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# SPECTROPHOTOMETRIC DETERMINATON OF AMLODIPINE BESYLATE AND ASPARTAME IN TABLETS

## AMLODİPİN BESİLAT VE ASPARTAMIN TABLETLERDE MİKTAR TAYİNİ

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*The purpose of this study was to develop analysis methods for the spectrophotometric determination of amlodipine besylate and aspartame that have primary aliphatic amine groups after derivatization with NQS which form coloured and stable derivatives with substances having those groups, and to apply the developed methods to the determination of these active substances in pharmaceutical preparations. The reaction for amlodipine besylate was completed at pH 8.5, in aqueous solution at 50°C within 20 min, with a reagent/amine mole ratio of 6. The reaction for aspartame was completed at pH 8.5 and 40°C in 20 mins, with a reagent/amine mole ratio of 10. The absorbances of the derivatives formed were measured at 462 nm and 444 nm for amlodipine besylate and aspartame respectively. The Lambert-Beer's Law was found to be valid at the concentration ranges of 10-80 µg/mL and 10-60 µg/mL, for the derivatives respectively. Results obtained from the developed methods applied to the determination of amlodipine besylate and aspartame in tablets were compared statiscally with the results obtained by UV-spectrophotometric and non-aqueous titration.*

*Bu çalışmanın amacı, primer alifatik amin grubu içeren maddelerle renkli ve dayanıklı türevler oluşturan NQS ile türevlendirildikten sonra bu grupları içeren amlodipin besilat ve aspartamın specktrofotometrik miktar tayini için analiz metotları geliştirmek ve geliştirilen metotları bu aktif maddelerin farmasötik preparatlarda miktar tayinine uygulamaktır. Amlodipin besilat için reaksiyon, sulu ortamda pH 8.5 da, 50°C de 20 dakikada, belirteç/amin mol oranı 6 olduğunda, aspartam için ise pH 8.5 da, 40°C de 20 dakikada, belirteç/amin mol oranı 20 olduğunda tamalanmaktadır. Oluşan türevlerin absorbansları sırasıyla 462 nm ve 444 nm de ölçülerek Lambert-Beer kanununun 10-80 µg/mL ve 10-60 µg/mL konsantrasyon aralıklarında geçerli olduğu bulunmuştur. Geliştirilen yöntemler amlodipin besilat ve aspartam içeren tabletlerde miktar tayinine uygulanmış, bulunan sonuçlar UV-spektrofotometrisi ve susuz ortamda titrasyon yöntemleriyle elde edilen sonuçlarla istatistiksel olarak kıyaslanmıştır.*

**Keywords** : *Amlodipine besylate; Aspartame; Spektrofotometric assay*

**Anahtar kelimeler:** *Amplodipin besilat; Aspartam; Spektrofotometrik miktar tayin*

## Introduction

Amlodipine besylate is a calcium channel blocker in dihydropiridine class that has antihypertansive and antiangineal activity(1). Several methods have been reported for the analysis of this drug substance in biological fluids (2-4) and tablets(5).

Aspartame is a dipeptid used as a synthetic sweetener (6). Analyses of aspatame by the methods of TLC (7), spectrophotometry (8), and HPLC (9.10) have been published.

1,2-Naphtoquinone-4-sulphonic acid (NQS) sodium salt was used for the qualitative and quantitative analyses of primary and secondary amines (11-14).

The present study describes, two simple and sensitive spectrophotometric methods for the analyses of amlodipine besylate and aspartame in tablets. The methods were based on the formation of

amine-NQ derivatives from the drug substances with NQS.

### Materials

*Instruments*: A Shimadzu UV-160 A model double-beam spectrophotometer with 1 cm path lenght glass cells and a Metrohm Herisau pH meter with combined glass electrode were used.

*Chemicals*: Amlodipine besylate, aspartame and their tablets were kindly supplied from Sanovel İlaç San. Ve Tic. A.Ş. Istanbul. NQS and other chemicals were obtained from E.Merck, Darmstadt, Germany. Solvents used were of analytical grade.

*Stock Solutions*

Amlodipine besylate: Accurately weighed amlodipine besylate, equivalent to 50 mg of anhydrous amlodipine base, was dissolved in 2.5 mL ethanol and diluted to 50 mL with water. Standard solutions (0.1-0.8 mg/mL) were prepared from this solution by appropriate dilutions with water.

Aspartame: Accurately weighed aspartame, equivalent to 50 mg of anhydrous aspartame, was dissolved in 50 mL water. Standard solutions (0.1-0.6 mg/mL) were prepared from this solution by appropriate dilutions with water.

*Sample Solutions*

Amlodipine besylate(**1**): Twenty tablets each containing amlodipine besylate equivalent to 10 mg amlodipine base were weighed and powdered. The powder equivalent to about 10 mg amlodipine base was accurately weighed and transferred into a 25 mL calibrated flask. After adding 15 mL of water:ethanol (95:5), the mixture was shaken for 30 mins, diluted to volume with the solvent mixture and filtered.

Aspartame(**2**): Twenty tablets of 20 mg aspartame were weighed and powdered. The powder equivalent to about 20 mg aspartame was accurately weighed and transferred into a 50 mL calibrated flask. After adding 30 mL water, the mixture was shaken for 10 mins, diluted to volume with water and filtered.

Reagent solution: For aspartame assay, $2.5 \times 10^{-2}$ M and for amlodipine besylate assay, $1.5 \times 10^{-4}$ M solutions of NQS in water were prepared freshly.

Buffer solution was prepared by dissolving 0.62 g $H_3BO_3$ and 0.75 g KCl in 50 mL of water. The solution was adjusted to pH 8.5 with 0.2 N NaOH solution, and then the volume was diluted to 200 mL with water.

### Methods

Amlodipine besylate: An aliquot of 0.5 mL of standard solution and sample solution were transferred into 10 mL tubes. After adding 0.5 mL of buffer solution and 0.5 mL of reagent solution, the mixture was allowed to stand at 50°C for 20 min. Then the mixture was cooled and 0.5 mL of 0.1 N HCl and 4 mL of $CHCl_3$:n-BuOH (1:1) were added. After shaking for 2 mins, with vortex mixer, the mixture was centrifuged. The organic phase was separated and the absorbance were measured spectrophotometerically at 462 nm against blank.

Aspartame: An aliquot of 0.5 mL of standard solution and sample solution were transferred into 10 mL tubes. After adding 0.5 mL of buffer solution and 0.5 mL of reagent solution, the mixture was allowed to stand at 40°C for 20 mins. Then the mixture was cooled and 1 mL of 0.1 N HCl and 4 mL of $CHCl_3$:n-BuOH (3:1) were added. After shaking for 2 mins with vortex mixer, the mixture was centrifuged. The organic phase was separated and absorbances were measured by a spectrophotometer at 444 nm against blank.

Calibration curves for amlodipine besylate and aspartame were constructed by plotting the absorbance values versus concentrations. Regression equations of the calibration curves were calculated by the method of least squares. The amounts of amlodipine besylate and aspartame in tablets were calculated from the regression equations of the calibration curves.

## Results and Discussion

The most suitable organic solvents for the extraction of the derivatives from aqueous phases were the mixtures of $CHCl_3$:n-BuOH (1:1) for **1** and (3:1) for **2** (Tables 1 and 2).

Table 1. Absorbance values and maximum aborbances ($\lambda_{max}$) of amlodipine-NQ in different solvents.

| Solvent | Absorbance | $\lambda_{max}$ (nm) |
|---|---|---|
| Chloroform | 0.358 | 448 |
| Chloroform-n.Butanol (3:1) | 0.390 | 450 |
| Chloroform-n.Butanol (1:1) | 0.385 | 462 |
| Dichloromethane | 0.357 | 448 |
| n. Butanol | 0.395 | 480 |
| Ethylacetate | 0.320 | 442 |

Table 2. Absorbance values and maximum aborbances ($\lambda_{max}$) of aspartame-NQ in different solvents.

| Solvent | Absorbance | $\lambda_{max}$ (nm) |
|---|---|---|
| Chloroform | 0.315 | 439 |
| Chloroform-n.Butanol (3:1) | 0.502 | 444 |
| Chloroform-n.Butanol (1:1) | 0.490 | 448 |
| Dichloromethane | 0.198 | 438 |
| n. Butanol | 0.498 | 478 |
| Ethylacetate | 0.451 | 443 |

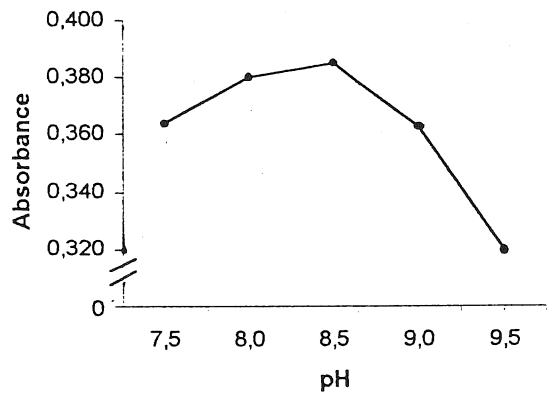


Fig.1. Effect of pH on the reaction of amlodipine with NQS.

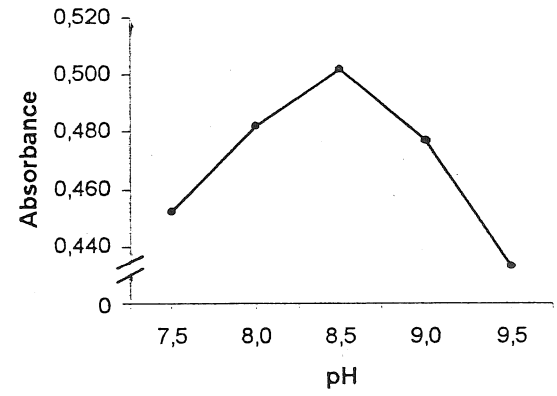


Fig.2. Effect of pH on the reaction of aspartame with NQS.

Addition of 0.1 N HCl was found to be necessary for a quantitative extraction. The absorbtion spectrums of derivatives showed maximums at 462 nm for **1** and at 444 nm for **2**. The rections between **1** and **2** with NQS was proceeded in alkaline medium. The best results for **1** and **2** were obtained at pH 8.5. (Figs 1, 2)

The effects of varying temperatures and reaction periods have been studied. For **1** and **2** maximum absorbance values were obtained at 50°C and 40°C in 20 mins respectively. The reaction with NQS required a reagent/amine mole ratio of 6 for **1** and 20 for **2** to proceed quantitatively (Figs. 3, 4). For **1** and **2** Beer's Law was obeyed in the

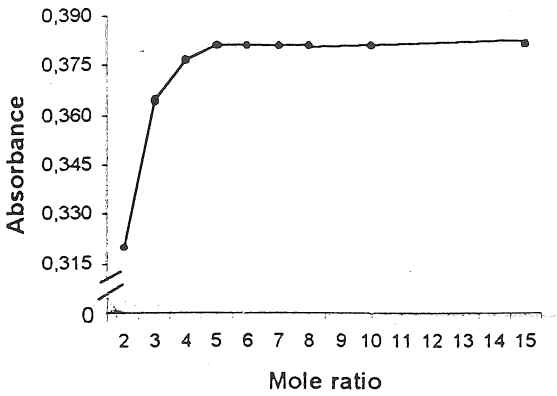


Fig.3. Effect of reagent concentration on the reaction of amlodipine with NQS.

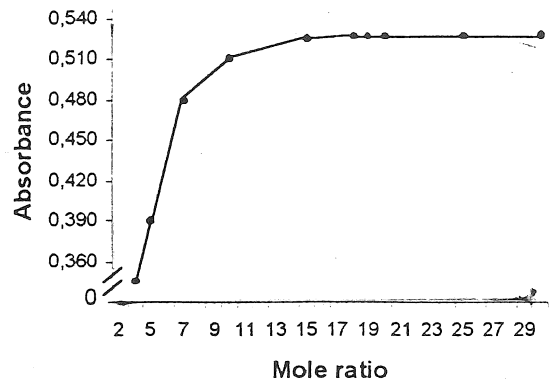


Fig.2. Effect of reagent concentration on the reaction of aspartame with NQS.

Table 3. Statistical evaluations of the results obtained by proposed and UV-spectrophotometric methods for the assay of amlodipine besylate in tablets (10 mg amlodipine/tablet).

| Statistical Values | Proposed Method | UV-spectrophotometeric Method |
|---|---|---|
| Mean (mg) | 9.67 | 9.77 |
| %recovery | 96.73 | 97.7 |
| n | 6 | 6 |
| Standard deviation | 0.086 | 0.089 |
| Relative standard deviation | 0.89 | 0.91 |
| Confidence limit | 9.67±0.078 | 9.77±0.081 |
| t test of significance | t=1.97 (p=0.05 t=2.23) | |
| F test of significance | F=1.07 (p=0.05 t=5.05) | |

Table 4. Statistical evaluations of the results obtained by proposed and titrimetirc methods for the assay of aspartame in tablets (20 mg aspartame/tablet).

| Statistical Values | Proposed Method | Titrimetric Method |
|---|---|---|
| Mean (mg) | 19.74 | 19.86 |
| %recovery | 98.72 | 99.3 |
| n | 6 | 6 |
| Standard deviation | 0.106 | 0.113 |
| Relative standard deviation | 0.54 | 0.57 |
| Confidence limit | 19.74±0.97 | 19.86±0.103 |
| t test of significance | t=1.89 (p=0.05 t=2.23) | |
| F test to significance | F=1.14 (p=0.05 t=5.05) | |

concentration ranges of 10-80 µg/mL and 10-60 µg/mL. The regression equations for **1** and **2** were A=0.0074 C + 0.0102 (r=0.9999) and A= 0.0125 C + 0.0288 (r=0.9999) respectively.

The results obtained by the proposed

spectrophotometeric methods applied to commercially available tablets were compared with those obtained by the UV-spectrophotometric method for **1** and titrimetric method for **2** in terms of t- and F- tests of significance at 95% confidence level. Statistical evaluations were shown in Tables 3 and 4.

As a conclusion, the proposed spectrophotometric methods are simple, sensitive and precise for routine pharmaceutical analyses of amlodipine besylate and aspartame in tablets.

**References**

1. Haria, M., Wagstaff, A. J.: Drugs 50(3) 560 (1995)
2. Shimooka, K., Sawada, Y., Tatematsu, H.: J.Pharm. Biomed. Anal. 7(11) 1267 (1989)
3. Pandya, K.K., Satia, M., Gandhi, T.P., Modi, I.A., Chakravarthy, B.K.: J. Chromatogr. Biomed. Appl. 667(2) 315 (1995)
4. Monkmann, S.C., Ellis, J.S., Cholerton, S., Thomasson, J.M., Seymour, R.A., Idle, J.R.: Ibid. 678, 360 (1996)
5. Chandrashekhar, T.G., Rao, P.S.N., Smrita, K., Vyas, S.K., Dutt, C.: J.Planar Chromatogr. Mod. TLC 7 (6) 458 (1994)
6. Martindale, The Extra Pharmacopoeia 30 th. Ed., The Pharmaceutical Sciences, London 1993
7. Regnoult, C., Delvordre, P., Bonnier, H., Postair, E.: J. Chromatogr. 607 (1) 159 (1992)
8. Atmaca, S., İskender, G., Bayer, E.: Acta Pharm. Turc. 31(1) 37 (1989)
9. Verzella, G., Mangia, A.: J. Chromatogr. 346, 417 (1985)
10. Verzella, G., Bagnasco, G., Mangia, A.: Ibid. 349 (1) 83 (1985)
11. Açıkkol, M., Atmaca, S., İskender, G.: Acta Pharm.Turc. 33, 29 (1991)
12. İskender, G., Yarenci, B.: Ibid. 37, 5 (1995)
13. Lindsay-Smith, J.R., Smart, A.U., Hancock, F.E., Twigg, M.E.: J.Chromatogr. 483, 341 (1989)
14. Molins-Lega, C., Campins-Falco, P., Sevillano-Cabeza, A.: J. Chromatogr. Biomed. Appl. 672 (1) 81 (1995)

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