İstanbul Ecz. Fak. Mec.
34 (1) (2001)

J. Fac. Pharm. Istanbul
34 (1) (2001)

DETERMINATION OF AMLODIPINE IN TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

S. TATAR and S. ATMACA

SUMMARY

In this study, a high-performance liquid chromatographic method (HPLC) has been developed for the assay of amlodipine. The method depends on the formation of a fluorophore between the drug and 4-chloro-7-nitrobenzofurazan (NBD-Cl). It was found that the derivatization reaction proceeded quantitatively at pH 8.5 and 70 °C within 30 min when the molar ratio of reagent to amlodipine was 100. Nortriptyline hydrochloride was selected as an internal standard. Amlodipine- and nortriptyline-NBD derivatives were analysed on a C_{18} column using methanol-water (80:20) as the mobile phase and they were detected spectrofluorometrically by monitoring the excitation at 459 nm and emission at 528 nm. The assay was linear over the concentration range of 0.04-10.72 ng/20 µL.

The proposed method was applied to the determination of amlodipine besylate in tablets. The results were compared statistically with those obtained by the UV-spectrophotometric method using t- and F-tests. There was no significant difference between the two methods in the respect of mean values and standard deviations at 95% confidence level.

ÖZET

Bu çalışmada, amlodipin için yüksek performanslı sıvı kromatografik (HPLC) bir tayin yöntemi geliştirilmiştir. Yöntem amlodipin ile NBD-Cl arasında bir fluorofor oluşmasına dayanmaktadır. Türevlendirme reaksiyonunun pH 8.5 da, 70 °C de 30 dakika içerisinde, belirteç/amlodipin mol oranı 100 olduğunda, kantitatif olarak yürüdüğü saptanmıştır. İnternal standart olarak nortriptilin hidroklorür seçilmiştir. Amlodipin- ve nortriptilin-NBD türevleri Bondapak C_{18} kolonda, metanol-su (80:20) mobil faz sistemi kullanılarak analiz edilmiştir. Türevler spektrofluorimetrik olarak 459 nm eksitasyon ve 528 nm emisyon dalga boylarında saptanmıştır. Doğrusallık 0.04-0.72 ng / 20µL amlodipin konsantrasyonları arasında gözlenmiştir.

Geliştirilen yöntem tabletlerde amlodipin besilat miktar tayinine uygulanmıştır. Sonuçlar UV-spektrofotometrik yöntem ile elde edilen sonuçlarla t- ve F- testleri kullanılarak istatistiksel olarak kıyaslanmış ve iki yöntem arasında ortalamalar ve standart sapmalar yönünden % 95 olasılık düzeyinde anlamlı bir fark bulunmamıştır.

Key words: Amlodipine determination, 4-chloro-7-nitrobenzofurazan, high-performance liquid chromatography.

INTRODUCTION

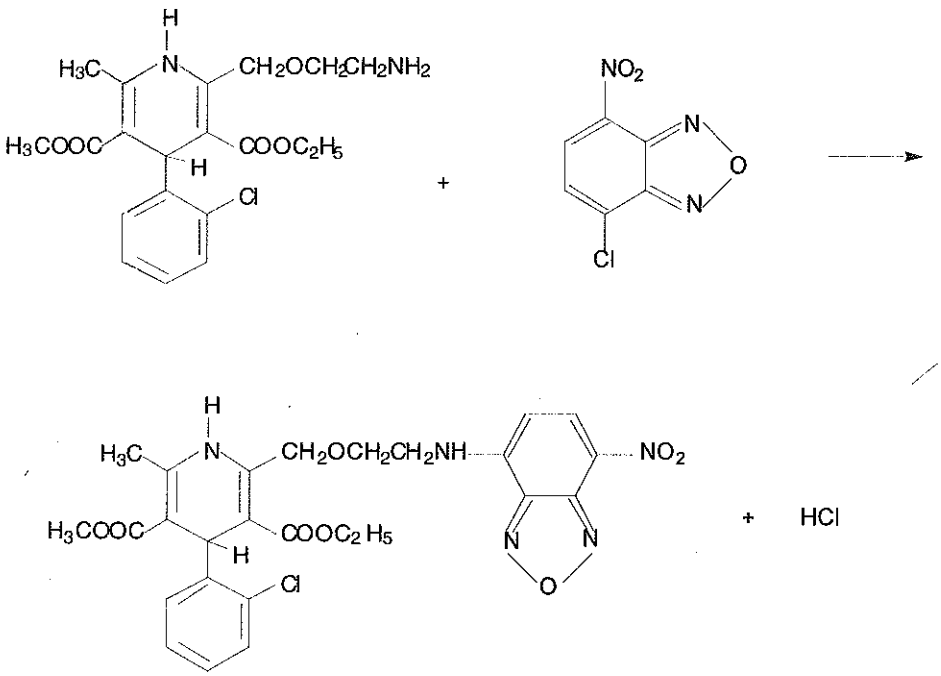
Amlodipine is a calcium channel blocking agent of the dihydropyridine derivative which is used in the treatment of hypertension and angina (1).

A few assay methods such as spectrophotometric (2), fluorimetric (3) and HPTLC (4) have been published for the quantitation of amlodipine in pharmaceutical dosage forms.

In this study an HPLC method was developed for the determination of this drug in tablets by means of the derivative formed with 4-chloro-7-nitrobenzofurazan (NBD-Cl) which is a specific reagent in the analysis of primary and secondary aliphatic amines.

RESULTS AND DISCUSSION

The reaction between amlodipine and NBD-Cl produced a highly fluorescent derivative (Reaction Sheme).



Reaction Sheme

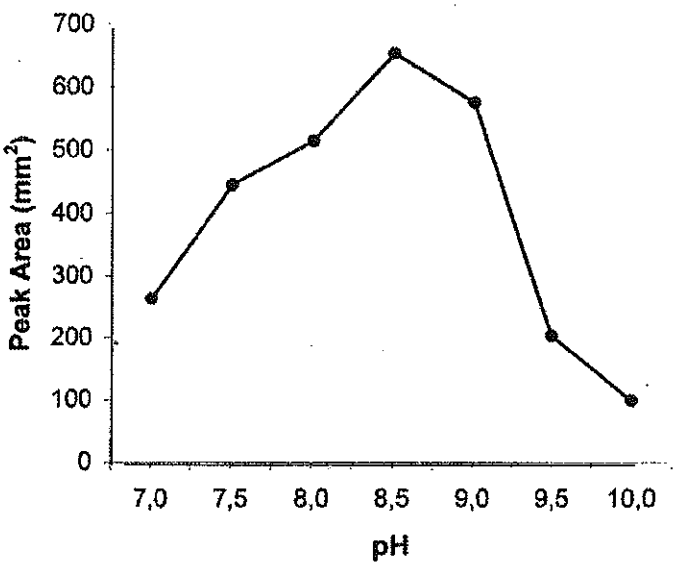


Figure 1 : Effect of pH on the reaction of amlodipine with NBD-Cl

Experimental parameters effecting the reaction such as pH, amount of the reagent, reaction temperature and time were optimised. The results shown in Fig.1 indicated that maximum fluorescence was obtained at pH 8.5. The reagent amount required was examined by changing the mole ratio of NBD-Cl to amlodipine. A 100 fold molar excess of the reagent was found to be necessary to complete the reaction (Fig. 2).

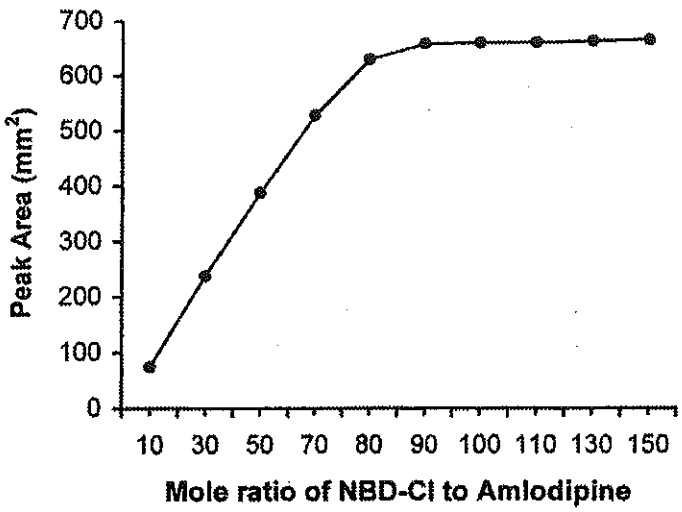


Figure 2 : Effect of reagent concentration on the reaction of amlodipine with NBD-Cl

Since the rate of the reaction was very slow at room temperature, the reaction was performed at different temperatures. The best results were obtained at 70 °C within 30 min. Reversed phase liquid chromatographic analyses were performed by using nortriptyline hydrochloride as an internal standard. The derivatives were detected by a fluorescence detector (λ_{eks}=459 nm, λ_{em}=528 nm). Fig. 3 shows a typical chromatogram for amlodipine- and nortriptyline-NBD derivatives with retention times of 7 min and 19 min respectively.

A linear detector response for the peak-area ratios of amlodipine to internal standard was observed in two concentration ranges between 0.04-0.20 ng/20 µL and 0.12-0.72 ng/20 µL with a correlation coefficients 0.9998 and 0.9999 respectively.

The proposed method was applied to the assay of amlodipine in tablets (Table) and the results were compared statistically with those obtained by the spectrophotometric method (5) using student t- and F- tests. There is no significant difference between

the two methods in the respect of mean values and standard deviations at 95% confidence level.

In conclusion, the present method provided a sensitive and specific analytical procedure for amlodipine. It can be applied for quality control testing and drug stability monitoring. Moreover, the method is sensitive enough for therapeutic monitoring of amlodipine in biological fluids. This subject is now under investigation.

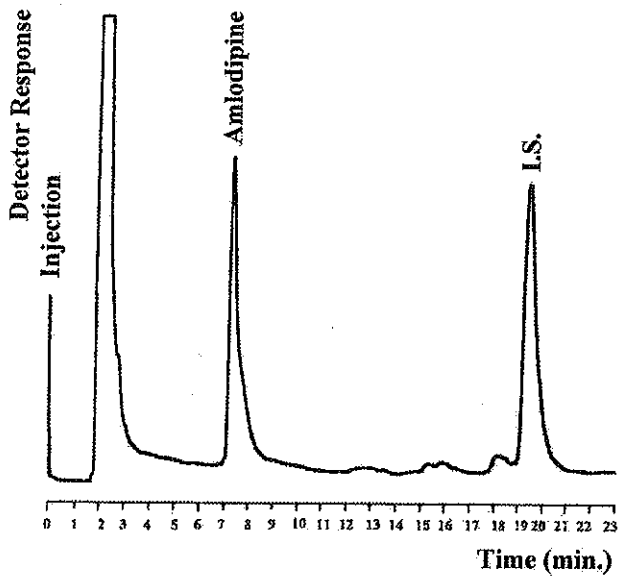


Figure 3 : Representative HPLC chromatogram of 0.48 ng/20µL of amlodipine and 30 ng/20 µL of internal standard (I.S.)
(Column: Bondapak C_{18}, mobile phase: methanol-water (80:20), detection: λ_{eks}=459 nm, λ_{em}=528 nm, flow rate: 0.8 mL/min)

EXPERIMENTAL

Materials and reagents

Amlodipine besylate and its tablets (Norvasc ®) were kindly supplied by Pfizer Ilaç San. A.Ş. (Istanbul, Turkey). Nortriptyline hydrochloride, the internal standard, was a generous gift of Mustafa Nevzat Ilaç San. A.Ş. (Istanbul, Turkey).

Solvents and other chemicals were of analytical reagent grade except methanol which was HPLC grade (Merck, Darmstadt, Germany).

Table : Comparison of the results obtained by the proposed and spectrophotometric methods for the assay of amlodipine in tablets (each tablet contains 5 mg of amlodipine as the besylate).

Statistical value	Proposed method	Spectrophotometric method
Mean	5.01	5.03
Recovery (%)	100.12	100.50
RSD (%)	1.27	0.91
Confidence limits	4.95-5.07	4.98-5.08
t-test of significance *	0.47	
F-test of significance *	1.96	
* n =6 p=0.05	t=2.23	F=5.05

Solutions

Standard solution: A stock solution of amlodipine was prepared by dissolving amlodipine besylate (equivalent to about 10 mg of free base) with 2 mL of ethanol and diluting it to 100 mL with water. This solution was diluted twice with water to give standard solution of 0.025 µg/mL.

Sample solution: Ten tablets were weighed and powdered. An accurately weighed portion of the powdered tablets, equivalent to about 10 mg of free base was shaken mechanically with about 50 mL of water-ethanol mixture (98:2) for 30 min and diluted to 100 mL with the same solvent mixture, mixed and filtered. A 1 mL volume of the filtrate was adjusted to 10 mL with water, then 25 µL of this solution was diluted to 10 mL.

Internal standard: Nortriptyline solution, (0.5 µg/mL) was prepared in water.

Reagent solution: NBD-Cl solutions (1.2 and 4.4 µg/mL) were prepared freshly in methanol.

Buffer solution: Borate buffer (0.1 M) was prepared from boric acid. The pH was adjusted to 8.5 with 0.1 M NaOH.

INSTRUMENTATION

The HPLC system (Waters, Milford, MA, USA) consisted of a Model A solvent-delivery system, a Rheodyne injection valve with a 20 µL loop and a Model 470 fluorescence detector at an excitation wavelength of 459 nm and emission wavelength of 528 nm. The detector was connected to a strip-chart recorder (Linear 355). Chromatographic separation was achieved isocratically on a 300 mm x 3.9 mm i.d. Bondapak C_{18} column (Waters), fitted with a 3 cm guard column packed with the same material. The mobile phase was methanol-water (80:20). After filtering, this mixture was degassed and delivered at a flow rate 0.8 mL/min.

Assay procedure

Accurate volumes of 20-100 µL and 60-360 µL of the standard solution for the concentration ranges of 0.04-0.20 and 0.12-0.72 ng/20µL, respectively, or 300 µL of the sample solution were transferred into 12 mL centrifuge tubes. After addition of 25 µL or 75 µL internal standard solution for the first and the latter concentration ranges, respectively, the volumes were made up to 1 mL with water.Then, 500 µL of 0.1 N NaOH solution was added to the each tube and the mixture was extracted with 5 mL of n-heptane:isopropanol (99:1). After mixing for 2 min on a vortex mixer and centrifugation at 1500g for 2 min, 4.5 mL of upper organic phase was transferred into another centrifuge tube for evaporation under nitrogen at 45 °C. The dried extracts were reconstituted for derivatization by adding 100 µL of buffer solution (pH: 8.5) and 100 µL of NBD-Cl solution. The tubes were vortex-mixed for 15 s and the reaction mixture was kept at 70 °C for 30 min. After cooling, 100 µL of 0.1 N HCl was added and the contents were extracted with 3x1.5 mL of ethyl acetate. The combined organic layers were dried on anhydrous sodium sulphate. A 4 mL aliquot of the extract was evaporated under nitrogen at 45 °C. The residue was dissolved in 200 µL of the mobile phase and 20 µL of this solution was injected into the HPLC system.

Calibration graphs for two concentration ranges were prepared by plotting the peak area ratios of amlodipine to internal standard against the concentration. The amount of amlodipine in tablets was calculated from the regression equation of the calibration graph over the concentration range and 0.12-0.72 ng/20µL of amlodipine.

Acknowledgement: The authors would like to thank the Research Fund of University of Istanbul for sponsorship of a part of this project (Project number : T-457/071197).

REFERENCES

1. Haria, M., Wagstaff, A. J., Drugs, **50** (1), 560 (1995).
2. Çetin, G., Sungur, S., Sci. Pharm., **63**, 93 (1995).
3. Mohamed, Y. E., Naglaa, M. E., Bahia, A. M., Nashwa, G. M., Bull. Fac. Pharm., **36** (1), 1(1998). – Ref: Chem. Abstr., **129**, 193792 (1998).
4. Chandrashekhar, T. G., Rao, P. S. N., Smrita, K., Vyas, S. K., Dutt, C., J.- Planar-Chromatogr.- Mod.- TLC, 7 (6), 458 (1994). – Ref: Chem. Abstr., **122** (8), 89616 p (1995).
5. Sanovel Ilaç San. A. Ş. Istanbul, Turkey (Interview).