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Spectrofluorometric determination of nimodipine in dosage forms and human urine

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A simple sensitive and specific spectrofluorometric method was developed for the determination of nimodipine (NDP) in pharmaceutical preparations and human urine. The method is based on reduction of nimodipine with Zn/HCl and measuring the obtained fluorescence at 425 nm after excitation at 360 nm. The factors affecting the development of the fluorophore and its stability were studied and optimized. The effect of some surfactants such as β-cyclodextrin (βCD), carboxymethylcelullose (CMC), sodium dodecyl sulphate (SDS) and Triton X-100, on the fluorescence intensity was studied. The fluorescence intensity-concentration plot is rectilinear over the range 0.1–5.0 μg/ml in presence of Triton X-100 with a minimum detectability limit of 0.06 μg/ml (1.62×10^{-7} M). The proposed method was successfully applied to commercial tablets containing NDP, the percentage recovery agreed well with those obtained using the official methods. The method was further extended to the *in vitro* determination of NDP in spiked human urine samples. The % recovery was 102.1 ± 2.54 ($n = 4$). A proposal of the reduction reaction pathway was postulated.

1. Introduction

Nimodipine, [1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid 2-methoxyethyl 1-methylethyl ester [1], is a dihydropyridine calcium-channel blocker that acts particularly on cerebral blood vessels. It is used in cerebrovascular disorders, particularly in the prevention and treatment of ischaemic neurological deficits caused by arterial vasospasm following subarachnoid haemorrhage [2]. The BP recommends a titrimetric method using Ce(IV) as titrant and ferroin as indicator for the raw material and HPLC for the formulations [3]. The therapeutic importance of NDP initiated several reports on its determination, both in formulations and in biological fluids, viz: spectrophotometry [4–6], voltammetry [4, 7, 8], TLC [9, 10], GC [11–13], HPLC [14–18], supercritical fluid chromatography [19, 20] and capillary electophoresis [21]. Most of these methods are either not sufficiently sensitive [4–10] or complicated and require highly dedicated and sophisticated instrumentation [12–22]. There is, therefore, a need for a simple and sensitive method for the determination of NDP. The proposed method is based on the fact that nimodipine has no native fluorescence in spite of the presence of a dihydropyridine ring structure which imparts strong fluorescence characteristics to the compound containing it [22]. This is due to strong quenching effect of the nitro group. Reduction of the nitro group to the corresponding amino group enabled NDP to retain its strong fluorescence. The addition of Triton X-100 enhanced the produced fluorescence substantially.

2. Investigations, results and discussion

The proposed spectrofluorometric method is based on reducing the NO_2 group into the corresponding amino group (NH_2) which enhances the fluorescence by allowing a high degree of resonance stability of the multiple conjugated double bonds present in the nimodipine molecule. Different reduction systems were attempted, viz Zn/NH_4Cl, Zn/formic acid and Zn/HCl. It was found that the Zn/HCl system gave the highest fluorescence intensity and, therefore, it was used throughout the study. The fluorescence spectrum of the reduction product of NDP is shown in the Fig.

The reaction conditions with respect to the amount of Zn powder, the volume of 1 M HCl and the reaction time at constant temperature were optimized to achieve maximum fluorescence. The first goal was to choose the most appropriate reduction system. The fluorescence intensities of the solutions as a function of reducing reagents were compared. Reduction systems investigated were: Zn/HCl, Zn/NH_4Cl or Zn/formic acid. Of all the reducing agents, the highest fluorescence intensity was observed with 0.1 g of Zn powder. The fluorescence of the solutions was investigated over a hydrochloric acid (1 M) volume range of 1–6 ml. The optimum fluorescence was achieved using 2 ml. The effect of time on the development of the fluorescent product and its stability was studied. The reaction product was formed immediately, reaches maximum development after 15 min and remains stable for more than 130 min and at least and for 3 days if kept in the refrigerator.

In order to examine the effect of macromolecules surfactants (having the concentrations of 1%, w/v in case of

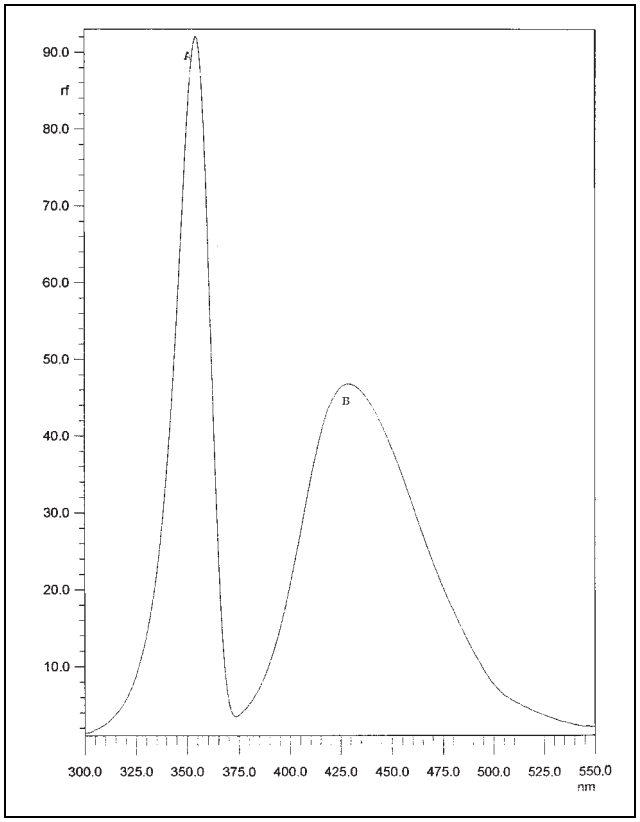

Fig.: Fluorescence spectra of the reduction product of nimodipine (6 μg/ml). A: Excitation spectrum; B: Emission spectrum.

βCD, SDS and CMC or 2%, v/v in case of Triton X-100) on the fluorescence intensity of the reduced NDP, their volumes were investigated over the range of 1–6 ml (Table 1). From the results, it is evident that the addition of Triton X-100 (2.0%, v/v) gave the highest readings, and therefore, it was used throughout the study.

The method was tested for linearity, specificity, precision and reproducibility. With the above fluorometric method, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the relative fluorescence intensity on the concentration of the drug in the range of 0.1–5.0 μg/ml in presence of Triton X-100. Statistical evaluation of the experimental data regarding standard deviation of the residuals ($S_{y/x}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) were calculated, and found to be 0.376, 0.411 and 0.605, respectively. The small values point out to the high precision of the method [23]. The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficient (close to 1).

The validity of the method could be proved by analyzing an authentic sample of NDP. The results obtained are in good agreement with those given by the official method [3]. The specificity of the method was investigated by observing that no interference was encountered from common tablet excipients.

Table 1: Effect of surfactants on the fluorescence intensity of nimodipine

Surfactant	% Change
β-CD [0.1%, w/v]	−3.290
CMC [0.6%, w/v]	+16.11
SDS [0.6%, w/v]	+60.21
Triton X-100 [0.6%, v/v]	+110.2

Under the described experimental conditions, the fluorescence intensity-concentration plot was rectilinear over the range of 0.1–5.0 μg/ml using Triton X-100.

Linear regression analysis of the data gave the following equation:

$$F = 18.986\,C - 2.074 \qquad R = 0.9966 \qquad (1)$$

where F is the fluorescence intensity and C is the concentration in μg/ml; R is the correlation coefficient.

The simplicity of the method and the stability of the reaction product permitted the determination of NDP in commercial tablets. The results obtained (Table 2) were statistically comparable with those given using the official method [3]. Common tablet excipients, such as talc powder, lactose, avisil, maize starch, hydrogenated vegetable oil, lactose and gelatin did not interfere with the assay.

The high sensitivity attained by the proposed method allowed its extension to the *in vitro* determination of NDP in spiked human urine samples with the aid of Triton X-100. NDP is orally administered in doses of 30 mg three times daily. This results in a urine level of concentration of about 0.6 μg/ml. This concentration lies within the working range of the proposed method. The results of analysis of urine samples are abridged in Table 3 and seem to be satisfactorily accurate and precise. A calibration graph was first constructed by plotting the fluorescence intensities versus increasing concentration of NDP in spiked human urine sample over the range 0.1–1.0 μg/ml.

Linear regression analysis of the data gave the following equation:

$$F = 21.1\,C + 0.95 \qquad R = 0.9965 \qquad (2)$$

where F is the fluorescence intensity; C is the concentration in μg/ml; R is the correlation coefficient.

The major advantage of the proposed method over the reported chromatographic methods as applied to urine is that it does not require a prior extraction step, thus, it is more simple and time saving.

Table 2: Application of the proposed spectroflourometric method to the analysis of nimodipine in tablets

No.	Concentration added (μg/ml)	Concentration found (μg/ml)	% recovery
1.	0.2	0.204	102.00
2.	0.3	0.311	103.67
3.	1.5	1.520	101.33
4.	2.0	2.056	102.80
5.	3.0	3.005	100.17
6.	4.0	4.055	101.4
$\bar{X}$			101.89%
SD			±1.12
t			1.584 (2.36)*
F			3.93 (5.79)*

* The figures in parenthesis are the tabulated values of t and F at 95% confidence limit.

Table 3: Application of the proposed spectroflourometric method to the determination of nimodipine (0.1–1.0 μg/ml) in spiked human urine

Amount added (μg/ml)	Amount found (μg/ml)	% recovery
0.25	0.263	105.2
0.5	0.483	101.6
0.75	0.775	103.3
1.0	0.983	98.30
$\bar{X}$		102.1
SD		±2.54

3. Experimental

3.1. Apparatus

The fluorescence intensities were measured using a spectrofluorimeter (Kontron, SFM 25, Italy), equipped with Xenon arc discharge lamb, excitation, emission grating monochromators and a 1×1 cm quartz cell. The apparatus was driven by a Pentium II PC Computer.

3.2. Reagents and materials

A reference standard sample of nimodipine (NDP, Batch # R-0191–02) was obtained from Payer Wuppertal, Germany. Commercial tablets containing NDP was obtained from the local market. Nimotop® tablets (Batch # R 111) labeled to contain 30 mg/tablet. Urine was obtained from healthy volunteers (males, around 35 years old).
Zinc powder (E. Merck, Darmstadt, Germany), minimum purity is 95%. Hydrochloric acid (BDH, Poole, UK), 1M aqueous solution. Ammonium chloride, 10% (w/v) aqueous solution. Formic acid, Surechem Products Limited (SCP), England., 98–100% (w/v). β-Cyclodextrin (βCD) (Sigma, St. Louis, MO, USA) 1%, (w/v) aqueous solution. Carboxymethycelullose sodium salt (CMC), (BDH, Poole, England), 1%, (w/v) aqueous solution. Sodium dodecyl sulphate (SDS) (Winlab, UK), 1%, (w/v) aqueous solution. Triton X-100 (Acros-New Jersey, USA), (1%, v/v) aqueous solution. Methanol (E. Merck, Darmstdt, Germany), spectroscopic grade.

3.3. Procedures

3.3.1. Preparation of standard solution.

A stock solution was prepared by dissolving 20 mg of NDP in 100.0 ml of methanol. Working standard solution was obtained by serial dilution with the same solvent. The solutions were stable for at least 4 days if kept in the refrigerator.

3.3.2. Construction of the calibration graph

Transfer quantitatively aliquot solutions of the working standard solution to a set of 10 ml volumetric flasks. Add 0.1 g Zn and 2 ml of 1 M HCl. Leave to stand for 15 min then add 3 ml of the Triton X-100 (2%, v/v aqueous solution) and complete to the mark with distilled water. Filter the solutions using filter paper and discard the first few ml of the filtrate. Measure the relative fluorescence of the filtrate at 360/425 nm. The calibration graph was obtained by plotting the fluorescence intensity versus the final concentration of NDP. Alternatively, the regression equation was derived.

3.3.3. Procedure for the tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 20 mg of NDP into a 100 ml volumetric flask; add about 60 ml of methanol and sonicate for 30 min. Complete to the mark with the same solvent. Centrifuge the mixture for 5 min and transfer the clear centrifugate into a small volumetric flask. Proceed as described under 3.3.2. Determine the nominal content of the tablets either from a previously plotted calibration graph or using the regression equation.

3.3.4. Procedure for the determination of NDP in spiked human urine

Transfer 1 ml of urine previously spiked with the drug into 10 ml volumetric flasks, add 0.1 g of Zn powder, 2 ml of 1 M HCl and leave to stand for 15 min. Add 3 ml of Triton X-100 (2%, v/v aqueous solution). Filter, measure the RF at 360/425 nm. The nominal content of NDP in urine was determined from the corresponding regression equation.

References

1 Budavari, S. (Ed.) The Merck Index, 12th ed., p. 1125 and 1126, Merck & Co., Whitehouse Station, NJ. 1996
2 Parfitt, K. (ed.): Martindale, The Complete Drug Reference. 33rd ed., p. 946, The Pharmaceutical Press, London 2002
3 The British Pharmacopoeia, p. 960, HMSO, The Pharmaceutical Press, London 2000
4 Squella, J. A.; Sturm, J. C.; Lenac, R.; Nunez-Vergara, L. J.: Anal. Lett. **25**, 281 (1992)
5 Reddy, M. N.; Murthy, T. K.; Rao-Kanna, K. V.; Gopal-Hara, A. V.; Sankar, D. G.: Indian Drugs **38**, 140 (2001)
6 Bharathi, S. N.; Prakash, M. S.; Nagarajan, M.; Kumar, K. A.: Indian Drugs **36**, 661 (1999)
7 Alvarez-Lueje, A.; Nunez-Vergara, L. J.; Squella, J. A.: Electoanal. **6**, 259 (1994)
8 Zeng, Y. H.; Zhou, Y. L.: Fenxi Huaxue **27,** 832 (1999)
9 Marciniec, B.; Brzeska, A.: Chem. Anal. (Warsaw) **44**, 849 (1999)
10 Zhu, O. H.; Yu, P. X.; Deng, Q. Y.; Zeng, L. M.: J. Planar. Chromatogr. Mod. TLC **14**, 137 (2001)
11 Rosseel, M. T.; Bogaert, M. G.; Huyghens, L.: J. Chromatogr. Biomed. Appl. **98,** 224 (1990)
12 Jakobsen, P.; Mikkelsen, E. O.; Laursen, J.; Jensen, F.: J. Chromatogr. Biomed. Appl. **47**, 383 (1986)
13 Olsen, H.; Gaarskaer, F. B.; Mikkelsen, E. O.; Jakobsen, P.; Voldby, B.: Chirality **12,** 660 (2000)
14 Patel, Y. P.; Patil, S.; Bhoir, I. C.; Sundaresan, M.: J. Chromatogr. A **828**, 283 (1998)
15 Tesarova, E.; Gilar, M.; Hobza, P.; Kabelac, M.; Deyl, Z.; Smolkova-Keulemanosova, E.: J. High Resolut. Chromatogr. **18**, 597 (1995)
16 Lopez, J. A.; Martinez, V.; Alonso, R. M.; Jimenez, R. M.: J. Chromatogr. A **870,** 105 (2000)
17 Hu, Y. S.; Tang, Q. H.; Du, Q. Y.: Sepu. **18**, 376 (2000)
18 Aymard, G.; Cayre-Castel, M.; Fernadez, C.; Lacomblez, L.; Diquet, B.: Ther. Drug. Monit. **20**, 422 (1998)
19 Patil, S. T.; Bhoir, I. C.; Sundaresan, M.: Indian Drugs. **36,** 698 (1999)
20 Bhoir, I. C.; Raman, B.; Sundaresan, M.; Bhagwat, A. M.: J. Pharm. Biomed. Anal. **17**, 539 (1998)
21 Christians, T.; Holzgrabe, U.: Electrophoresis **21**, 3609 (2000)
22 Beses, J.; Bartos, J.; in: Colorimetric and fluorometric determination of organic compounds and drugs. Marcel Dekker, NY 1974
23 Miller, J. C.; Miller, J. N., in: Statistics for Analytical Chemistry, chapter 4, p. 83, Johny Wiley and Sons, New York 1983