

COLORIMETRIC DETERMINATION OF AMLODIPINE BESYLATE AND TAMSULOSIN HYDROCHLORIDE

¹Ruba N. Shdeed and ²Imad Shdeed

¹Pharmaceutical Sciences Department, Lebanese International University, Beirut, Lebanon.

²Resident for Cardiology and Angiology, Maerkisches Klinikum Leudenscheid, Germany.

Article Received on
26 July 2018,

Revised on 16 August 2018,
Accepted on 06 Sept. 2018

DOI: 10.20959/wjpr201817-12764

*Corresponding Author

Ruba Shdeed

Pharmaceutical Sciences
Department, Lebanese
International University,
Beirut, Lebanon.

ABSTRACT

This study presents new spectrophotometric and spectrofluorimetric methods for the estimation of amlodipine besylate and tamsulosin hydrochloride in bulk and in pharmaceutical formulations. The methods depend on the coupling reaction between 4-chloro-7-nitrobenzofurazan [4-chloro-7-nitro-2,1,3-benzoxadiazole] (NBD-Cl) with amlodipine besylate and tamsulosin hydrochloride, respectively, resulting in stable colored products which can be measured spectrophotometrically and spectrofluorimetrically. For amlodipine besylate, its determination was successful by measuring the spectrophotometric absorbance, first derivative and second derivative

amplitudes at 471 nm, 500.6 nm, and 495/510 nm, respectively; while the relative fluorescence intensity and its first derivative amplitudes at 533 nm and 502 nm, respectively, was used to determine amlodipine besylate. On the other hand, only spectrophotometric techniques were successful in estimating tamsulosin hydrochloride using the amplitudes of the absorbance, first derivative and second derivative at 513 nm, 512.2 nm and 507.6 nm, respectively. The described methods are simple, sensitive, selective and inexpensive and can be used in quality control analysis.

KEYWORDS: Amlodipine besylate; Tamsulosin Hydrochloride; NBD-Cl; Spectrophotometry; Spectrofluorimetry.

INTRODUCTION

Amlodipine besylate is an oral long-acting calcium channel blocker (dihydropyridine class) used as anti-hypertensive and in the treatment of angina. Its literature reveals various methods for the determination of amlodipine in dosage forms including spectrophotometry^[1-4],

spectrofluorimetry^[5], high performance liquid chromatography^[6-8] and differential-pulse voltammetry.^[9] In biological fluids, amlodipine detection was carried out by electrophoresis^[10], liquid chromatography^[11-19], high performance liquid chromatography^[20-24], and gas chromatography.^[25]

Tamsulosin hydrochloride is an alpha-adrenergic blocking agent taken orally to treat signs and symptoms of benign prostatic hyperplasia (BPH). Upon literature screening of tamsulosin, its determination in pharmaceuticals was done including only two methods: electrophoresis^[26] and high performance liquid chromatography.^[27] In biological fluids, tamsulosin determination was performed using performance liquid chromatography^[28,29], liquid chromatography-electron spray ionization-mass spectroscopy^[30-32], and liquid chromatography-mass spectroscopy.^[33,34]

In this study, amlodipine besylate and tamsulosin hydrochloride were determined spectrophotometry and spectrofluorimetry. These methods depend on the coupling reaction between 4-chloro-7-nitrobenzofurazan [4-chloro-7-nitro-2,1,3-benzoxadiazole] (NBD-Cl) with amlodipine besylate and tamsulosin hydrochloride, respectively, resulting in stable colored products which can be measured spectrophotometrically and spectrofluorimetrically.

NBD-Cl is a reagent widely used for the derivatization of aliphatic thiols, primary and secondary aliphatic amines^[35] as well as several amino acids as cysteine, cystine^[36], histidine, tyrosine, phenyl alanine and tryptophan.^[37] Several pharmaceutical drugs^[38-40] have been determined through this approach.

NBD-Cl is non-fluorescent until it reacts with amino groups such as amines, amino acids, peptides, and proteins to form highly fluorescent compounds. NBD-Cl also reacts with thiol group to form fluorescent adducts.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were carried out on a JascoV-530 double beam UV-Vis spectrophotometer with 1-cm quartz cells, connected to a computer loaded with Jasco UVPC software and supported with Jasco Spectra Manager software for GULLIVER Ver. 1.53, and HP Deskjet 5652 printer.

MATERIALS AND REAGENTS

Pharmaceutical grades of amlodipine besylate and tamsulosin hydrochloride were kindly supplied by (Pharco Pharmaceuticals, Alexandria, ARE) and certified to contain 99.98 and 99.99%, respectively. All the chemicals were of analytical reagent grade.

Reagents

- *NBD-Cl solution* (0.01 M): Prepared by dissolving 200 mg in 100 ml methanol.
- *Borate Buffer* (Clark and Lub's Borate)^[41]
 - *pH 8.8*: Prepared by mixing 50 ml of a mixture of 6.2 g boric acid and 7.46 g KCl per liter with 16.3 ml of 0.1 M NaOH then diluted to 100 ml with distilled water.
 - *pH 9.4*: Prepared by mixing 50 ml of a mixture of 6.2 g boric acid and 7.46 g KCl per liter with 32 ml of 0.1 M NaOH then diluted to 100 ml with distilled water.
- *2 M Hydrochloric acid*

Standard Solutions

- Amlodipine besylate standard solution: Prepared to contain 0.3 mg/ml methanol and stored refrigerated at 4 °C for at least 4 days.
- Tamsulosin hydrochloride standard solution: Prepared to contain 0.6 mg/ml methanol and stored refrigerated at 4 °C for at least 4 days.

CONSTRUCTION OF CALIBRATION CURVES

For Amlodipine Besylate

A. Spectrophotometric Measurements

Accurately measured volumes (0.2 - 1 ml) of the methanolic standard solution of amlodipine besylate were transferred into a set of 10-ml volumetric flasks and the volumes were adjusted to 1 ml with methanol. Each flask was treated with 2 ml borate buffer pH 8.8, followed by 2 ml NBD-Cl reagent and mixed well. The flasks were heated in a thermostatically controlled water bath maintained at 70 °C for 40 minutes. The reaction was quenched by cooling under tap water; 1 ml of 2 M hydrochloric acid was added and the solutions were diluted to volume with methanol. The absorbance, D_1 and D_2 values of each of the resulted colored solutions were measured at 471 nm, 500.6 nm, and 495/510 nm against a similarly prepared blank for A, D_1 and D_2 , respectively, and the calibration curves were constructed (Fig. 1-3).

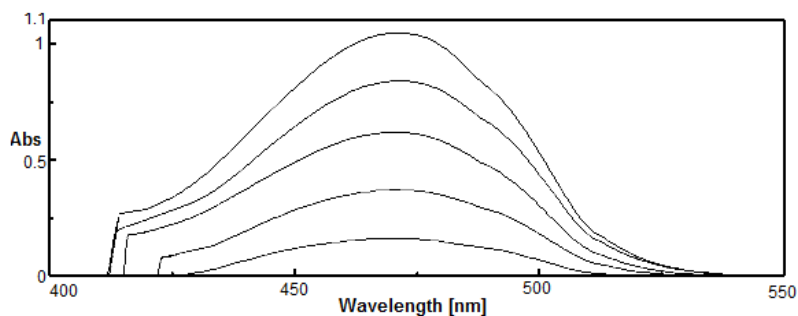


Figure 1: Absorption spectra of the reaction products of NBD-Cl with 6, 12, 18, 24 and 30 µg/ml amlodipine besylate.

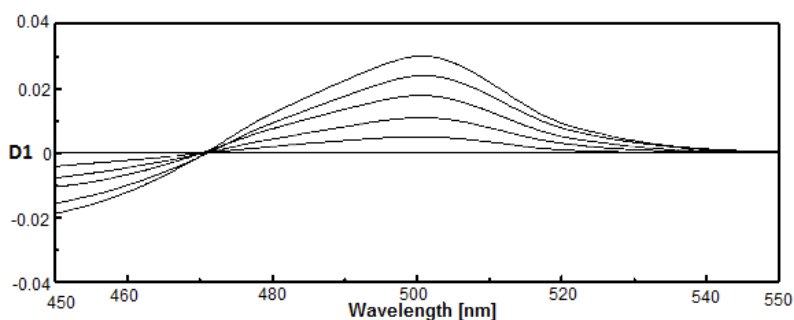


Figure 2: First derivative spectra of the reaction products of NBD-Cl with 6, 12, 18, 24 and 30 µg/ml amlodipine besylate.

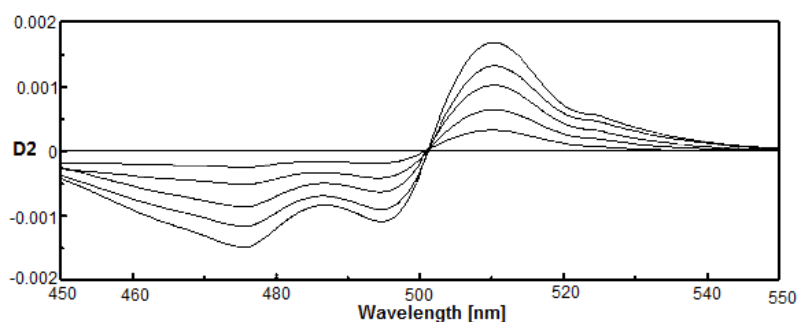


Figure 3: Second derivative spectra of the reaction products of NBD-Cl with 6, 12, 18, 24 and 30 µg/ml amlodipine besylate.

B. Spectrofluorimetric Measurements

An accurately measured 1 ml of the colored solutions prepared for the spectrophotometric measurements were transferred into a set of 10-ml volumetric flasks and the volumes were completed with methanol. The fluorescence intensity of the resulting solutions was measured at the excitation and emission wavelengths ($\lambda_{ex}/\lambda_{em}$) 467/533 nm against reagent similarly prepared blanks (Fig. 4), then the first derivative spectra (D_1) were recorded and the peak amplitudes were measured at 502 nm (Fig. 5).

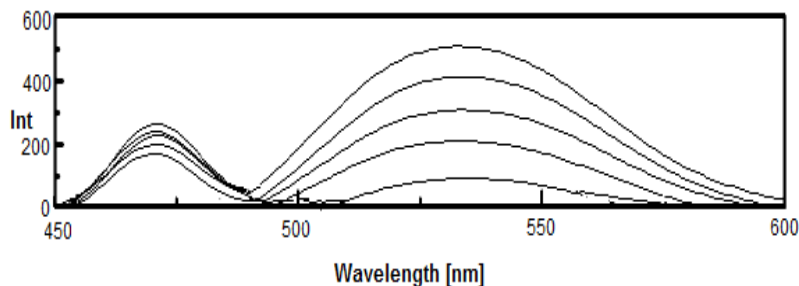


Figure 4: Fluorescence spectra of the reaction products with NBD-Cl with 0.6, 1.2, 1.8, 2.4 and 3 µg/ml amlodipine besylate.

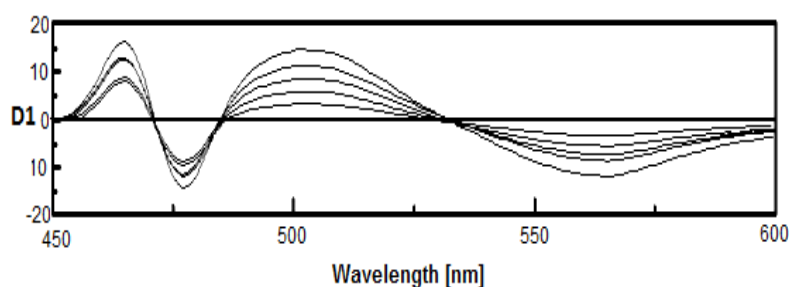


Figure 5: First derivative of fluorescence spectra of the reaction products of NBD-Cl with 0.6, 1.2, 1.8, 2.4 and 3 µg/ml amlodipine besylate.

C. Assay of Amlodipine Besylate in Lowrac® Capsules

The powder of a total of 15 capsules were emptied and mixed well. An accurately weighed amount of powder containing an equivalent amount of 15 mg amlodipine besylate, was transferred into a 50-ml volumetric flask mixed with about 30 ml methanol and stirred for 30 minutes, completed to volume with methanol and then filtered. Aliquot volumes of this solution were treated as under “Amlodipine Besylate”.

For Tamsulosin Hydrochloride

A. Spectrophotometric Measurements

Appropriate volumes (0.2 – 1 ml) of the methanolic standard solution of tamsulosin hydrochloride were transferred into a set of 10-ml volumetric flasks and the volumes were adjusted to 1 ml with methanol. Each flask was treated with 2 ml buffer solution pH 9.4, followed by 2.5 ml NBD-Cl reagent and mixed well. The flasks were heated in a thermostatically controlled water bath maintained at 70°C for 30 minutes, then cooled under tap water. Each flask was completed to volume with methanol. The absorbance, D_1 and D_2 values of each of the resulted colored solutions were recorded at 513 nm, 507.6 nm and 512.2

for A, D₁ and D₂, respectively, against a similarly prepared blank, and calibration curves were constructed (Fig. 6-8).

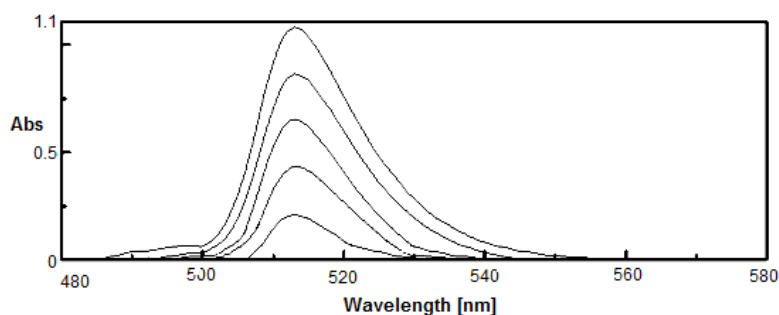


Figure 6: Absorption spectra of the reaction products of NBD-Cl with 12, 24, 36, 48 and 60 µg/ml tamsulosin hydrochloride.

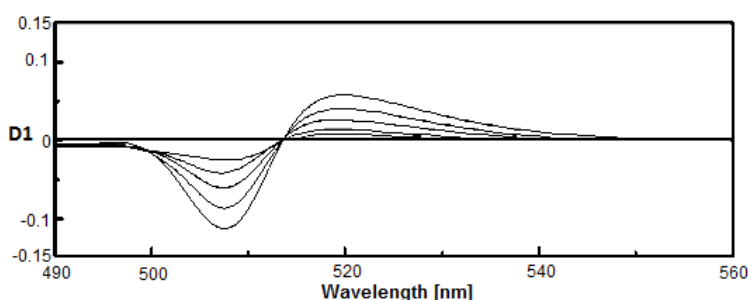


Figure 7: First derivative spectra of the reaction products of NBD-Cl with 12, 24, 36, 48 and 60 µg/ml tamsulosin hydrochloride.

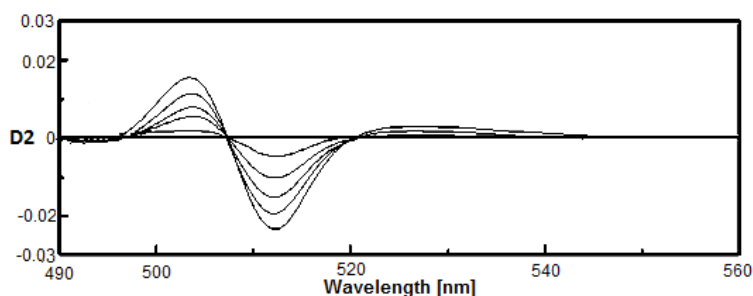


Figure 8: Second derivative spectra of the reaction products of NBD-Cl with 12, 24, 36, 48 and 60 µg/ml tamsulosin hydrochloride.

B. Assay of Tamsulosin Hydrochloride in Omnic Ocas[®] tablets

A total of 15 tablets was mashed and finely powdered. An accurately weighed amount of the powder containing the equivalent amount of 3 mg tamsulosin hydrochloride, was transferred into a 50-ml volumetric flask mixed with about 30 ml methanol and stirred for 30 minutes,

completed to volume with methanol and then filtered. Aliquot volumes of this solution were treated as under “Tamsulosin Hydrochloride”.

RESULTS AND DISCUSSION

- Each of amlodipine besylate and tamsulosin hydrochloride reacted with NBD-Cl in a slightly alkaline borate buffer solution to form a yellow colored product with maximum absorbance at 471 nm and 513 nm, respectively (Fig. 1&6). Only amlodipine besylate product exhibited fluorescence emission in methanol at 533 nm (Fig. 3). The colored product of tamsulosin hydrochloride showed no fluorescence, this may be attributed to the inhibiting effect of the sulfur dioxide group present within the molecule.
- The first derivative of the fluorescence spectra of amlodipine besylate colored product was also measured at 502 nm (Fig. 5).
- The first and the second derivatives of the absorption spectra of the produced colored products of the selected drugs were recorded. The D_1 and D_2 amplitudes for amlodipine besylate colored product were measured at 500.6 nm and 495/510 nm, respectively (Fig. 2&3). For tamsulosin hydrochloride the D_1 and D_2 amplitudes were measured at 512.2 nm and 507.6 nm, respectively (Fig. 7&8).

Optimum Reaction Conditions

Different experimental parameters affecting the development of the produced colored products and their stability were carefully studied and optimized. Each parameter was changed individually while keeping the others constant.

A. Buffer pH and its Volume

- The dependence of the reaction on pH was studied using borate buffer in the pH range 7.8-10.4. Below pH 7.8, no color was formed in both drug treatments, while upon increasing the pH, higher absorbance values were observed with maximum absorbance at pH 8.8 for amlodipine besylate and pH 9.4 for tamsulosin hydrochloride (Fig. 9). At higher pH values, a net decrease in the absorbance of the resulted colored products of both drug solutions was observed. Other buffers with the same pH values, such as phosphate buffer and 0.1 M sodium bicarbonate, were tried and compared with the borate buffer. It was found that the borate buffer is the most suitable, since it resulted in more stable colored solutions.

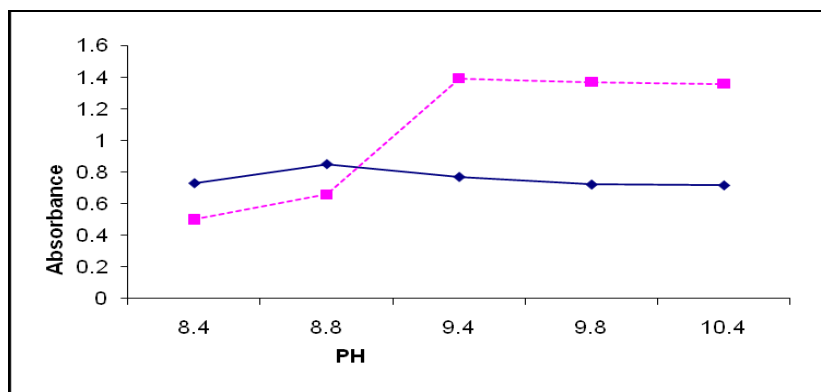


Figure 9: Effect of pH on absorbance values of 0.3 mg/ml amlodipine besylate (—◆—) and 1 mg/ml tamsulosin hydrochloride (-■-).

- The effect of the volume of the borate buffer was also studied and it was observed that 2 ml resulted in the highest color intensity for amlodipine besylate and tamsulosin hydrochloride (Fig. 10).

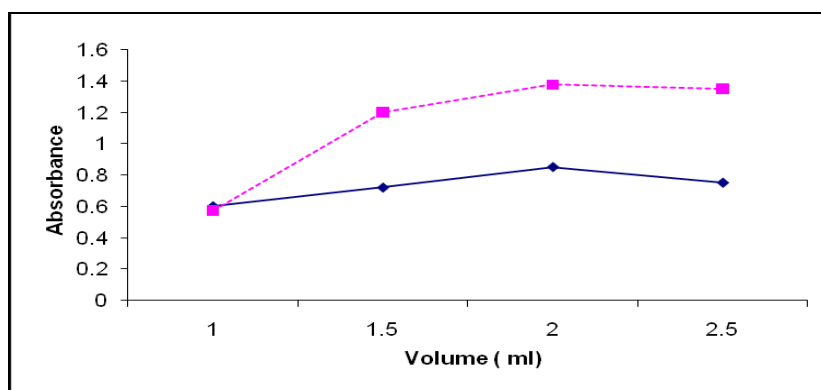


Figure 10: Effect of volume of buffer solution on absorbance values of 0.3 mg/ml amlodipine besylate (—◆—) and 1 mg/ml tamsulosin hydrochloride (-■-).

- For amlodipine besylate, the absorbance of the hydrolysis product of NBD-Cl was quenched by decreasing the pH of the reaction medium to a $\text{pH} < 1$.^[42] Therefore, acidification of the reaction mixtures was essential before the absorbance measurements. This resulted in a remarkable decrease in the reagent blank color intensity due to the destruction of NBD-OH without affecting the drug reagent adduct, hence the sensitivity was increased. This step was accomplished by the addition of 1 ml of 2 M hydrochloric acid solution to the reaction with amlodipine besylate. While for tamsulosin hydrochloride the decrease in pH resulted in destructing of the drug reagent adduct, thus no acidification was carried out.

B. Reagent Volume

The volume effect of 0.01 M NBD-Cl reagent was also studied and was revealed that increasing the volume of reagent increases the color intensity until it reaches 2 ml and 2.5 ml for amlodipine besylate and tamsulosin hydrochloride treatment, respectively, above which a decline in intensity was detected (Fig. 11).

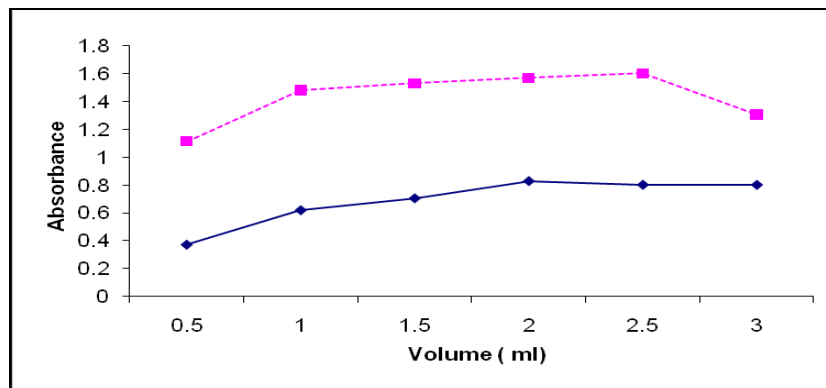


Figure 11: Effect of reagent volume on the absorbance values of 0.3 mg/ml amlodipine besylate (—◆—) and 1 mg/ml tamsulosin hydrochloride (---■---).

C. Temperature and Heating Time

Preliminary studies reported that the reaction rate was very slow at room temperature. In this study, the derivatization reaction was performed at different temperatures and at various periods. As it was found that the reaction was completed at 70°C within 40 minutes for amlodipine besylate and 30 minutes for tamsulosin hydrochloride (Fig. 12). Increasing the temperature to 80°C resulted in an apparent decrease in the reaction rate (Fig. 13).

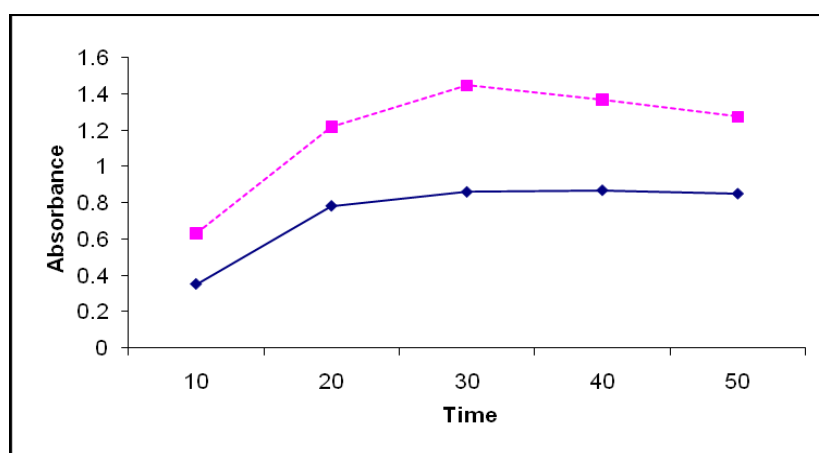


Figure 12: Effect of heating time on absorbance values of 0.3 mg/ml amlodipine besylate (—◆—) and 1 mg/ml tamsulosin hydrochloride (---■---)

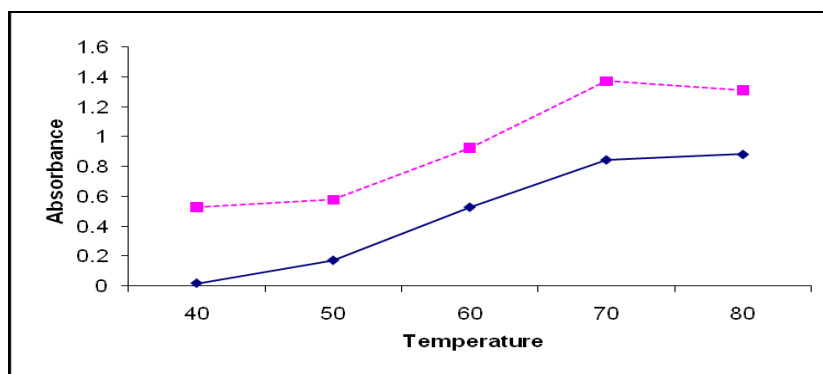


Figure 13: Effect of temperature on the absorbance values of 0.3 mg/ml amlodipine besylate (◆) and 1 mg/ml tamsulosin hydrochloride (■).

D. Absorbance Stability

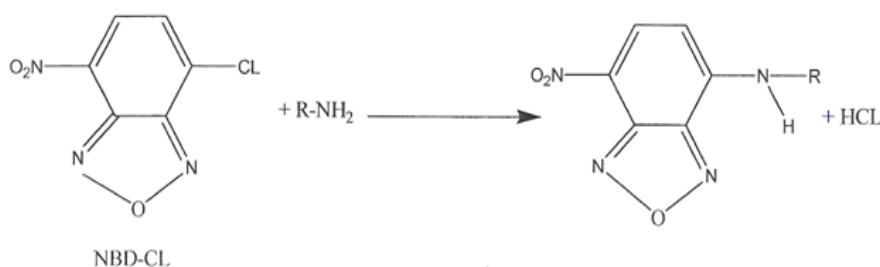
Measuring the absorbance of the colored products produced at different time intervals revealed their stability for more than 30 minutes.

Spectrofluorimetric Method

- Several solvents were tried as diluents for the color formed between the investigated drug and NBD-Cl prior to the spectrofluorimetric measurements.
- Methanol was found to be the most suitable and solutions showed the highest fluorescence intensities.
- Water was found unsuitable as a diluent since the aqueous solutions showed the least fluorescence intensity. This possibly could be due to the loss of excited state energy through the hydrogen bonding with water molecules.^[36]

Mechanism of the Reaction

The reaction mechanism could be considered as nucleophilic substitution^[36] in which one molecule of NBD-Cl condenses with one molecule of drug through the primary aliphatic amino group as illustrated in the following scheme:



Scheme 1: The condensation of primary aliphatic amine molecule with NBD-Cl.

The first and the second derivatives of the absorption spectra of the produced colored products with each of amlodipine besylate and tamsulosin hydrochloride were recorded. The absolute values were measured at 500.6 nm and 495/510 nm, for D₁ and D₂, respectively, for amlodipine besylate colored product (Fig. 2&3); and at 507.6 nm and 512.2 nm, for D₁ and D₂, respectively, for tamsulosin hydrochloride colored product (Fig. 6&7).

Under the optimum reaction conditions, the absorbance and the relative fluorescence intensity were linearly correlated to amlodipine besylate concentration over the range of 6-30 µg/ml and 0.6-3 µg/ml, respectively; while only the absorbance intensity for tamsulosin hydrochloride was linear over the concentration range of 12-60 µg/ml. Data recorded in tables 1 and 2 summarizes the characteristics of the calibration graphs.

Table 1: Assay parameters for the determination of amlodipine besylate using NBD-Cl reagent by spectrophotometric and spectrofluorimetric methods.

Parameters	Spectrophotometric Methods			Spectrofluorimetric Methods	
	A	D ₁	D ₂	Relative Fluorescence	D ₁
λ (nm)	471	500.6	495/510	533	502
Conc (µg/ml)	6.0-30.0	6.0-30.0	6.0-30.0	0.6-3.0	0.6-3.0
a	-2.13×10^{-2}	-1.12×10^{-3}	-6.7×10^{-5}	-4.98×10^{-2}	-0.117
b	1.0734	3.15×10^{-2}	2.85×10^{-3}	5.83×10^{-3}	0.222
r	0.9999	0.9997	0.9999	0.9993	0.9929
S _a	3.78×10^{-3}	2.75×10^{-4}	1.7×10^{-5}	4.38×10^{-2}	0.144
S _b	5.69×10^{-3}	4.15×10^{-4}	2.57×10^{-5}	1.25×10^{-4}	1.53×10^{-2}
(S _b) ²	3.24×10^{-5}	1.72×10^{-7}	6.60×10^{-10}	1.56×10^{-8}	2.34×10^{-4}
F	35502.6	5749.36	12294.72	2162.02	210.89
Sig-F	3.3×10^{-7}	5.06×10^{-6}	1.62×10^{-6}	2.19×10^{-5}	7.08×10^{-4}

Table 2: Assay parameters for the determination of tamsulosin hydrochloride using NBD-Cl reagent by spectrophotometric methods.

Parameters	Spectrophotometric Methods		
	A	D ₁	D ₂
λ (nm)	513	512.2	507.6
Conc (µg/ml)	12.0-60.0	12.0-60.0	12.0-60.0
a	-3.64×10^{-3}	-2.95×10^{-4}	-6.4×10^{-5}
b	0.935	0.117	2.36×10^{-2}
r	0.9995	0.9993	0.9993
S _a	1.26×10^{-2}	1.70×10^{-3}	3.44×10^{-4}
S _b	1.77×10^{-2}	2.57×10^{-3}	5.18×10^{-4}
(S _b) ²	3.13×10^{-4}	6.60×10^{-6}	2.68×10^{-7}
F	2782.08	2070.53	2071.34
Sig-F	1.5×10^{-5}	2.34×10^{-5}	2.34×10^{-5}

VALIDATION OF THE PROPOSED METHODS

Linearity

Under the studied experimental conditions, the absorbance and the fluorescence intensity values were found to be proportional to drug concentrations over the ranges stated in tables 27 and 28. The values of the correlation coefficients and the variances indicate the good linearity of the calibration graphs. The values of the intercepts did not differ significantly from the theoretical value, zero. Statistical parameters such as standard deviation of the intercept (S_a), the standard deviation of the slope (S_b), F-values and sig-F values, are also given in the tables 1 and 2.

Precision and Accuracy

Five replicate determinations at different concentrations of amlodipine besylate and tamsulosin hydrochloride were carried out to test precision and accuracy of the proposed methods. As shown in tables 3 & 4, Rec %, RSD % and Er % values indicate good repeatability and accuracy of the proposed methods. The ruggedness of the methods was studied by the inter-day and intra-day precision by the analysis of five replicates a day for three consecutive days at the concentration levels presented in tables 5 & 6. The intra-day and inter-day percentages of relative standard deviations were satisfactory.

Analysis of Dosage Forms

The applicability of the proposed methods was tested by the determination of each drug in its commercial dosage form. The good recovery results obtained and the low RDS % values indicate high accuracy and precision of the proposed methods for the analysis of these compounds in dosage forms (Tables 7&8). The results show that the developed methods are unaffected by the inactive materials in the dosage forms. Therefore, the proposed methods can be used successfully for the routine analysis of these drugs in quality control laboratories.

Table 3: Accuracy for the determination of amlodipine besylate by NBD-Cl reagent.

Method	Taken ($\mu\text{g/ml}$)	Found \pm SD*	Recovery %*	RSD %	Error %
A 471 nm	12.0	11.94 \pm 0.32	99.46	0.32	-0.53
	18.0	18.10 \pm 0.27	100.53	0.27	0.53
	24.0	24.09 \pm 0.23	100.37	0.23	0.38
D ₁ 500.6 nm	12.0	12.01 \pm 0.29	100.12	0.29	0.12
	18.0	18.08 \pm 0.33	100.44	0.33	0.44
	24.0	24.06 \pm 0.36	100.25	0.36	0.24
D ₂ 495/510 nm	12.0	11.98 \pm 0.24	99.87	0.24	-0.23
	18.0	18.04 \pm 0.21	100.23	0.21	0.23

	24.0	24.02 ± 0.35	100.09	0.35	0.1
Intensity 533 nm	1.2	1.19 ± 0.94	99.17	0.95	-0.83
	1.8	1.83 ± 1.18	101.67	1.16	1.67
	2.4	2.42 ± 1.51	100.83	1.5	0.83
D ₁ 502 nm	1.2	1.22 ± 1.71	101.67	1.68	1.67
	1.8	1.79 ± 1.22	99.44	1.23	-0.56
	2.4	2.38 ± 0.97	99.17	0.98	-0.83

*Each result is the mean of five determinations

Table 4: Accuracy for the determination of tamsulosin hydrochloride by NBD-Cl reagent.

Method	Taken (µg/ml)	Found ± SD*	Recovery %*	RSD %	Error %
A 513 nm	24.0	24.11 ± 0.56	100.45	0.56	0.55
	36.0	36.08 ± 0.62	100.21	0.62	0.21
	48.0	47.94 ± 0.34	99.88	0.34	-0.12
D ₁ 512.2 nm	24.0	23.99 ± 0.42	99.99	0.42	-0.01
	36.0	36.10 ± 0.54	100.28	0.54	0.28
	48.0	48.07 ± 0.29	100.14	0.29	0.15
D ₂ 507.6 nm	24.0	24.06 ± 0.48	100.24	0.48	0.24
	36.0	36.10 ± 0.41	100.27	0.41	0.27
	48.0	47.92 ± 0.37	99.83	0.37	-0.17

*Each result is the mean of five determinations

Table 5: Precision of the proposed methods for the determination of amlodipine besylate using NBD-Cl reagent.

Frequency of Analysis	Method of Analysis	Used (µg/ml)	Found* (µg/ml)	Found* % ± SD	RSD %	Error %
Intra-day	A	12.0	11.99	99.92 ± 0.12	0.12	-0.08
	D ₁	18.0	18.02	100.11 ± 0.20	0.20	0.11
	D ₂	24.0	24.01	100.06 ± 0.09	0.09	0.06
	Relative Fluorescence	1.8.0	1.81	100.56 ± 0.92	0.92	0.56
	D ₁	2.4.0	2.42	100.83 ± 0.75	0.75	0.83
Inter-day	A	12.0	12.01	100.10 ± 0.29	0.29	0.10
	D ₁	18.0	17.99	99.93 ± 0.25	0.25	-0.07
	D ₂	24.0	24.04	100.18 ± 0.13	0.13	0.18
	Relative Fluorescence	1.8	1.79	99.44 ± 0.97	0.98	-0.56
	D ₁	2.4	2.39	99.56 ± 1.12	1.12	-0.44

*Each result is the mean of five determinations

Table 6: Precision of the proposed methods for the determination of tamsulosin hydrochloride using NBD-Cl reagent.

Frequency of Analysis	Method of Analysis	Used ($\mu\text{g/ml}$)	Found* ($\mu\text{g/ml}$)	Found* % \pm SD	RSD %	Er %
Intra-day	A	24.0	23.97	99.86 \pm 0.45	0.45	-0.15
	D ₁	36.0	36.08	100.22 \pm 0.57	0.57	0.22
	D ₂	48.0	48.03	100.07 \pm 0.23	0.23	0.08
Inter-day	A	24.0	24.03	100.13 \pm 0.29	0.29	0.13
	D ₁	36.0	36.06	100.17 \pm 0.31	0.31	0.17
	D ₂	48.0	47.92	99.83 \pm 0.13	0.13	-0.18

*Each result is the mean of five determinations

Table 7: Assay results for the determination of amlodipine besylate in Lowrac® by the proposed methods.

Method	Conc. Added $\mu\text{g/ml}$	Conc. Found $\mu\text{g/ml}$	% Rec
A 471 nm	6.0	6.10	101.67
	12.0	12.11	100.92
	18.0	17.94	99.67
	24.0	23.88	99.50
	30.0	30.15	100.50
Mean \pm RSD %			100.45 \pm 0.90
D ₁ 500.6 nm	6.0	6.08	101.33
	12.0	12.12	101.00
	18.0	18.05	100.28
	24.0	23.91	99.63
	30.0	30.18	100.60
Mean \pm RSD %			100.57 \pm 0.66
D ₂ 495/510 nm	6.0	5.95	99.17
	12.0	12.14	101.17
	18.0	18.08	100.44
	24.0	24.04	100.17
	30.0	30.12	100.40
Mean \pm RSD %			100.27 \pm 0.72
Relative Fluorescence 467/533 nm	0.6	0.59	98.17
	1.2	1.21	101.10
	1.8	1.82	101.17
	2.4	2.39	99.58
	3.0	3.02	100.67
Mean \pm RSD %			100.42 \pm 1.27
D ₁ 502 nm	0.6	0.59	98.50
	1.2	1.21	101.17
	1.8	1.82	100.94
	2.4	2.39	99.45
	3.0	3.02	100.77
Mean \pm RSD %			100.16 \pm 1.15

Table 8: Assay results for the determination of tamsulosin hydrochloride in Omnic Ocas® by the proposed methods.

Method	Conc Added $\mu\text{g/ml}$	Conc. Found $\mu\text{g/ml}$	% Rec
A 513 nm	12.0	12.19	101.58
	24.0	23.86	99.42
	36.0	36.09	100.25
	48.0	48.12	100.25
	60.0	59.91	99.85
Mean \pm RSD %			100.27 \pm 0.81
D ₁ 507.6 nm	12.0	12.14	101.17
	24.0	23.78	99.08
	36.0	36.15	100.42
	48.0	48.13	100.27
	60.0	59.87	99.78
Mean \pm RSD %			100.14 \pm 0.78
D ₂ 512.2 nm	12.0	12.13	101.08
	24.0	24.11	100.56
	36.0	36.19	100.53
	48.0	48.17	100.35
	60.0	60.09	100.14
Mean \pm RSD %			100.53 \pm 0.35

CONCLUSION

Simple, sensitive, selective and inexpensive spectrophotometric and spectrofluorimetric methods were developed for the analysis of amlodipine besylate and tamsulosin hydrochloride in commercial drug capsules and tablets, respectively. The applicability of the developed methods was evaluated through the determination of both drugs in pharmaceutical formulations with good accuracy and precision. Thus it can be readily applied for the routine quality control testing and drug stability monitoring.

REFERENCES

1. Malesuik, MD.; Cardoso, SG.; Bajerski., L. and Lanzasova, FA.; *J. AOAC Int.*, 2006; 89(2): 359-64.
2. Rahman, N. and Nasrul Hoda, M.; *J. Pharm Biomed Anal.*, 26, 2003; 31(2): 381-92.
3. Basavaiah, K.; Chandrashekar, U. and Prameela, HC.; *Farmaco.*, 2003; 58(2): 141-8.
4. Rahman, N. and Azmi, SN.; *Farmaco.*, 2001; 56(10): 731-5.
5. Abdel-Wadood, HM.; Mohamed, NA. and Mahmoud, AM.; *Spectrochim Acta A Mol Biomol Spectrosc.*, 2008; 70(3): 564-70.
6. Malesuik, MD.; Cardoso, SG.; Bajerski, L. and Lanzasova, FA.; *J. AOAC Int.*, 2006; 89(2): 359-64.

7. Bhushan, R.; Gupta, D. and Singh, SK.; *Biomed Chromatogr.*, 2006; 20(2): 217-24.
8. Alsarra, IA.; *J. Chromatogr Sci.*, 2009; 47(10): 863-7.
9. Altiokka, G.; Dogrukol-Ak, D.; Tunçel, M. and Aboul-Enein, HY.; *Arch. Pharm. (Weinheim)*, 2002; 335(2): 104-108.
10. Miks, P.; Maráková, K.; Marák, J.; Nemeč, I.; Valásková, I. and Havránek, E.; *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 1, 2008; 875(1): 266-72.
11. Zou, Q.; Zhan, Y.; Ge, Z.; Wei, P. and Ouyang, P.; *Arzneimittelforschung.*, 2009; 59(8): 383-91.
12. Ramani, AV.; Sengupta, P. and Mullangi, R.; *Biomed. Chromatogr.*, 2009; 23(6): 615-22.
13. Ma, Y.; Qin, F.; Sun, X.; Lu, X. and Li, F.; *J. Pharm. Biomed. Anal.*, 12, 2007; 43(4): 1540-5.
14. Bhatt, J.; Singh, S.; Subbaiah, G.; Shah, B.; Kambli, S. and Ameta, S.; *Biomed. Chromatogr.*, 2007; 21(2): 169-75.
15. Nirogi, RV.; Kandikere, VN.; Mudigonda, K.; Shukla, M. and Maurya, S.; *Biomed. Chromatogr.*, 2006; 20(9): 833-42.
16. Massaroti, P.; Moraes, LA.; Marchioretto, MA.; Cassiano, NM.; Bernasconi, G.; Calafatti, SA.; Barros, FA.; Meurer, EC. and Pedrazzoli, J.; *Anal. Bioanal. Chem.*, 2005; 382(4): 1049-54.
17. Baranda, AB.; Mueller, CA.; Alonso, RM.; Jiménez, RM. and Weinmann, W.; *Ther. Drug Monit.*, 2005; 27(1): 44-52.
18. Chen, XY.; Luan, Y.; Zhong, DF. And Du, ZM.; *Yao Xue Xue Bao.*, 2001; 36(1): 51-4.
19. Yasuda, T.; Tanaka, M. and Iba, K.; *J. Mass Spectrom.*, 1996; 31(8): 879-84.
20. Zarghi, A.; Foroutan, SM.; Shafaati, A. and Khoddam, A.; *Farmaco.*, 2005; 60(9): 789-92.
21. Bahrami, G. and Mirzaeei, Sh.; *J. Pharm. Biomed. Anal.*, 21, 2004; 36(1): 163-8.
22. Tatar, S. and Atmaca, S.; *J. Chromatogr. B Biomed. Sci. Appl.*, 15, 2001; 758(2): 305-10.
23. Josefsson, M. and Zackrisson, AL.; *Norlander, B.; J. Chromatogr. B. Biomed. Appl.*, 20, 1995; 672(2): 310-3.
24. Pandya, KK.; satia, M.; Gandhi, TP.; Modi, IA.; Modi, RI. and Chakravarthy, BK.; *J. Chromatogr. B. Biomed. Appl.*, 19, 1995; 667(2): 315-20.
25. Beresford, AP.; Macrae, PV.; Stopher, DA. and Wood, BA.; *J. Chromatogr.*, 4, 1987; 420(1): 178-83.
26. Kavalírová, A.; Pospíšilová, M. and Karlíček, R.; *Farmaco.*, 2005; 60(10): 834-9.

27. Chandorkar, JG.; Kotwal, VB.; Dhande, NS.; Gurav, SG.; Pande, VV. and Yadav, PV.; *Pak. J. Pharm. Sci.*, 2008; 21(3): 307-10.
28. Macek, J.; Klíma, J. and Ptáček, P.; *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 5, 2004; 809(2): 307-11.
29. Matsushima, H.; Takanuki, KI.; Kamimura, H.; Watanabe, T. and Higuchi, S.; *J. Chromatogr B Biomed. Sci. Appl.*, 1, 1997; 695(2): 317-27.
30. Keski-Rahkonen, P.; Pärssinen, O.; Leppänen, E.; Mauriala, T.; Lehtonen, M. and Auriola, S.; *J. Pharm. Biomed. Anal.*, 17, 2007; 43(2): 606-12.
31. Ramakrishna, NV.; Vishwottam, KN.; Manoj, S.; Koteswara, M.; Wishu, S. and Varma, DP.; *Biomed. Chromatogr.*, 2005; 19(10): 709-19.
32. Din, L.; Li, L.; Tao, P.; Yang, J. and Zhang, Z.; *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 5, 2002; 767(1): 75-81.
33. Ksycińska, H.; Rudzki, PJ. and Sarosiek, A.; *Acta. Pol. Pharm.*, 2006; 63(5): 417-9.
34. Qi, M.; Wang, P. and Liu, L.; *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 5, 2004; 805(1): 7-11.
35. Lawrence, J.F. and Frei, R.W.; *Anal. Chem.*, 1972; 44(12): 2046-2049.
36. Akinyele, A.F.; Okogun, J.I. and Faboya, O.P.; *J. Agric. Food. Chem.*, 1999; 47: 2303.
37. Saleh, H.M. and Al-Ghannam, S.M.; *Alex. J. Pharm. Sci.*, 2000; 14: 25-29.
38. Amin, A.S.; Ragab, GH. and Saleh, H.M.; *J. of Pharm. and Biom. Anal.*, 2002; 30: 1347-1353.
39. El-Enany, N.; *J. of AOAC Int.*, 2003; 86(2): 209-214.
40. Hassan, E.M.; Belal, F.; A-Deeb, A.O. and Khalil, N.Y.; *J. of AOAC Int.*, 2001; 84(4): 1017-1024.
41. Documenta Geigy "Scientific Tables", Konrad Diem., 6th edition, J.R. Geigy S.A., Basle, Switzerland, 1957; 14-315.
42. Imai, K.; Toyóoka, T. and Miyano, H.; *Analyst*, 1984; 109-1365.