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Determination of Vigabatrin in Tablets by High Performance Liquid Chromatography

Vigabatrinin Tabletlerde Yüksek Performanslı sıvı Kromatografisi ile Tayini

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Abstract

A new high-performance liquid chromatographic method (HPLC) was developed for the determination of vigabatrin in tablets by means of the derivative formed with 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) which is a specific reagent in the analysis of primary and secondary aliphatic amines. It was found that the derivatization reaction proceeded quantitatively at pH 9.0 and 60 ^{0}C within 20 min when the molar ratio of reagent to vigabatrin was 50. Aspartame was selected as an internal standard. Vigabatrin- and aspartame-NQ derivatives were analysed on a C_{18} column using 10 mM phosphoric acid-acetonitrile (75:25) as the mobile phase and were detected at 451 nm. The method was linear over the concentration range of 0.0576 - 2.1600 μg / 20μl. Under the experimental conditions, the lower limit of quantitation was found to be 43.2 ng/20μl and the lower limit of detection was 10.8 ng / 20μl at a signal-to-noise ratio of 5. The proposed method was applied to the determination of vigabatrin in tablets. The results were compared statistically with those obtained by the HPLC method reported previously using t-and F-tests.

Key words: Vigabatrin; 1,2 – naphthoquinone – 4 – sulfonic acid sodium salt ; high-performance liquid chromatography

Introduction

Vigabatrin is a new antiepileptic drug and its mechanism involves increasing the concentration of γ-aminobutyric acid (GABA), one of the brain's inhibitory neurotransmitters (Riekkinen *et al.*, 1989) . A few assay methods such as spectrophotometry (Olgun *et al.*, 2002, Al-Zehouri *et al.*, 2001), spectrofluorimetry (Olgun *et al.*, 2002, Belal *et al.*, 2002) and HPLC with UV- detection (Chen *et al.*, 1987) have been reported for the determination of vigabatrin in pharmaceutical preparations. In the present study, an HPLC method was developed for the determination of vigabatrin in tablets by means of the derivative formed with 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) which is a

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specific reagent for the analysis of primary and secondary aliphatic amines (Hernando *et al.*, 1999, Iskender and Sagirli, 2000).

Materials and Method

Apparatus : UV-visible spectrophotometer (Shimadzu UV-160A), HPLC system (Therma Separation Products Liquid Chromatograph with Model Spectra System®) consisted of P 4000 solvent-delivery system equipped with an injection valve with a 20 µl loop and UV 3000 detector set at 451 nm (Therma Separation, Texas, USA). Chromatographic separation was achieved isocratically on a C_{18}, 5 µm Shim-Pack CLS-ODS column (250 x 4 mm I.D., Shimadzu) fitted with guard column (4 x 3 mm I.D., Phenomenex) packed with the same material and maintained at ambient temperature. The mobile phase was 10 mM phosphoric acid – acetonitrile (75:25, v / v) with a flow rate of 1.0 ml/min.

Chemicals : Vigabatrin and Vigabatrin tablet 500 mg (Sabril®) were supplied by Aventis Pharma (Istanbul, Turkey). Aspartame was obtained from Sanecta (Maastricht, Netherlands). NQS was purchased from Sigma (St. Louis, MO, USA). Solvents and other chemicals were of analytical reagent grade, acetonitrile HPLC grade (Merck, Darmstadt, Germany).

Stock solution: Accurately weighed 5 mg vigabatrin was dissolved in 10 ml of water. Standard solutions (4, 5, 25, 50, 75, 100, 125, 150 µg/ml) were prepared from stock solution by dilutions with water.

Sample solution: Ten tablets were weighed and powdered. A portion of the powdered tablets, equivalent to 500 mg of vigabatrin was accurately weighed and transferred into a 100 ml volumetric flask and 50 ml of water was added and shaken mechanically for 30 min. The volume was diluted with water, mixed and filtered and 1.5 ml of the filtrate was made up to 10 ml with water in a calibrated flask. Then 1 ml of this solution was diluted to 10 ml in a calibrated flask.

Internal standard aspartame (1.5 mg/ml) and *reagent* NQS (3.02 mg/ml) solutions were prepared freshly in water.

Buffer solution: 0.620 g boric acid and 0.750 g potassium chloride were dissolved in 100 ml of water, pH was adjusted to 9.0 with 0.1 N NaOH and the volume was made up to 200 ml with water.

Assay procedure: 100 µl of each standard or 100 µl of the sample solution was added into 500 µl of water in glass stoppered tubes followed by the addition of 50 µl of internal standard, 500 µl of buffer and NQS solutions, the mixtures were heated at 60°C in a water bath for 20 min. The mixtures were then cooled and acidified with 250 µl of 0.1 N HCl. The derivatives were extracted 2 times with 2.5 ml of $CHCl_3$: n-butanol (4:1) on a vortex mixer and the combined organic layers were dried on anhydrous sodium sulphate. 4.5 ml aliquot of the extracts was evaporated under nitrogen at 30 °C. The residue was dissolved in 125 µl of the mobile phase and 20 µl of this solution was injected into the HPLC system. The calibration graph was plotted and the regression equation was calculated therefrom. The amount of vigabatrin in tablets was then calculated from this regression equation.

Results and Discussion

Experimental parameters affecting the reaction such as pH, amount of the reagent, reaction temperature and time were determined spectrophotometrically. The reaction was proceeded in alkaline medium. The pH dependence of the system was studied in the range of 7.0-11.0 using buffer solutions at different pH values. The results indicated that maximum absorbance was obtained at pH 9.0 (Fig.1).

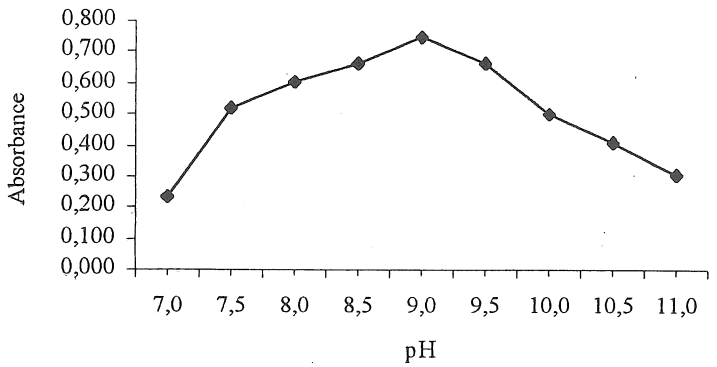

Fig.1. Effect of pH on the reaction of vigabatrin with NQS.

The reagent amount required was examined by changing the mole ratio of NQS to vigabatrin. 50 fold molar excess of the reagent was found to be necessary to complete the reaction (Fig.2). A greater excess showed no further improvement.

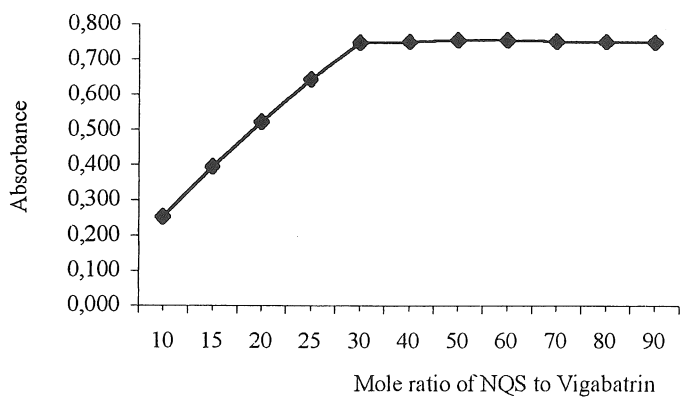

Fig.2. Effect of reagent concentration on the reaction of vigabatrin with NQS.

Since the rate of reaction was very slow at room temperature, the derivatization reaction was performed at different temperatures and periods. As it is seen in Fig.3 the best results were obtained at 60 °C within 20 min.

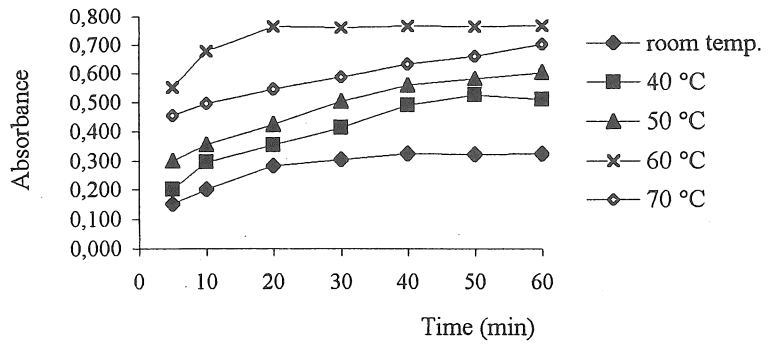

Fig.3. Effect of heating time on the reaction of vigabatrin with NQS.

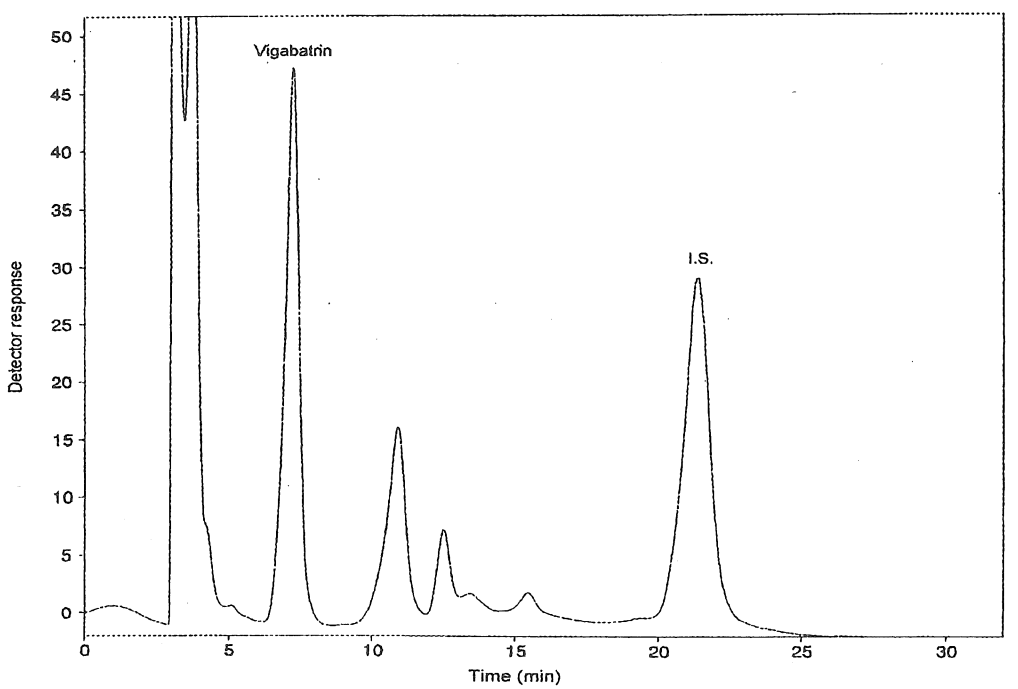

Fig.4. HPLC chromatogram of 1.08 μg of vigabatrin and 10.8 μg of aspartame (I.S.) in 20 μl injection volume.

The absorbance values were influenced also by the solvent used. Several solvents such as chloroform, dichloromethane, ethyl acetate, ethyl acetate : n-hexane (1:1), n-butanol and chloroform : n-butanol (4:1) were examined. The derivative showed maximum absorbance in chloroform : n-butanol (4:1) at 451 nm which was stable for at least 24 h at 4 °C in dark.

Reversed-phase liquid chromatographic analyses were performed by using aspartame as an internal standard. The derivatives were detected at 451 nm. Fig.4 shows a typical chromatogram for vigabatrin-NQ and aspartame-NQ derivatives with retention times of 8.1 min and 22.3 min, respectively. Under these experimental conditions the lower limit of quantitation was found to be 43.2 ng/20µl and the lower limit of detection was 10.8 ng/20µl at a signal-to-noise ratio of 5.

A linear detector response for the peak area ratios of vigabatrin to internal standard (I.S.) was observed in the concentration range of 0.0576 – 2.1600 µg/20µl. The regression equation was $A_{vigabatrin} / A_{I.S.} = 0.06122\ c + 0.002578$ ($r = 0.9999$).

The proposed method was applied to analysis of vigabatrin in tablets (Table 1) and the results were compared statistically with those obtained by the HPLC method (Chen *et al.*, 1987) reported previously. Using the t- and F-tests at 95% confidence level, no significant difference was found between the mean values and standard deviations of the two methods.

Table 1. Statistical evaluations of the results obtained by the proposed and comparison methods for the assay of vigabatrin in tablets (each tablet contains 500 mg of vigabatrin).

Statistical value	Proposed method	Comparison method
Mean	501.91	500.87
Recovery (%)	100.38	100.17
RSD (%)	0.77	0.39
Confidence limits	497.88 – 505.94	498.77-502.97
t-test of significance*	t= 0.78	
F-test of significance*	F= 3.69	

* n=6 p=0.05 t=2.23 F=5.05

In conclusion, the present method provided a specific and alternative analytical procedure for the determination of vigabatrin in tablets. It can be applied for quality control testings and drug stability controls. Moreover, this method is sensitive enough for therapeutic monitoring of vigabatrin in biological fluids.

Özet

Vigabatrinin tabletlerdeki miktar tayini için primer ve sekonder alifatik aminlerin analizinde spesifik bir belirteç olan 1,2-naftokinon-4-sülfonik asit sodyum tuzu (NQS) ile türev oluşumuna dayanan yeni bir yüksek performanslı sıvı kromatografisi yöntemi geliştirildi. Türevlendirme reaksiyonunun kantitatif olarak pH 9.0 da, 60 ^{0}C de 20 dakika içerisinde, belirteç / vigabatrin mol oranı 50 olduğunda, kantitatif olarak yürüdüğü saptandı. Aspartam internal standart olarak seçildi. Vigabatrin- ve aspartam-NQ türevleri C_{18} kolonda, 10 mM fosforik asit-asetonitril (75 : 25) mobil faz sistemi kullanılarak analiz edildi ve türevler 451 nm dalga boyunda saptandı. Doğrusallık 0.0576 - 2.1600 µg/20µl arasında gözlendi. Deneysel koşullarda tayin sınırı 43.2 ng/20µl ve teşhis sınırı sinyal-gürültü oranı 5 olduğunda 10.8 ng/20µl olarak bulundu. Geliştirilen metod tabletlerde vigabatrin tayinine uygulandı. Sonuçlar, literatürde kayıtlı bir HPLC yöntemi ile elde edilen sonuçlarla t- ve F- testleri kullanılarak istatistiksel olarak kıyaslandı.

References

Al-Zehouri, J., Al-Madi, S., Belal, F. (2001). Determination of the antiepileptics vigabatrin and gabapentin in dosage forms and biological fluids using Hantzsch reaction. *Arzneimittel-Forschung*, 51 : 97-103.

Belal, F., Abdine, A., Al-Majed, A., Khalil, N.Y. (2002). Spectrofluorimetric determination of vigabatrin and gabapentin in urine and dosage forms through derivatization with fluorescamine. *J. Pharm. Biomed. Anal.* 27: 253-260.

Chen, T.M., Contario, J.J., Fike, R.R.(1987). High-performance liquid chromatographic assay for vigabatrin and its primary degradation product in a pharmaceutical tablet formulation. *J. Chromatogr.* 398: 351-354.

Hernando, D., Saurina, J., Hernandez-Cassou, S. (1999). Liquid chromatographic determination of aniline in table-top sweeteners based on pre-column derivatization with 1,2-naphthoquinone-4-sulfonate. *J. Chromatogr. A* 859 : 227-233.

Iskender, G., Sagirli, O. (2000). Spectrophotometric determination of amlodipine besylate and aspartame in tablets. *Acta Pharm. Turc.* 42 : 1-5.

Olgun, N., Erturk, S., Atmaca, S. (2002). Spectrofluorimetric and spectrophotometric methods for the determination of vigabatrin in tablets. *J. Pharm. Biomed. Anal.* 29 : 1-5.

Riekkinen, P.J., Pitkanen, A., Ylinen, A., Sivenius, J., Halonen, T. (1989). Specificity of vigabatrin for the GABAergic system in human epilepsy. *Epilepsia*, 30 (Suppl.3): 18-22.

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