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SPECTROPHOTOMETRIC DETERMINATION OF AMLODIPINE BESYLATE IN TABLETS WITH TRINITROBENZENE SULPHONIC ACID

TABLETLERDEKİ AMLODİPİN BESİLAT'IN TRİNİTROBENZEN SÜLFONİK ASİT İLE SPEKTROFOTOMETRİK TAYİNİ

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ÖZET

Amlodipin besilatın (AB) tayini için hızlı ve basit bir spektrofotometrik metod geliştirildi. Metod etken madde ile trinitrobenzensülfonik asitin renkli bir ürün oluşturmasına dayanmaktadır. Reaksiyon oda sıcaklığında alkali ortamda (pH 10) yürümektedir. Oluşan ürün kloroform fazına ekstre edildikten sonra 337 nm de absorbansı ölçülmektedir. Ekstraksiyonlu işlemde Beer kanunu 6.0-30.0 $\mu g.ml^{-1}$ AB aralığında geçerlidir. Metoddaki bütün değişkenler optimize edilmiş ve metodun uygulanabilirliği AB içeren tabletler analiz edilerek sınanmıştır. Sonuçların tekraredilebilirliği bağıl standart sapma olarak % 1.34 tür ve analiz edilen tabletlere ilave edilen saf AB için geri kazanım % 99.7 dir. Sonuçlar daha önce geliştirilen kloranil metoduyla istatistiksel olarak uyumludur.. Elde edilen veriler geliştirilen metodun tabletlerdeki AB nin analizinde kullanılabileceğini göstermektedir.

Anahtar kelimeler *: Amlodipin besilat tayini, trinitrobenzensülfonik asit, renk reaksiyonu, UV-Görünür bölge spektrofotometrisi, ilaç analizi.*

ABSTRACT

A rapid and simple spectrophotometric method for the determination of amlodipine besylate was developed. The method was based on the formation of a colored derivative between the drug and trinitrobenzenesulphonic acid. The reaction proceeds at room temperature in alkaline media (pH 10). The product was extracted into chloroform and the absorbance was measured at 337 nm. The Beer law limits for the procedure with extraction step were between 6.0-30.0 $\mu g\text{-}ml^{-1}$ AB. All variables in the method were optimized and the applicability of the method was examined by analyzing tablets containing AB.

Reproducibility of the results was 1.34 % as relative standard deviation and the recovery for pure AB added to preanalyzed tablets was 99.7 %. The results were statistically in concordance with that of chloranil method, which was formerly developed. Data obtained shows that the developed method can be used for determination of AB in tablets.

Keywords : Amlodipine besylate determination, trinitrobenzenesulphonic acid, color reaction, UV-vis spectrophotometry, drug analysis.

INTRODUCTION

Amlodipine besylate (AB), chemically known as 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)- l,4-dihydro-6-methyl- 3,5-pyridinedicarboxylic acid, 3-ethyl, -5-methylester besylate is a long acting calcium antagonist of dihydropyridine group. It was introduced for treatment of hypertension and angina pectoris (1-3). Very few methods have been published for the quantification of this drug. It has been determined in human plasma by HPLC (4) and GC (1,5), in rabbit plasma by LC (6) and in dosage forms by spectrophotometry (7-9). This paper describes a very simple and rapid spectrophotometric method, which is based on the formation of a colored derivative of AB with trinitrobenzene sulphonic acid (TNBS) at room temperature. TNBS has been previously reported to be a sensitive color reagent for amines, amino acids and peptides (10-15)

MATERIALS AND METHODS

Apparatus

A Shimadzu 1601 UV-Visible spectrophotometer connected to an IBM-PC and a Lexmark 1020 printer was used for the absorbance measurements. The measurements were made with 1-cm quartz cells. Operating conditions : Slit-width 2 nm, scan range 250-500 nm, scan speed 2 nm min^{-1}.

Chemicals and materials

Amlodipine besylate (AB) and Norvasc ® tablets (contains amlodipine besylate equal to 10 mg amlodipine) were kindly provided from Pfizer, Istanbul, Turkey.

2,4,6-trinitrobenzenesulphonic acid (TNBS) and other chemicals used were of analytical reagent grade. They were purchased from Merck.

Tablet solutions were filtered by Schleicher & Schuell FB 030/2 disposable filters (porewidth 0.45 μm).

Solutions

- Standard solution of AB (250 $\mu g ml^{-1)}$ was prepared in distilled water.

- 0.5 % TNBS solution was prepared by 1/10 dilution of the reagent with water. The reagent is prepared freshly everyday and must be protected against light (15).

- Buffer solution of pH=10 was prepared according to USP (16) using 0.2 M H_3B0_3 + KG mixture and 0.2 M NaOH.

Procedure I

Aliquots of standard AB solution (0.1 - 1.0 ml) were transferred to 10-ml glas-stoppered test tubes (n = 6). 1.0 ml of buffer solution (pH=10) and 0.1 ml of TNBS solution were added to each tube. They were vortexed for 10 sec after being capped and allowed to stand for 20 min in the dark. Following this period, 1.0 ml of methanol was added to each tube and they were filled to 10 ml with the buffer solution (2.5 - 25.0 $\mu g ml^{-1}$ AB). The absorbances of the working solutions were measured at 346 nm against blank. Calibration-graph was plotted using absorbance-values versus concentration.

Procedure II (with extraction step)

Following the reaction time of 20 min in Procedure I, 1.0 ml of methanolic HCl and 4 ml of chloroform were added to each tube. The mixture was vortexed for 1 min and allowed to stand for separation. After 5 min, the absorbance of lower layer (organic phase) was measured at 337 nm against blank (6.0 -30.0 $\mu g.ml^{-1}$ AB). Calibration-graph was plotted using absorbance-values versus concentration.

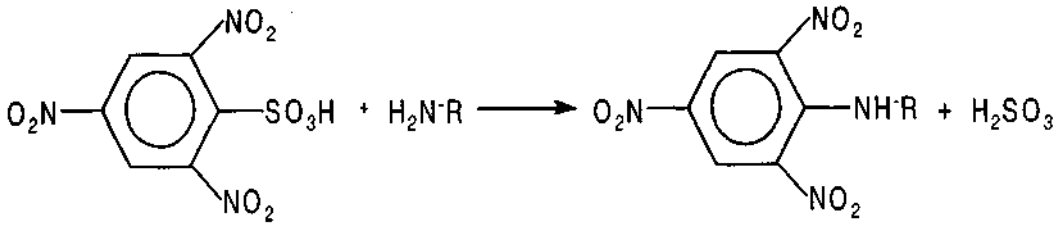
Sample preparation

20 tablets were weighed and powdered. Powder equivalent to about 10 mg of AB was accurately weighed and transferred into a 100-ml calibrated flask with about 50 ml distilled water. The mixture was shaken mechanically for 20 minutes and diluted to volume with the same solvent. The solution was filtered and 1.0 ml* of the filtrate was pipetted into a 10-ml test tube. It is treated as in "Procedure I" (*In the Procedure II (with extraction step), 0.5 ml of the filtrate was pipetted into a 10-ml test tube. It is treated as in "Procedure II"). The amount of AB in the sample was calculated from the corresponding regression equation.

RESULTS AND DISCUSSION

TNBS forms coloured derivatives with amines according to the reaction below (Scheme 1).

Scheme I



The absorption spectrum cf the Amlodipine besylate -TNBS derivative after extraction-step was shown in Figure 1.

Figure 1. Absorption spectrum of AB -TNBS derivative after extraction.

Since the reagent is light-sensitive, the reaction should be carried out by protecting against light. The optimum conditions for the development of the colored product was established by varying the parameters such as pH of the reaction media, amount of buffer added, reaction time, amount of reagent added and type of extraction solvent.

The effect of pH was studied in the range of 8-11. The maximum absorbance was obtained at pH=10 (Table 1).

Table 1. The effect of pH on color intensity.

No.	A B S O R **B** A N **C** **E**			
	pH = 8	pH = 9	**pH=10**	**pH=11**
1	0,232	0,400	0,449	0,430
2	0,265	0,388	0,450	0,442
3	0,267	0,399	0,445	0,409
4	0,260	0,393	0,441	0,437
5	0,245	0,405	0,440	0,413
Mean	0,254	0,397	0,445	0,426
SD	0,015	0,007	0,005	0,015
RSD %	5,91	1,76	1,12	3,52

*Absorban :ce was measured at 346 nm.

1.0 ml of buffer solution was sufficient to keep the pH of the solution at 10. Color formation occurs at room temperature. Color intensity was not affected by heating. In order to determine the equilibrium time of the reaction, color intensities after 10-20-30-40 min were compared. 20 min was found to be sufficient (Table 2).

The optimum amount of reagent needed was determined by carrying out the reaction with 0.05-0.1-0.2-0.4-0.6 ml of 0.5 % TNBS solution. The color density of the derivative increased with increasing amounts of the reagent. But blank absorbance exceeded accepted limits (17), when more than 0.1 ml was used (Table 3). Additionally, optimum reproducibility was achieved with this volume.

Under these conditions, the maximum absorption of the derivative was at 346 nm (Є = 16700). A linear relationship exists between absorbance and concentration of amlodipine besylate over the range of 2.5 - 25.0 $\mu g\text{-}ml^{-1}$. The regression equation was $A = 2.9 . 10^{-2} C + 8.4 .10^{-3}$ ($r = 0.9999$). But the color of the derivative was stable only for 15 min.

Table 2. The effect of reaction-time (min) on color intensity at room temperatur (20°C).

No.	A B S O R B A N C E			
	20°C x **10'**	**20°C** x **20'**	**20°C** x **30'**	**20°C x 40'**
1	0,438	0,449	0,437	0,453
2	0,421	0,451	0,427	0,425
3	0,417	0,444	0,428	0,433
4	0,433	0,455	0,447	0,418
5	0,427	0,454	0,444	0,439
Mean	0,427	0,451	0,437	0,434
SD	0,009	0,004	0,009	0,013
RSD %	2,11	0,89	2,06	3,00

*Absorbance was measured at 346 nm.

To increase the stability, the derivative was attempted to extract into an organic solvent. For this purpose, chloroform, methylene chloride, n-buthanol, ethylacetate and hexane were tested and chloroform was found to be superior to others. 4 ml of the solvent was sufficient for complete extraction of the derivative. After extraction, the maximum absorption has shifted to 337 nm (€ = 29500). A linear relationship between absorbance and concentration of the extracted derivative was observed over the range of 6.0 - 30.0 $\mu g.ml^{-1}$ AB .

Table 3. The effect of reagent volume (0.5 % TNBS) on color intensity.

No.	A B S O R B A N C E For 0.5 % TNBS added (ml)				
	0,05	0,1	0,2	0,4	0,6
1	0,435	0,453	0,495	0,436	0,600
2	0,426	0,449	0,483	0,427	0,557
3	0,419	0,453	0,475	0,393	0,419
4	0,430	0,445	0,489	0,385	0,477
5	0,429	0,443	0,500	0,382	0,512
Mean	0,428	0,450	0,490	0,405	0,513
SD	0,006	0,006	0,012	0,025	0,070
RSD %	1,40	1,33	2,44	6,17	13,64
Blank	0,213	0,355	0,663	1,290	1,805

Absorbance was measured at 346 nm.

The regression equation was A = 3.4110"^{2} C + 3.38. 10^{-2} (r = 0.9985). The sensitivity has increased compared with TNBS I method and the color of the extracted derivative was stable during 2 hours observed.

The method with and without extraction step was applied to the determination of amlodipine besylate in commercially available Norvasc tablets and the results were compared with a method, which was based on a charge-transfer complex formation reaction between AB and chloranil (Chloranil method = TCQ) (9) (Table 4). According to statistical data, the difference between the results is not significant for p=0.05 and n = 6. As an additional demonstration of accuracy, recovery experiments were performed by adding pure AB (equivalent to 10 mg of amlodipine) to the preanalysed tablet-samples. The recovery of AB for TNBS I and II were 100.2 % and 99.7 %, respectively. The reproducibility (precision) of the results for TNBS I and II were 1.13 % and 1.34 %, as relative standard deviation, respectively. Regarding the data for accuracy and precision, the method can be successfully applied to the determination of amlodipine besylate in bulk powder and tablets. If large sets of samples are to be analyzed, procedure with extraction step should be chosen, which was relatively time-consuming but more stable.

Table 4. Assay results for amlodipine in Norvasc® tablets using TNBS I method, TNBS II method (with extraction) and Chloranil method (TCQ).

METHOD	*FOUND ± RSD % (n=6)	**t- and F- values for	***ADDED (mg)	RECOVERY (%)
TNBSI	9.80 ± 1.13	TNBS I - TNBS II t= 1.09, F= 1.41	10.0	100.2
TNBS II	9.88 ±1.34	TNBS II - TCQ t=0.23, F=2.17	10.0	99.7
TCQ	9.90 ±0.91	TNBSI - TCQ t= 1.55, F=1.54	10.0	100.5

* Label claim was 10 mg amlodipine as amlodipine besylate.

** t_{teo} = 2,23 and F_{teo}=5,05 for p=0.05 and n=6.

***10 mg amlodipine as amlodipine besylate was added to the preanalysed formulation.

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