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Spectrofluorimetric determination of vigabatrin and gabapentin in urine and dosage forms through derivatization with fluorescamine

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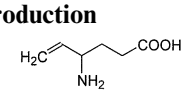
Abstract

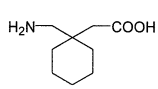
A stability-indicating, sensitive, simple and selective spectrofluorimetric method was developed for the determination of vigabatrin (VG) and gabapentin (GB). The method is based on the reaction between the two drugs and fluorescamine in borate buffer of pH 8.2 to give highly fluorescent derivatives that are measured at 472 nm using an excitation wavelength of 390 nm for both drugs. The optimum conditions were ascertained and the method was applied for the determination of VG and GB over the concentration range of 0.20–4.00 and 0.1–1.0 μg/ml, respectively with detection limits of 0.05 μg/ml (2.9×10^{-7} M) and 0.06 μg/ml (2.3×10^{-7} M) for VG and GB, respectively. The suggested method was applied, without any interference from the excipients, to the determination of the two drugs in their pharmaceutical formulations. Furthermore, the method was extended to the in-vitro determination of both drugs in spiked human urine. Interference from endogenous amino acids could be eliminated through selective complexation with copper acetate, the % recovery ($n = 4$) is 98.0 ± 7.05. Co-administered drugs such as lamotrigine, phenobarbitone, valproic acid, clopazam, carbamazepine, clonazepam and cimitidine did not interfere with the assay. The method is also stability-indicating; as the degradation product of vigabatrin: 5-vinylpyrrolidin-2-one, produced no interference with its analysis.

Keywords: Vigabatrin; Gabapentin; Fluorescamine; Dosage forms; Urine

1. Introduction



Vigabatrin
(VG)



Gabapentin
(GB)

Vigabatrin (VG): 4-amino-5-hexenoic acid, and gabapentin (GB): 1-(aminomethyl)cyclohexane acetic acid, are structurally related to the inhibitory neurotransmittor γ-aminobutyric acid (GABA). They constitute an important group of compounds that are used in the treatment of epilepsy [1]. The two drugs are relatively new, and therefore, are not yet official in any pharmacopoeia. A large literature exists for both VG and

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GB. However, all the published reports concentrate on chromatographic methods, using either gas chromatography [2–6] or high-performance liquid chromatography [7–21]. These methods require sample clean-up procedures and selective detectors, which limit their use in quality control and routine clinical studies. Spectrofluorimetric analysis constitutes a widespread, effective technique to improve analysis selectivity and sensitivity. The sensitivity of the fluorimetric method is comparable to that of analytical methods that employ radioactive isotopes as tracers [22]. Recently, both drugs were determined spectrofluorimetrically using either 4-chloro-7-nitrobenzo-2-oxal-1,3-diazole (NBD-Cl) [23] or through Hantzsch reaction [24].

The aim of the present work is to develop a simple, sensitive, and selective spectrofluorimetric procedure for the determination of vigabatrin (VG) and gabapentin (GB) in dosage forms and spiked urine samples. The method is based on the reaction between these two drugs and fluorescamine. The proposed method has distinct advantages over the reported chromatographic (20–22) and fluorimetric [23,24] methods regarding, simplicity, sensitivity and limit of detection.

2. Experimental

2.1. Instrumentation

Kontron Spectrofluorimeter, SFM 25A, equipped with a 150 W Xenon-high pressure lamp and driven by a PC Pentium II Computer.

2.2. Materials and reagents

All chemicals and solvents were of Analytical Reagent grade. Fluorescamine was purchased from Sigma, Saint Louis, MO. Solutions containing 0.2 and 1.0 mg ml^{-1} were prepared in acetone. The solutions are stable for at least 7 days if kept in the refrigerator. Borate buffer solution of pH 8.2 was used. The pH of the solutions was adjusted by using pH-Meter. Copper acetate (BDH, Poole, UK): 7% aqueous solution was prepared.

Vigabatrin was obtained from Hoechst-Marion Roussel, Winnersh, Berkshire, UK. Gabapentin was obtained from Parke-Davis, Cambridge, Bedfordshire, UK. Pharmaceutical preparations containing the two drugs were purchased from the local market. Urine samples were obtained from healthy volunteers.

2.3. Preparation of the standard solutions

Stock solutions of VG and GB were prepared by dissolving 1.0 mg/ml from each powder in water and were further diluted with the same solvent as appropriate. The solutions are stable for 2 days if kept in the refrigerator.

2.4. Construction of calibration graphs

To two sets of 10 ml volumetric flasks, increasing volumes from the stock solutions of the two drugs were quantitatively transferred, then the volume was adjusted to about 4 ml with water. To each flask, 5 ml of borate buffer solution of pH 8.2 followed by 1 ml fluorescamine solution (0.2 mg/ml) were added. The solutions were made up to the mark and mixed well. The fluorescence of the resulting solutions were measured after 5 min at 472 nm after excitation at 390 nm. The observed fluorescence was corrected by subtracting the fluorescence intensity measured using a reagent blank. The % relative fluorescence was plotted vs the final concentration to get the calibration graph. Alternatively, the corresponding regression equation was derived.

2.5. Assay of dosage forms

Accurate weights of powdered 20 tablets (VG) or mixed contents of 20 capsules (GB) equivalent to 50 mg of each drug were transferred quantitatively to 100 ml volumetric flasks with the aid of $\sim$ 50 ml of water. The contents of each flask were shaken for 10 min. The flasks were completed to volume with water and filtered. The filtrates were then diluted with water to suit the procedure mentioned under construction of calibration graphs (2.4). The nominal contents of the tablets or capsules were calculated using either the calibration graph or the corresponding regression equation.

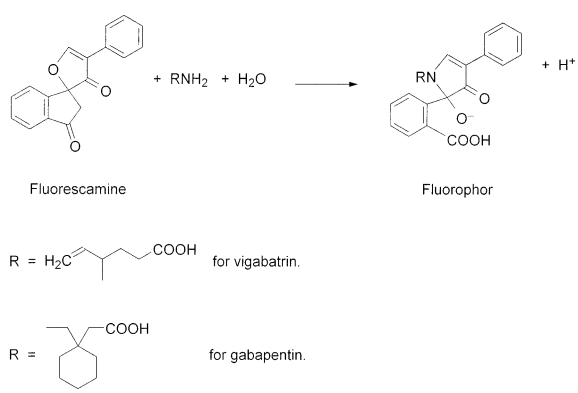
2.6. Assay of urine

Aliquots of spiked urine were transferred into stoppered test tubes. 0.3 ml of copper acetate solution was added and solution was heated in a boiling water-bath for 15 min. The solution was filtered into 10-ml measuring flask. The test tube and filter were washed with 2 ml of water, the washings were passed into the same flask. 4 ml of borate buffer pH 8.2 were added followed by 1 ml of fluorescamine solution (1.0 mg/ml). The solution was completed to the mark with water. The fluorescence of the solution was measured at 390/472 nm. The concentration of the drug was determined from the corresponding regression equation.

3. Results and discussion

Vigabatrin and gabapentin exhibit a very low UV absorption, with $A^{1\%}_{1\,cm}$ at 276 nm = 6.5 for both compounds. As a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods, and this problem is more aggravated if it is needed to estimate these drugs in biological fluids.

Both vigabatrin (VG) and gabapentin (GB) contain a primary aliphatic amino group which is known to react immediately with fluorescamine in alkaline-medium, to give a highly fluorescent yellow adduct. By analogy to previous reports [25,26], the reaction is proposed to proceed as shown in Scheme 1.



Scheme 1. Proposal of the reaction pathway between fluorescamine and the studied drugs.

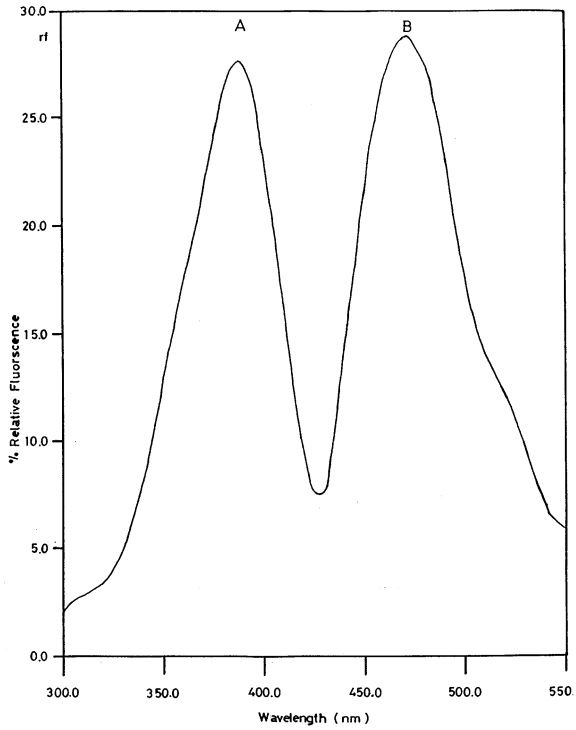


Fig. 1. Fluorescence spectra of the reaction product of vigabatrin (2.0 μg/ml) with fluorescamine. (A) absorption spectrum; and (B) emission spectrum.

Fig. 1 shows the fluorescence spectra of the reaction is proposed to proceed as shown int of vigabatrin (1.0 μg/ml) as a model example.

3.1. Optimization of the reaction conditions

Reaction of fluorescamine with primary amines has been shown to be strongly pH-dependent [27]. Thus, the pH of the reaction medium was varied to observe the relative fluorescence of VG and GP. First, the pH was varied over the whole pH range (2.1–12) in Britton-Robinson buffers. It was noticed that the fluorescence is developed only in alkaline medium and disappeared completely in acid media, therefore, the study of the pH was restricted to the range 7.2–9.5 using borate buffer. As shown in Fig. 2, the highest fluorescence readings for both drugs were obtained at pH 8.2. After studying a series of differ-

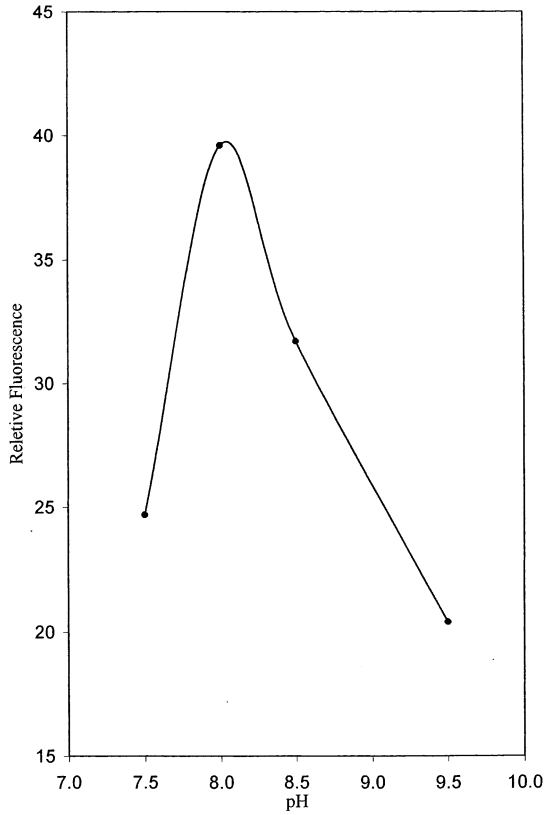


Fig. 2. Effect of pH on the development of the reaction product of vigabatrin (3.0 μg/ml) with fluorescamine.

ent buffer systems having the same pH value, it was found that borate buffer was superior, since the net fluorescence intensity was the highest. The influence of the reagent concentration was also studied by carrying out the fluorimetric procedure with constant concentration of the drug while varying the fluorescamine concentration over the range 1–30 μg/ml (final concentration). It was found that the fluorescence intensity increases linearly upon increasing the reagent concentration up to 20 μg/ml, after which a negligible increase was observed, therefore, 1 ml of 0.2 mg/ml solution was used throughout this study.

Regarding the reaction time and stability of the reaction products, it was found that, the fluorophore is formed immediately, reached maximum intensity after 5 min and remained stable for at least 3 h, after which it began to decrease very slowly.

3.2. Stoichiometry of the reaction

The stoichiometry of the reaction was studied using the limiting logarithmic method [28]. The two straight lines obtained upon using increasing concentrations of the drug while keeping the con-

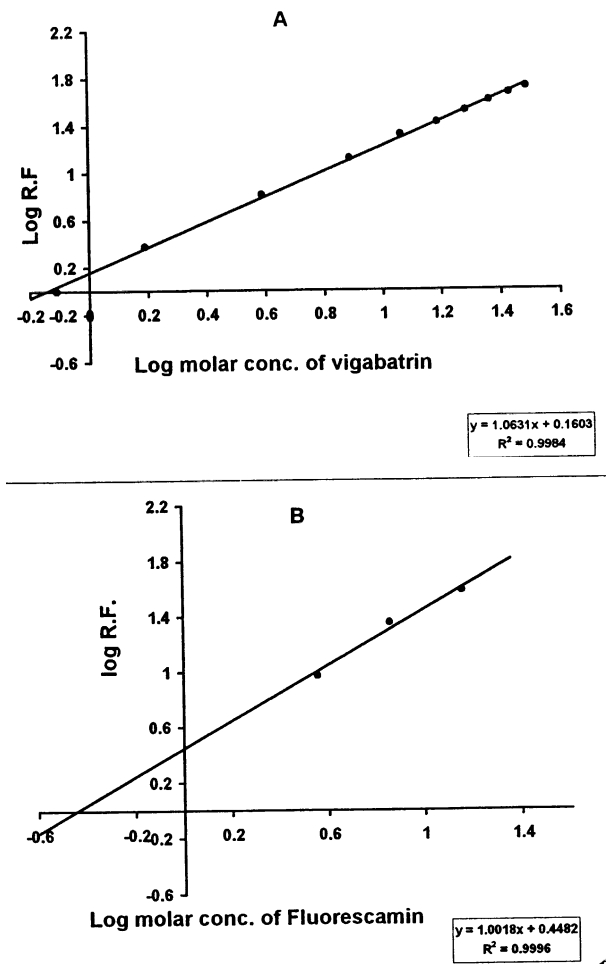


Fig. 3. Limiting logarithmic plots for the molar reactivity of vigabatrin with fluorescamine. (A) log R.F. versus log [vigabatrin] with [fluorescamine] kept at 7.2×10^{-5} M; (B) log R.F. versus log [fluorescamine] with [vigabatrin] kept at 3.1×10^{-5} M.

Table 1
Analytical parameters for the determination of vigabatrin and gabapentin using the proposed method

Parameter	Vigabatrin	Gabapentin
λ_{max}, Wavelength (nm)	390/472 nm	390/472 nm
Concentration range (μg/ml)	0.2–4.00	0.1–1.0
Regression equation		
Intercept (a)	-1.389×10^{-3}	0.479
Slope (b)	13.334	42.68
Correlation coefficient (r)	0.9997	0.9973
$S_{y/x}$	0.5099	1.5337
S_a	0.6425	4.0668
S_b	0.1216	1.9664
Detection limits (μg/ml)	0.05(2.9×10^{-7} M)	0.06 (2.3×10^{-7} M)

centration of the reagent constant (Fig. 3(A)) and upon using increasing concentrations of the reagent while keeping the concentration of the drug constant (Fig. 3(B)). The two lines gave two slopes with the values of 1.063 and 1.002 respectively, therefore the molar reactivity of the reaction is 1.063:1.002, i.e. 1:1. Hence the reaction pathway in Scheme 1 was proposed.

3.3. Validation

The method was tested for linearity, specificity, precision and reproducibility. By using the above fluorimetric procedure, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the relative fluorescence intensity on the concentration of both drugs over the ranges given in Table 1. The table also shows the detection limits and the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear least squares treatment of the results along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$). The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficients and standard deviations [29].

Table 2
Application of the proposed method to the determination of the studied compounds in dosage forms

Pharmaceutical preparation	% Recovery[c] ± SD	
	Proposed method	Reference method [23,24]
Sabril tablets[a] (500 mg vigabatrin/tablet).	100.21 ± 1.22	99.43 ± 0.49
	$t = 1.33$	
	$F = 6.2$	
Neurontin capsules[b] (400 mg gabapentin/capsule)	99.61 ± 0.98	100.00 ± 1.2
	$t = 0.56$	
	$F = 1.499$	

[a] Product of Marrion/Merrel Dow, Batch No. 98897.
[b] Product of Parke Davis, Batch No. 34059.
[c] Mean recovery of five separate determinations.
Theoretical values of t and F at $P = 0.05$ are 2.31 and 6.39, respectively.

The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets or capsule mass. It was shown that these compounds do not interfere with the proposed method (Table 2). It was also found that other co-administered drugs such as lamotrigine, phenobarbitone, valporic acid, carbamazepine, clonazepam, clopazam and cimetidine, do not interfere. The tolerance limits (concentration of interfering substance causing $< 3\%$ relative error) of VG as a model example are shown

Table 3
Effect of co-administered drugs and degradation product on the determination of 4.0 μg/ml of vigabatrin under the optimum conditions

Drug	Tolerance limit (μg/ml)
Carbamazepine	96.4
Cimetidine	96.4
Clonazepam	7.4
Clopazam	16.1
Phenobarbital	96.4
Valproic acid	32.1
Lamotrigine	96.4
5-Vinylpyrrolidin-2-one[a]	20.0

[a] Degradation product of VG.

Table 4
Within-day and between-day precision of the proposed method for the determination of vigabatrin

% Recovery	
Within-day	Between-days
99.79	100.06
100.83	100.29
98.76	99.70
99.79	99.70
98.55	
99.59	
100.62	
$\overline{X} = 99.70$	$\overline{X} = 99.94$
SD = ±0.79	SD = ±0.25
RSD% = 0.79	RSD% = 0.25

Each result is an average of seven separate determinations.

in Table 3. At the same time and because of the dependence of the reaction on the presence of a primary aliphatic amino group in the drug molecule, the degradation product of VG, 5-vinylpyrrolidin-2-one [8] in a concentration five times that of VG did not interfere, therefore the proposed method could be considered as stability indicating assay.

To examine the ruggedness of the procedure, the within-day and between-day precisions were evaluated by analysis of a 4.0 μg/ml sample of VG seven times a day and for 4 consecutive days. As shown in Table 4, the precision of the proposed method is fairly high, as indicated by the low values of RSD% for both drugs. The robustness of the method is demonstrated by the versatility of the experimental factors that affect the fluorescence intensity.

3.4. Applications

3.4.1. Assay of dosage forms

The applicability of the proposed method was tested by the determination of VG and GB in their dosage forms. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery and SD < 2 (Table 2). Tablets or capsules common excipients, such as talc, lactose, starch, avisil, gelatin or magnesium stearate did not interfere with the assay. Statistical analysis of the results obtained by the proposed method and those given by reference methods [23,24] was performed using the Student's t-test and the variance ratio F-test. The calculated values did not exceed the theoretical ones, indicating no significance difference between the compared methods regarding accuracy and precision, respectively (Table 2).

3.4.2. Application to spiked urine

The high sensitivity of the proposed method allowed the determination of the studied compounds in spiked human urine. Regarding vigabatrin, the absolute bioavailability is still unknown, however, 80% of the dose is found in the urine unchanged. After 1.5 g of the oral racemic mixture, peak concentrations of the S (+) enantiomer range from 50 to 100 mmole/l within 2 h [30]. As for gabapentin, it is rapidly absorbed after oral administration and its elimination half-life time ranges from 5 to 7 h. After 1 week of 400 mg dose given three times daily to patients, a Cp_{max} of 3.6–8.6 μg ml^{-1} and $Cp_{min.}$ of 2–4.8 μg ml^{-1} were observed [31]. In both cases, the drug level in urine is above the working range of the proposed method (Table 1).

The interference arising from the endogenous amino acids has been overcome by copper acetate (a modification of the Smithers et al. method) [10]. The amino acids are precipitated as their copper salts and removed by filtration.

Calibration graphs prepared from data obtained from the analysis of spiked urine were linear over the range 0.8–3.2 μg ml^{-1} (Fig. 4). Linear regression analysis of the data gave the following regression equation:

$$F = 9.32 + 26.64\ C\ (R^2 = 0.9756),$$

where F = fluorescence intensity and C = concentration (μg/ml).

The percentage recovery of VG, as a representative example, was 98.0 ($n = 4$) with a coefficient of variation of 7.19%, with a limit of detection of 0.076 μg ml^{-1}. Within-day precision was evaluated by replicate analysis of urine samples containing VG at concentration range 0.8–3.2 μg ml^{-1}.

Compared with the other reported fluormetric methods [23,24]. The proposed method is more simple, as it is conducted immediately at room temperature while that involving the use of NBD-Cl involves heating of the solution at 70 °C for 30 min [23], and that based on Hantzsch reaction [24] requires, heating at 100 °C for 20 min. In addition, the detection limit of the proposed method is lower than that given by either methods; for GB: the detection limit obtained by the proposed method is 2.9×10^{-7} M compared with 5.7×10^{-6} M and 5.8×10^{-7} M for NBD-Cl and Hantzsch method, respectively.

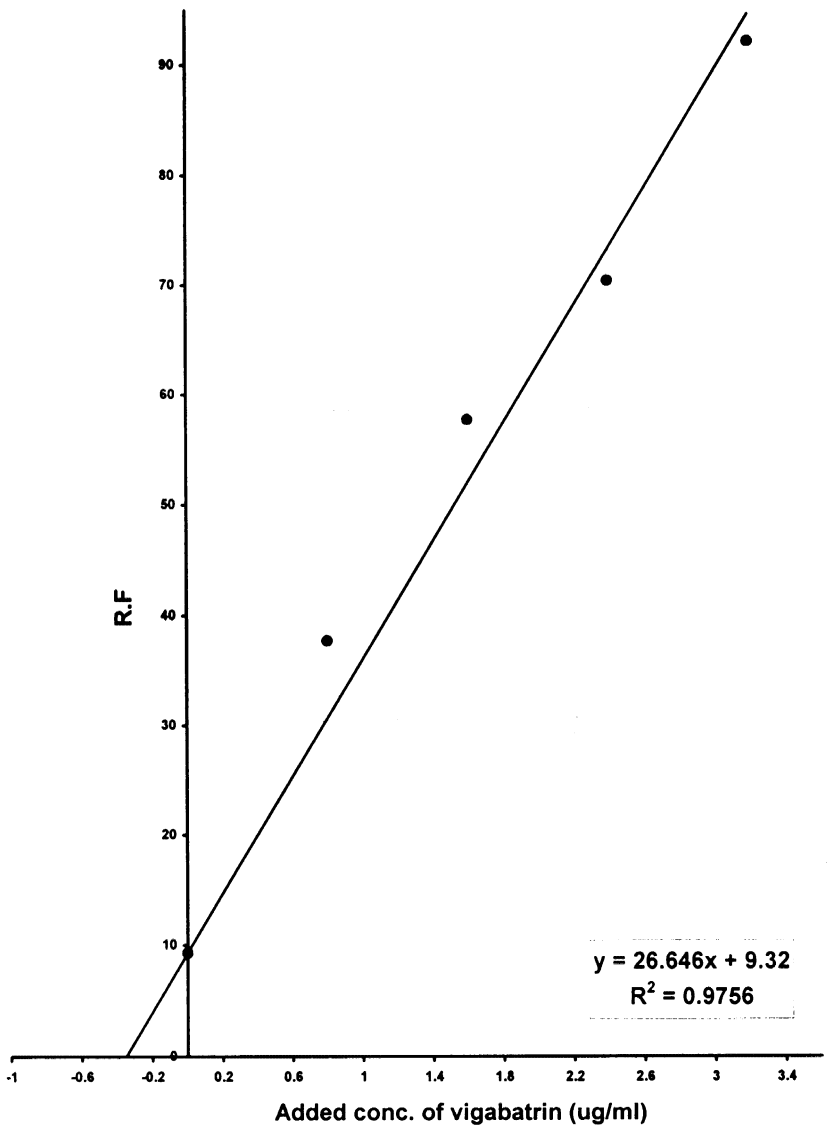


Fig. 4. Standard addition graph for the determination of vigabatrin in urine.

4. Conclusion

The proposed fluorimetric method is accurate, sensitive, rapid and less tedious than the reported chromatographic methods. It provides a simple solution for the problem of low absorptivity of vigabatrin and gabapentin and can be considered of real interest for reliable and practical quality control of these drugs in their pharmaceutical formulations and in biological fluids. Moreover, it is a stability-indicating assay, as the degradation product does not interfere.

References

[1] I.E. Leppik, Epilepsia 34 (4) (1994) S29–S40.
[2] W.D. Hooper, M.C. Kavanagh, R.G. Dickinson, J. Chromatogr. Biomed. Appl. 94 (1990) 167–174.
[3] T.M. Schramm, G.E. Mickinnon, M.J. Eadie, J. Chromatogr, Biomed. Appl. 127 (1993) 39–44.
[4] C.E. Wolf, J.J. Saady, A. Poklis, J. Anal. Toxicol. 20 (1996) 498–501.
[5] F. Van-Lente, V. Gatautis, Clin. Chem. 44 (1998) 2044–2045.
[6] M.M. Kushnir, J. Crossett, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1999) 1–6.
[7] K.D. Haegele, J. Schoun, R.G. Aiken, J. Huebert, J. Chromatogr. 274 (1983) 103–115.
[8] T.M. Chen, J.J. Contario, J. Chromatogr. 314 (1984) 495–498.
[9] J. Grove, R.G. Alken, P.J. Schechter, J. Chromatogr. 306 (1984) 383–387.
[10] J.A. Smithers, J.F. Lang, R. Okerholm, J. Chromatogr. 341 (1985) 232–238.
[11] T.M. Chen, J.J. Contario, R.R. Fike, J. Chromatogr. 398 (1987) 351–354.
[12] M.J. Cope, J.E. Davidson, Analyst 112 (1987) 417–421.
[13] T.M. Chen, R.R. Fike, Chromatography 11 (1990) 4–5.
[14] G.L. Lensmeyer, T. Kempf, B.E. Gidal, D.A. Wiebe, Ther. Drug Monit. 17 (1995) 251–258.
[15] G. Forrest, G.J. Sills, J.P. Leach, M.J. Brodie, J. Chromatogr. Biomed. Appl. 681 (1996) 421–425.
[16] U.H. Juergens, T.M. May, B. Rambeck, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1459–1471.
[17] T.A.C. Vermeij, P.M. Edelbroek, J. Chromatogr. Biomed. Appl. 716 (1998) 233–238.
[18] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monitr. 20 (1998) 430–434.
[19] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. Biomed. Appl. 727 (1999) 125–129.
[20] Q. Jiang, S. Li, J. Chromatogr. Biomed. Appl. 727 (1999) 119–123.
[21] S. Georege, L. Gill, R.A. Braithwaite, Ann. Clin. Biochem. 37 (2000) 338–342.
[22] G. Rapoport, M.F. Glatron, M.M. Lacadet, Acad. Sci. Ser. D 265 (1967) 639.
[23] E.M. Hassan, F. Belal, O.A. Al-Deeb, N.Y. Khalil, J. Assoc. Off. Anal. Chem. Int. (in press).
[24] J. Al-Zehouri, S. Al-Madi, F. Belal, Arzeneim. Forsch. 51 (2001) 97–103.
[25] M. Hefnawy, Y. El-Shabrawy, F. Belal, J. Pharm. Biomed. Anal. 21 (1999) 703–707.
[26] K.C. Chan, G.M. Muschik, H.J. Issaq, Electrophoresis 21 (2000) 2062–2066.
[27] S. Stein, P. Bohlen, J. Stone, W. Dairman, S. Undenfriend, Arch. Biochem. Biophys. 155 (1973) 203.
[28] J. Rose, Advanced Physico-Chemical Experiments, Sir Isaac Pitman and Sons, London, 1964, p. 67.
[29] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Wiley, New York, 1984.
[30] A. Richens, in R. Levy; R. Mattson, B. Meldrum (Eds.), 'Antiepileptic Drugs', third ed., Raven Press, New York, 1989, pp. 937–940.
[31] N.M. Gravens, G.B. Holmes, J.E. Leppik, Pharmacotherapy 9 (1989) 196.