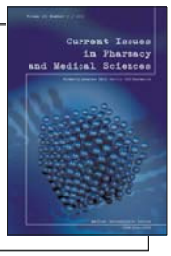
2012 © Curr. Issues Pharm. Med. Sci. Vol. 25, No. 3, Pages 299-302

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy



Validation of densitometric and videodensitometric TLC methods for analysis of vigabatrin in pharmaceutical formulations

BEATA PAW

Chair and Department of Medicinal Chemistry, Medical University, Lublin, Poland

ABSTRACT

Two new, simple, precise and accurate thin-layer chromatography methods with densitometric and videodensitometric detection were developed for the determination of vigabatrin in pharmaceutical preparations. The analysis was performed on silica gel 60 F_{254} plates in horizontal chambers with acetone-butan-2-one-formic acid-water (40:45:5:10, v/v), as mobile phase. The method is based on the reaction of the primary amino group of vigabatrin with ninhydrin reagent producing a colored product which absorbs maximally at 483 nm. Videodensitometric assay was performed at visible light. Calibration plots were constructed in the range 2.0 - 20.0 μg/spot with good correlation coefficients r=0.9991 and r=0.9949 for densitometry and videodensitometry, respectively. The LOD was 0.6 μg and 0.8 μg per spot for densitometric and videodensitometric assay, respectively; the LOQ was 1.5 μg per spot for both methods. The active substance was extracted from tablets with methanol. The mean SD recovery from commercially available tablets was 100.22 0.61 % (n=6) for the both methods. Tablet excipients did not interfere with the chromatography. Finally, the methods were compared statistically in respect of precision and accuracy. No significant differences were observed.

Keywords: TLC, densitometry, videodensitometry, vigabatrin, tablets

INTRODUCTION

Vigabatrin (4-amino-5-hexenoic acid, Fig. 1) is one of the newer generation of antiepileptic drugs. It is used for the treatment of partial seizures and difficult-to-use syndromic epilepsies. It is a structural analogue of the major inhibitory neurotransmitter of the brain γ-aminobutyric acid (GABA) leading to increasing levels of GABA in blood for antiepileptic activity [7,8].

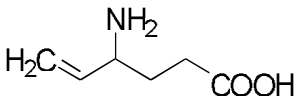


Fig. 1. Chemical structure of vigabatrin

Vigabatrin has been determined in pharmaceutical preparations (tablets, sachets) by high-performance liquid chromatography (HPLC) [1,4,5], spectrofluorimetry [2,3,6,10], spectrophotometry [2,10] and capillary electrophoresis (CE) [9,11]. All of these methods involve derivatization step before quantitative determination of vigabatrin. There is no data in the literature on the application of TLC for the analysis of this drug in pharmaceutical dosage forms and in biological material. Vigabatrin is anticonvulsant widely used for the treatment of seizures. Therefore, rapid and simple analytical methods are continually required for the determination of its concentration in different matrices. This paper describes the application of new densitometric and videodensitometric methods for quantitative analysis of vigabatrin in pure and pharmaceuticals. The results obtained by the both techniques were compared statistically in respect of precision and accuracy.

Corresponding author
* Chair and Department of Medicinal Chemistry, Medical University, 4 Jaczewskiego Str., 20-090 Lublin, Poland
e-mail: beata.paw@umlub.pl

MATERIALS AND METHODS

Chemicals. Vigabatrin as racemate (R,S)-4-amino-5--hexenoic acid was purchased from Sigma (St. Louis, MO, USA). Sabril tablets (Marion Merrell Dow, France) containing 500 mg vigabatrin per tablet were purchased from a local pharmacy. Methanol, butan-2-one from Merck (Darmstadt, Germany) and acetone, formic acid from POCh (Gliwice, Poland) were of analytical reagent grade.

Standard solutions. Stock standard solutions of vigabatrin (2.0 mg/mL) were prepared in methanol. These solutions were stored in a refrigerator at the temperature of 4°C and were stable for at least 6 weeks.

Samples Preparation. Extraction of the active substance from tablets was performed with methanol. The average mass of twenty tablets was determined. The tablets were ground and amounts of about 0.20 g were transferred to 25-mL volumetric flasks containing approximately 15 mL methanol. The mixtures were shaken mechanically for 15 min, diluted to volume with methanol, and filtered. Then, 2.0 mL of each solution were transferred to 10-mL volumetric flasks and diluted to volume with methanol. The resulting solutions were used for chromatographic analysis. Ten microliter of each solution were applied to TLC plates, developed, air dried, visualized by spraying with a solution of ninhydrin and scanned. The peak areas were recorded. The procedure was repeated six times, individually weighing the tablet powder each time.

Chromatographic procedure. Chromatography was performed on 20 cm x 10 cm silica gel 60 F_{254} TLC plates (Merck, Darmstadt, Germany). Varying volumes of standard solutions (1.0-10.0 µL, corresponding to 2.0-20.0 µg vigabatrin per spot) and six tablet solutions (10.0 µL, corresponding to 10.0 µg) were applied to the plates by means of a 25-µL microsyringe (accuracy 0.5 µL; Hamilton, Switzerland). Chromatograms were developed to a distance of 9 cm in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) with acetone-butan-2-one-formic acid-water (40:45:5:10, v/v), as mobile phase. After development the plates were dried at room temperature and visualized by spraying with a solution of ninhydrin (2% w/v in ethanol). Then, the plates were heated for 10 min at 70°C. The chromatograms obtained were analyzed densitometrically by means of a Desaga (Heidelberg, GerCD 60 densitometer controlled by Desaga ProQuant software. They were scanned at λ=483 nm with slit dimensions of 2.0 mm x 8.0 mm.

In videodensitometric analysis the chromatograms were analyzed at visible light by use of a Mitsubishi color-video CCD camera controlled by Desaga ProViDoc videodocumentation VD 40 system. Quantitative assay was performed using Desaga ProResult software. In both methods peak areas were used for calculations.

Calibration curves were constructed by plotting peak area against amount of the drug spotted. The amount of the substance analyzed in each tablet was calculated by use of appropriate regression equations.

RESULTS AND DISCUSSION

Vigabatrin exhibits a very low UV absorption. It is a small organic molecule without any chromophore or aromatic system. Thus, derivatization of the drug is necessary if measurement of vigabatrin is intended by UV/Vis detection. The elaborated method is based on the reaction of the primary amino group of vigabatrin with ninhydrin in ethanol to form a violet-red reaction product which absorbs light at a wavelength of 483 nm. The wavelength 483 nm was selected for densitometric evaluation, because at this wavelength there was a maximum of the absorption spectrum of colored product.

Videodensitometric detection was performed at visible light.

The mobile phase acetone-butan-2-one-formic acid-water (40:45:5:10, v/v) was selected as optimal for obtaining well-shaped, symmetrical single spots of vigabatrin. The horizontal technique and a migration distance of 9 cm were chosen as the best for chromatogram development. The average hR_F value was calculated from ten chromatograms developed with mobile phases prepared separately for each development. The hR_F value of the drug was 45±0.75 (mean ± SD; n=10). The development time was 26.4±2.35 min (mean ± SD; n=10).

The limits of detection (LOD) and quantification (LOQ) of vigabatrin were obtained experimentally, by investigating the signal-to-noise ratio (S/N). LOD and LOQ were established as 3 x S/N and 10 x S/N, respectively. The LOD of the method, the amount for which the signal-to-noise ratio was 3:1, was 0.6 µg and 0.8 µg per spot for densitometric and videodensitometric assay, respectively; the LOQ, the amount for which the signal-to-noise ratio was 10:1, was 1.5 µg per spot for both methods.

Calibration was carried out using six points. For each point, five measurements were made to improve the precision of the analytical procedure. The data were averaged and calibration curves were calculated. The plot of the peak area versus concentration of vigabatrin was found to be linear in the range 2.0-20.0 µg per spot. The calibration curves were represented by the following linear regression equations:

$y_{Dens} = 95.6486x + 9.2616$ $(r = 0.9991)$

and

$y_{Videodens} = 18.5082x + 46.9151$ $(r = 0.9949)$.

The densitogram and chromatogram recorded for the standard solutions are presented in Figures 2 and 3.

The intra-day and inter-day precision of the method were estimated by performing five determinations of small (2.0 µg per spot), medium (12.0 µg per spot), and large (20.0 µg per spot) amounts of vigabatrin. For densitometry the intraday precisions expressed as RSD were 1.54 and 0.76 % for the lowest and the highest concentrations. The respective values for the interday precision were 1.77 and 0.78 %. For videodensitometry the intraday RSD values ranged from 1.43-0.80 % for the lowest and the highest concentrations; the interday RSD values were 1.61 and 0.84, respectively. The results obtained are listed in Table 1.

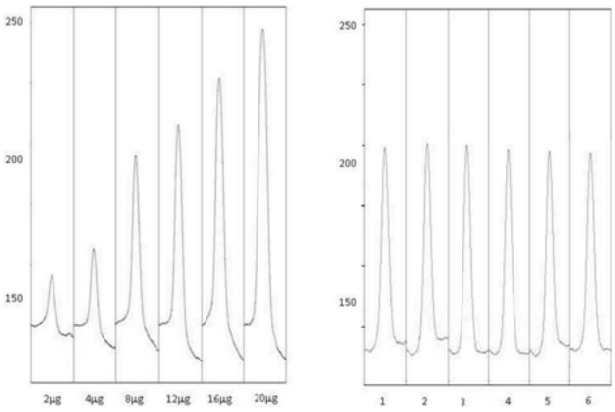


Fig. 2. Densitograms obtained from analysis of vigabatrin standard solutions in calibration range 2.0 – 20.0 µg per spot and tablet samples (1-6)

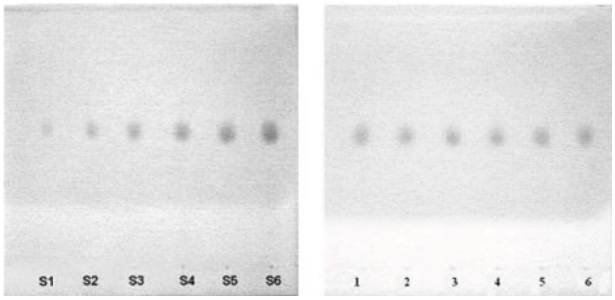


Fig. 3. Chromatograms obtained from analysis of vigabatrin calibration solutions (S1-S6) and tablet samples (1-6) on silica gel TLC plates developed with acetone-butan-2-one-formic acid-water (40:45:5:10, v/v), as mobile phase and visualized by spraying with a solution of ninhydrin (2% w/v in ethanol)

Table 1. Intra-day and inter-day precision of the TLC-system

Amount (µg/spot)	n	Intra-day precision RSD (%)		Inter-day precision RSD (%)	
		Densito-metry	Videodensi-tometry	Densito-metry	Videodensi-tometry
2.0	5	1.54	1.43	1.77	1.61
12.0	5	1.12	1.16	1.08	1.23
20.0	5	0.76	0.80	0.78	0.84

Accuracy of the method was assessed on the basis of determination of vigabatrin in the laboratory-prepared mixtures at 3 levels of addition (50, 100, and 150% of the drug concentration in tablets). For densitometry, the recovery results ranged from 99.27 to 100.36% for the lowest and the highest concentrations of the drug, with RSD values ranging from 1.95 to 0.87 %. For videodensitometry, the recovery results ranged from 100.27 to 100.54% for the lowest and the highest concentrations of the drug, with RSD values ranging from 2.05 to 1.17% (Table 2).

The densitometric and videodensitometric methods were successfully applied for the determination of vigabatrin in Sabril tablets. A single spot at R_F = 0.45 was observed in the chromatogram obtained from the drug sample extracted from tablets. There was no interference from the excipients present in the formulation. Results from analysis of vigabatrin in the pharmaceutical product were evaluated statistically; the results are shown in Table 3. The mean RSD values obtained for analysis the drug in tablets using classical densitometry and videodensitometry were not significantly different; they were 0.61 and 0.60%, respectively. For densitometry and videodensitometry total recovery from tablets was found to be 100.22%. Use of *Student's t*-test showed there was no significant difference between the measured and declared content (Table 3). Densitogram and chromatogram obtained during analysis of pharmaceutical formulation are shown in Figures 2 and 3.

Table 2. Accuracy data in the laboratory-prepared mixtures[a)]

Level of addition (%)	Densitometry		Videodensitometry	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
50	99.27	1.95	100.27	2.05
100	100.13	1.04	99.41	0.98
150	100.36	0.87	100.54	1.17

[a)] Results are the average of five determinations

Table 3. Statistical evaluation of results obtained from determination of vigabatrin in pharmaceutical preparation

	Densitometry	Videodensitometry
Amount claimed [mg]	500	500
Mean amount found [mg]	501.12	501.12
Recovery [%]	100.22	100.22
Variance	9.2801	9.1343
Standard deviation	3.0463	3.0223
Relative standard deviation [%]	0.61	0.60
95% Confidence interval	497.34–504.90	497.36–504.87
Difference between the declared and found amounts (t-Student test)	TV = 0.8221 $<t_{95\%,4}$ = 2.776	TV = 0.8257 $<t_{95\%,4}$ = 2.776

TV = the tested value

Comparison between the densitometry and videodensitometry was performed by Snedecor's F-test (precision) and Student's t-test (accuracy). Results are shown in Table 4. There were no significant differences between the two elaborated methods.

Table 4. Comparison of precision and accuracy between densitometry and videodensitometry

Test	Sabril tablets
F-test (precision)	1.016 (p = 0.988)
T-test (accuracy)	0.002 (p = 0.998)

CONCLUSION

The proposed densitometric and videodensitometric methods are simple, precise and accurate. No statistical differences in accuracy and precision between the two elaborated methods were observed. The results showed that the both methods are suitable for quantitative analysis of vigabatrin for different pharmaceutical purposes.

Acknowledgments: The paper was developed with the use of the equipment purchased within the Project "The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases" within the Operational Program

Development of Eastern Poland 2007- 2013, Prority Axis I Modern Economy, Operations I.3 Innovation Promotion.

REFERENCES

1. Al-Majed A.: A direct HPLC method for the resolution and quantitation of the R-(-)-and S-(+)-enantiomers of vigabatrin (ă-vinyl-GABA) in pharmaceutical dosage forms using teicoplanin aglycone chiral stationary phase. *J. Pharm. Biomed. Anal.*, 50, 96, 2009.
2. Al-Zehouri J., Al-madi S., Belal F.: Determination of the antiepileptics vigabatrin and gabapentin in dosage forms and biological fluids using Hantzsch reaction. *Arzneim. Forsch. Drug Res.*, 51, 97, 2001.
3. Belal F. et al.: Spectrofluorimetric determination of vigabatrin and gabapentin in urine and dosage forms through derivatization with fluorescamine. *J. Pharm. Biomed. Anal.*, 27, 253, 2002.
4. Cetin S.M. and Atmaca S.: Determination of vigabatrin in tablets by high performance liquid chromatography. *Acta Pharmaceutica Turcica*, 44, 57, 2002.
5. Chen T.M., Contario J.J., Fike R.R.: High-performance liquid chromatographic assay for vigabatrin and its primary degradation product in a pharmaceutical tablet formulation. *J. Chromatogr.*, 398, 351, 1987.
6. Ekram M.H. et al.: Spectrofluorimetric determination of vigabatrin and gabapentin in dosage forms and spiked plasma samples through derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole. *J.AOAC Int.*, 84, 993, 2001.
7. Johannessen B.D. et al.: Therapeutic drug monitoring of the newer antiepileptic drugs. *Ther. Drug Monit.*, 25, 347, 2003.
8. Johannessen S.I. and Tomson T.: Pharmacokinetic Variability of Newer Antiepileptic Drugs. *Clin. Pharmacokinet.*, 45, 1061, 2006.
9. Lin F.M. et al.: Capillary electrophoresis analysis of gabapentin and vigabatrin in pharmaceutical preparations as ofloxacin derivatives. *Analytica Chimica Acta*, 523, 9, 2004.
10. Olgun N., Erturuk S., Atmaca S.: Spectrofluorimetric and spectrophotometric methods for the determination of vigabatrin in tablets. *J. Pharm. Biomed. Anal.*, 29, 1, 2002.
11. Shafaati A. and Lucy C.: Application of capillary zone electrophoresis with indirect UV detection to the determination of a model drug, vigabatrin, in dosage forms. *J. Pharm. Pharmaceut. Sci.*, 8, 190, 2005.