 nm [11]. The developed study is a very sensitive fluorimetric technique for the analysis of BDP in tablets and spiked plasma samples.

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2. RESULTS AND DISCUSSION

BDP containes a secondary amino group which is easily reacting with NBD-F to produce a yellow-colored fluorescence in alkaline medium. The resulting derivative exhibits the highest fluorescence intensities by excitation at 465 nm and emmission at 550 nm in dichloromethane (Figure 2).

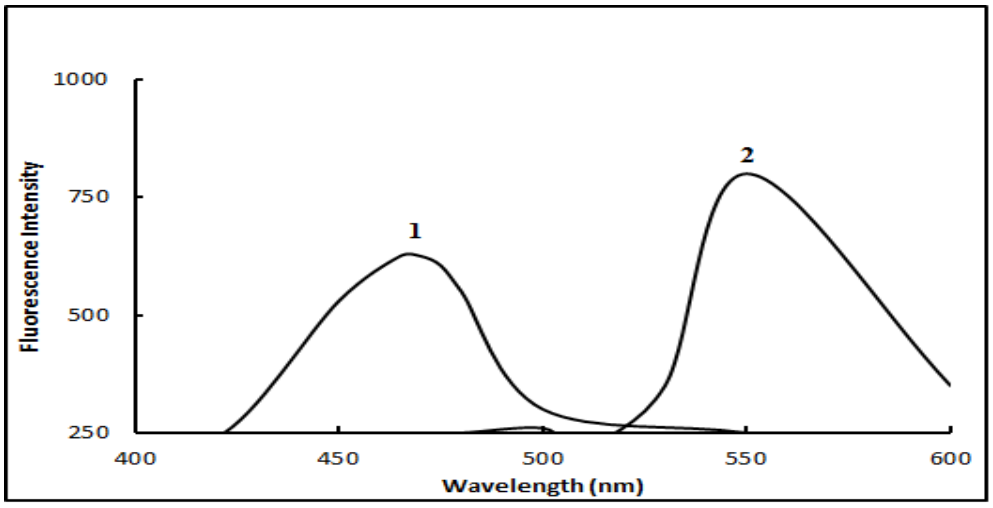

Figure 2. Fluorescence spectrum of the derivatization product of BDP with NBD-F: (1) Excitation spectrum and (2) Emission spectrum.

The experimental parameters have been investigated and optimized for the efficiency of the reaction product. Each parameter has been changed separately while the other parameters were constant. These parameters are as follows; diluting solvent, volume of the buffer, pH, reaction time, concentration of NBD-F and temperature.

In order to choose optimum pH, different pH values between 7 to 11 were studied using borate buffer. pH 8.5 was choosen as optimum pH (Figure 3). Before measurement, the acidification was made with 0.2 mL of 1.0 N HCl because of produce of NBD-OH (its maximum absorbance at 465 nm). 0–3 mL volumes of pH 8.5 borate buffer were trialed. 0.2 mL borate buffer addition is found to be suitable.

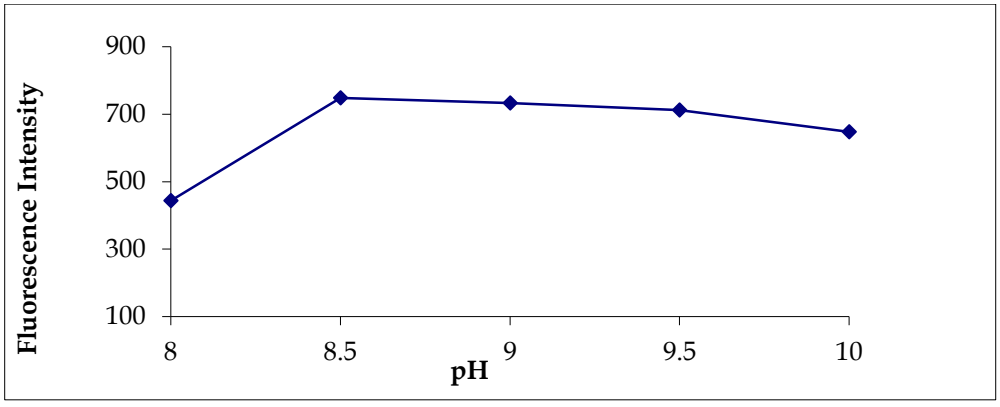

Figure 3. The results of pH studies on the derivatization process between BDP with NBD-F.

Temperature from 60 to 80 °C range was investigated to found optimum temperature on the reaction procedure. the highest intensity obtained at 80°C (Figure 4). As shown in Figure 4, the temperature on the BDP and NBD-F reaction was examined at a range of 60 to 80°C and the highest intensity was obtained at 80°C at 3 min. The obtained colour remained stable for 12 h.

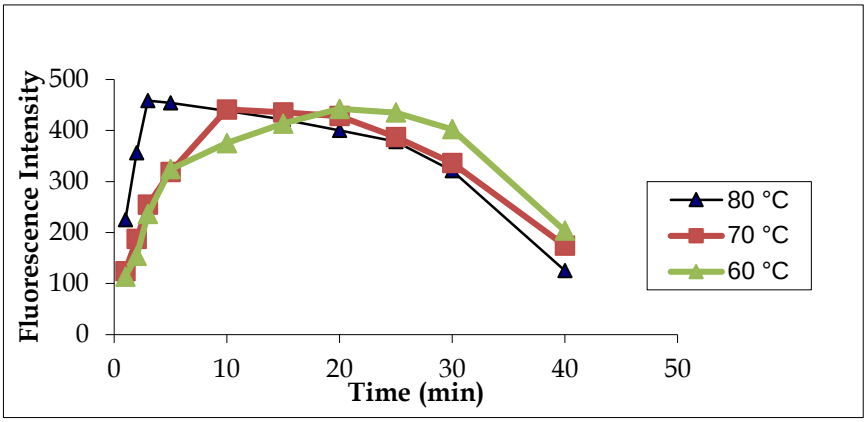

Figure 4. The optimum temperature and heating time selection.

The NBD-F concentration has been also invesitigated. As shown in Figure 5, 0.2 mL of 0.5% NBD-F solution was choosen. Organic solvents, (i.e. dichloromethane, acetonitrile, methanol, chloroform and ethyl acetate) were studied. Dichloromethane gave the highest absorbance.

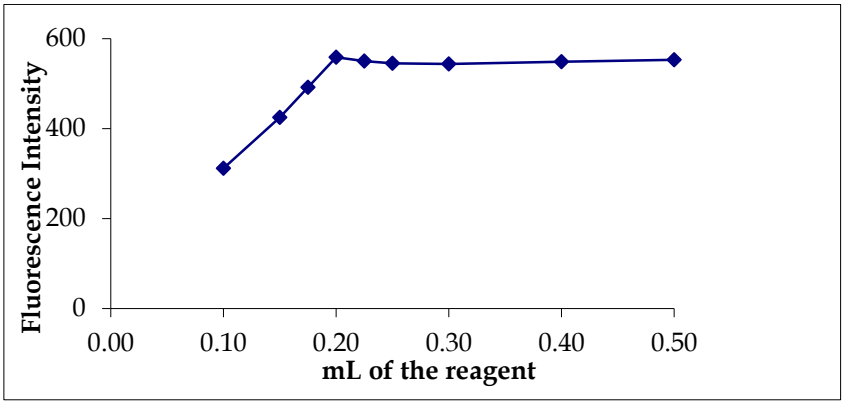

Figure 5. The results of volume of NBD-F on the derivatization process between BDP with NBD-F.

Different solvents for selecting the most suitable one for dilution were invesitaigated such as; methanol, acetonitrile, ethanol and acetone. Methanol gave best results. The molar ratio of NBD-F to BDP was investigated based on the continuous variation method of Job [12]. The reaction stoichiometry was obtained to be 1:1 ratio using equimolar solutions of BDP and NBD-F approving that one molecule of BDP reacts with one molecule of NBD-F (Figure 6).

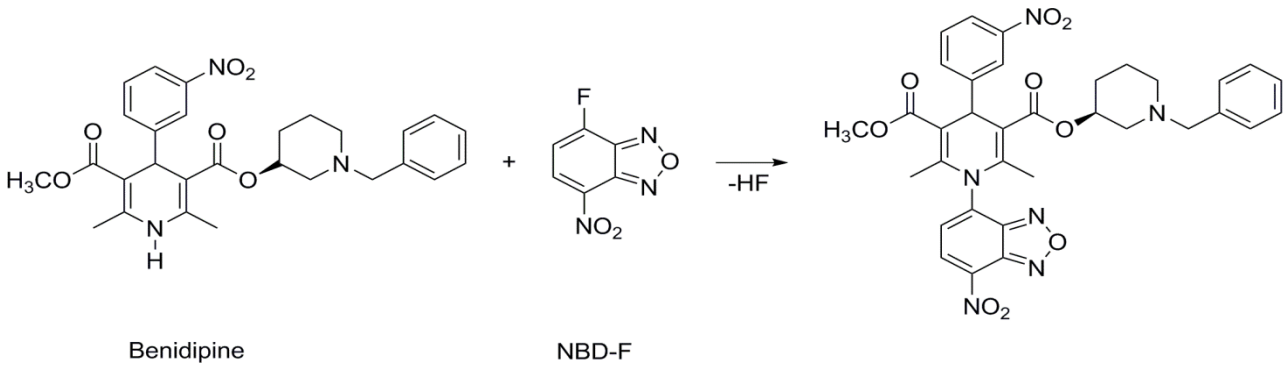

Figure 6. Proposed reaction pathway between benidipine and NBD-F reagent.

Method validation has been performed according to linearity, accuracy, precision and robustness parameters (followings ICH recommendations) [13].

The calibration curves were plotted the relative fluorescence intensities against the drug concentrations (ng mL^{-1}). The analytical parameters of the technique were presented at Table 1. The relative fluorescence intensity values and the drug concentrations were linear within the range of 10-200 ng mL^{-1}. The regression equation has been shown as:

$$I_f = 4.4465C + 102 \ (r^2 = 0.9974) \quad \text{(Eq. 1)}$$

Where I_f is the fluorescence intensity, C is the concentration of the drug in ng mL^{-1} and r^2 is the coefficient of determination (n=5).

Table 1. The analytical parameters.

Parameters	Values Found
Wavelength (nm)	λex: 465, λem:550
Concentration range [a] (ng mL^{-1})	10-200
Regression equation	I_f= 4.4465C + 102
Intercept± SD	102±0.59
Slope± SD	4.4465±0.041
Determination coefficient (r^2)	0.9974
Precision	
Intra-day [a], RSD %	0.87
Inter-day[b], RSD %	1.25
LOD (ng mL^{-1})	0.445
LOQ (ng mL^{-1})	1.348

[a] n=5
[b] five different days

The LOQ and LOD were measured following formula (according to ICH Q2 (R1) recommendation [13], The formula has been shown as;

$$LOD = 3.3Sa/slope \quad LOQ = 10Sa/slope \quad \text{(Eq. 2)}$$

where Sa is the standard deviation of the intercept. LOD was 0.445 1.348 ng mL^{-1} and LOQ was 1.348 ng mL^{-1}.

The precisions trials were performed by analysis of BDP at the three diffent concentrations (20, 100, 200 ng mL^{-1}) (each n=5) for five consecutive days. The RSD values were found as 0.87%, for intra-day precision and 1.25%, inter-day precision that was indicated to high precision. (Table 1).

The standard addition technique was used for proving accuracy of the method. The 20 ng mL^{-1} of sample solution were added to 10, 100, 150 ng mL^{-1} concentration of the standard solution and analyzed. The results of recovery study were given in Table 2. The mean recoveries were found as 99.80%.

Table 2. The recovery results.

Amount taken[a] (ng mL^{-1})	Amount added (ng mL^{-1})	Total amount found [b] (ng mL^{-1}) (Mean± S.D.[c])	Recovery (%)	RSD (%)
20	10	29.92±0.12	99.20	0.40
	100	120.05±0.85	100.05	0.71
	150	170.24±1.15	100.16	0.68

[a] Coniel® (4 mg)
[b] Five independent analyses.
[c] Standard deviation

Robustness was tested by measuring the effect of little modifications. In this research, one parameter was modified whereas the other was kept constant (time (optimum ± 1 min), temperature (optimum ± 1°C),

pH (optimum ± 0.1), and HCl concentration (optimum ± 5 mM)) and the recovery percentage was measured. Obtained data proved the robustness of the proposed method.

The applicability of the proposed method was analysed by the determination of BDP in tablets. The results obtained are satisfactory with the excellent % recovery and SD<2 (Table 3). Experiments showed that there was no interference due to the additions and excipients.

Table 3. The results of BDP in tablets using proposed fluorimetric technique (n=5).

Label claim [a] (mg/per tablet)	Mean[b] ± S.D	Recovery (%)	RSD (%)
4	4.03 ± 0.027	100.75	0.68

[a] Coniel® (4 mg)
[b] Five independent analyses.

The analysis were trialed as described the general analysis procedure to understand for applicability of the proposed method to spiked plasma samples. The results were shown in Table 4 which has precise and accurate.

Table 4. The results of recoveries of BDP using plasma samples (*n*=5).

Added (ng mL^{-1})	Determined ± S.D (ng mL^{-1})	Recovery (%)	RSD (%)[a]
20.0	14.52±0.12	72.60	0.83
100.0	78.26±0.54	78.26	0.69
200.0	168.37±0.89	84.18	0.53

[a] Five independent analyses.

4. CONCLUSION

The proposed fluorimetric technique has high sensitivities and simple analytical process in order to analysis of BDP in bulk, tablet formulations and spiked plasma. Therefore, the proposed technique is suggestible for a routine quality control and clinical laboratories.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Solutions

Benidipine was kindly supplied by Pure Chem LTD (Gujarat, India) and its tablets (Coniel®) was taken from local drugstore. NBD- F and other chemicals were from Fluka. Analytical grade chemicals were used. benidipine stock solution was prepared as 1 mg mL^{-1} in methanol and dilution were made with the same solvent (5 µg mL^{-1}). Methanol was used for preparing NBD-F solution (0.5% solution). Buffer solution were prepared as follow: 0.375 g potassium chloride and 0.310 g of boric acid were weighed and 50 mL of water was added. The pH was brought to 8.0 using 0.1 N sodium hydroxide solutions.

4.1.2. Apparatus

Hitachi spectrofluorometer (Model U-2900) were used forf luorescence measurements Xenon lamps and 1 cm light path cells were used. The excitation wavelength was 465 nm and emission wavelength was of 550 nm. The pH was measures using the WTW pH 526 digital pH meter. (The sensitivity of the pH meter is: −2.00-16.00 pH, accuracy: 0.01 at room temperature. pH meter has been calibrated using calibration buffers have pH of 4.01, 7.00.)

4.2. Methods

4.2.1. General procedures

0.010-0.2 mL standard series of BDP solutions has been added to 10 mL tubes. These solution has been diluted with methanol to 0.2 mL and mixed with 0.2 mL of borate solution. After addition of 0.2 mL NBD-F solution, the system was heated at 80 °C for 3 min. The cooling was done in the ice batch and then mixture was acidified using 0.2 mL of 1 N HCl solutions. The extraction was made with 5 mL of dichloromethane on a vortex mixer and centrifugated. The fluorescence intensities were measured against a blank prepared similarly (λ_{ex}: 465 nm and λ_{em}: 550nm). Sodium fluorescein was used as a reference standart solution.

4.2.2. Procedure for tablet formulation

Ten tablets were taken into 250 mL volumetric flask. After addition of 200 mL of methanol, the content was mixed 20 minutes mechanically and sonicated for 20 minutes. The volume of flask was completed to 250 mL with water. The 20 mL of the filtrate was removed. This filtrate was further diluted using methanol to get working solutions then processed as detailed under the preparation of calibration curve. The nominal contents of the tablets were determined using the calibration graph.

4.2.3. Procedures for plasma samples

5.0 mL of human blood sample has been used and the sample has been centrifuged at 3000 rpm for 30 min. (Ethics committee approval was taken by BVU Ethics Clinical Research Commite, number: 13/02/2019-2792). A 100 µL of plasma samples has been spiked with three different concentration (20, 100 and 200 ng mL^{-1}) of drug and added 1 mL of 5 N NaOH followed by 1-min liquid–liquid extraction with 5 mL of diethyl ether [10]. The organic layer was evaporated through evaporated to dryness under nitrogen at room temperature. After adding 100 µl methanol to residue, method was performed according to described in Section 2. was followed and measured the fluorescence of solution (λ_{ex}: 465 nm and λ_{em}: 550nm). Measurement in the plasma were done a previously plotted calibration graph and using the corresponding regression equation.

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