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Quantitative analysis of felbamate in pharmaceutical preparation by thin-layer chromatography with densitometric UV detection

Analiza ilościowa felbamatu w preparacie farmaceutycznym metodą chromatografii cienkowarstwowej z detekcją densytometryczną

Felbamate (2-phenyl-1,3-propanediol dicarbamate, Fig. 1) is an antiepileptic drug, which is similar in structure to meprobamate. It is recommended for treatment of partial seizures with or without secondary generalization in adults, and for Lennox-Gastaut syndrome in children. Because of the risk of aplastic anemia and hepatotoxicity, felbamate is not recommended as first-line treatment [1, 2]. Felbamate is a lipophilic, water-insoluble, nonionic compound. It is believed to be an antagonist at the strychnine-insensitive glycine receptor site of the N-methyl-D-aspartate (NMDA) receptor-ionophore complex. Antagonism of this site blocks the effects of the excitatory amino acids and suppresses the occurrence of seizures [3].

Literature data on determination of felbamate in pharmaceuticals describe only one HPLC [4] and ion-selective electrode [3] methods. Because there are no papers describing quantitation of the drug in pharmaceutical preparations and in biological material by TLC and densitometry, we decided to establish this method as a continuation of our earlier work.

This study presents a simple, rapid and accurate densitometric method for a quantitative analysis of felbamate in tablets.

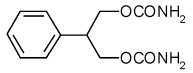

Fig. 1. Structure of felbamate

MATERIAL AND METHODS

Chemicals. Felbamate pure substance was purchased from Sigma (St. Louis, MO, USA). Taloxa® tablets containing 400 mg felbamate per tablet were obtained commercially. Methanol from Merck (Darmstadt, Germany) and chloroform, acetone, acetic acid from POCh (Gliwice, Poland) were of analytical reagent grade.

Standard solution. Stock standard solution of felbamate (3.0 mg/mL) was prepared by dissolving 30.0 mg pure substance in 10.0 mL methanol. The solution was stored in a refrigerator at the temperature of 4^0C and was stable for at least 6 weeks.

Tablet samples. Extraction of the active substance from tablets was performed with methanol.

The average mass of 20 Taloxa 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. The resulting solutions were used for chromatographic analysis. Five microliter of each solution was applied to TLC plates, developed, dried, 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 solution (2.0–20.0 µL, corresponding to 6.0–60.0 µg felbamate per spot) and six tablet solutions (5.0 µL, corresponding to 25.0 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-chloroform-acetic acid (59:40:1, v/v), as mobile phase. After development, the plates were dried at room temperature. The chromatograms obtained were analyzed densitometrically by means of a Desaga (Heidelberg, Germany) CD 60 densitometer controlled by Desaga ProQuant software. The chromatograms were scanned at λ=205 nm with slit dimensions 0.4 mm x 6.0 mm.

Calibration curve was constructed by plotting peak area against the amount of the drug spotted. The amount of the substance analyzed in each tablet was calculated using the appropriate regression equation.

RESULTS AND DISCUSSION

The mobile phase acetone-chloroform-acetic acid (59:40:1, v/v) was selected as optimal for obtaining well-shaped, symmetrical single spots of felbamate. The horizontal technique and a migration distance of 9 cm were chosen as the best for chromatogram development. The R_F value of the drug was 0.61±0.02 (mean ± SD; n=30). The wavelength 205 nm was selected for densitometric evaluation, because at this wavelength there was a maximum of the absorption spectrum of felbamate.

The detection limit (LOD) and quantification limit (LOQ) were determined visually by establishing the minimum levels at which the analyte could be reliably detected and quantified with acceptable accuracy and precision. In a densitometric assay the LOD and LOQ for felbamate were found to be 2.0 and 4.0 µg per spot (Fig. 2).

Calibration was done using six points. For each point, five measurements were made to improve the precision of the analytical procedure. The data were averaged and calibration curve was calculated. The plot of the peak area versus concentration of felbamate was found to be linear in the range 6.0–60.0 µg per spot. The calibration curve was represented by the following linear regression equation:

$$y = 32.8792x + 2.0564 \qquad (r = 0.9990).$$

The densitogram recorded for the standard solutions is presented in Figure 3A.

The intra-day and inter-day precision of the method were estimated by performing five determinations of small (6.0 µg per spot), medium (36.0 µg per spot), and large (60.0 µg per spot) amounts of felbamate. The intraday precisions for felbamate expressed as RSD were 0.78 and 0.81% for the lowest and the highest concentrations. The respective values for the interday precision were 0.84 and 1.08%. The results obtained are listed in Table 1.

Accuracy of the method was assessed on the basis of determination of felbamate in the laboratory-prepared mixtures at 3 levels of addition (50, 100, and 150% of the drug concentration in tablets). For felbamate, the recovery results ranged from 100.17 to 100.46% for the lowest and the highest concentrations of the drug, with RSD values ranging from 0.84 to 0.45% (Table 2).

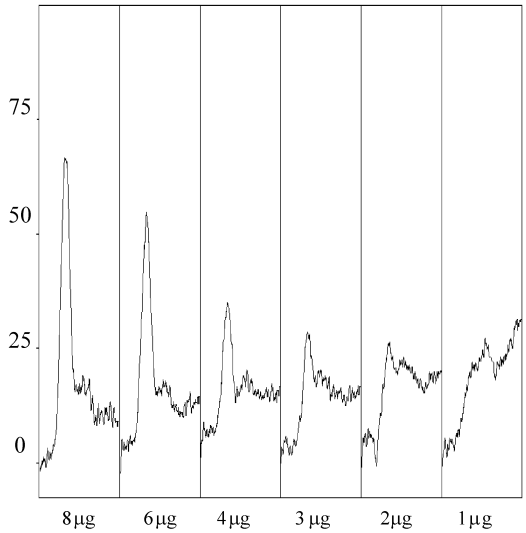

Fig. 2. Evaluation of the detection limit by analysis of a series of solutions containing decreasing concentrations of felbamate

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

Amount (µg/spot)	n	Intra-day precision RSD (%)	Inter-day precision RSD (%)
6.0	5	0.78	0.84
36.0	5	1.22	1.34
60.0	5	0.81	1.08

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

Level of addition (%)	Felbamate	
	Recovery (%)	RSD (%)
50	100.17	0.84
100	99.93	0.71
150	100.46	0.45

[a)] Results are the average of five determinations

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

	Taloxa tablets
Amount claimed (mg)	400
Mean amount found (mg)	400.18
Recovery (%)	100.05
Variance	2.0424
Standard deviation	1.4291
Relative standard deviation (%)	0.36
95% Confidence interval	398.41–401.96

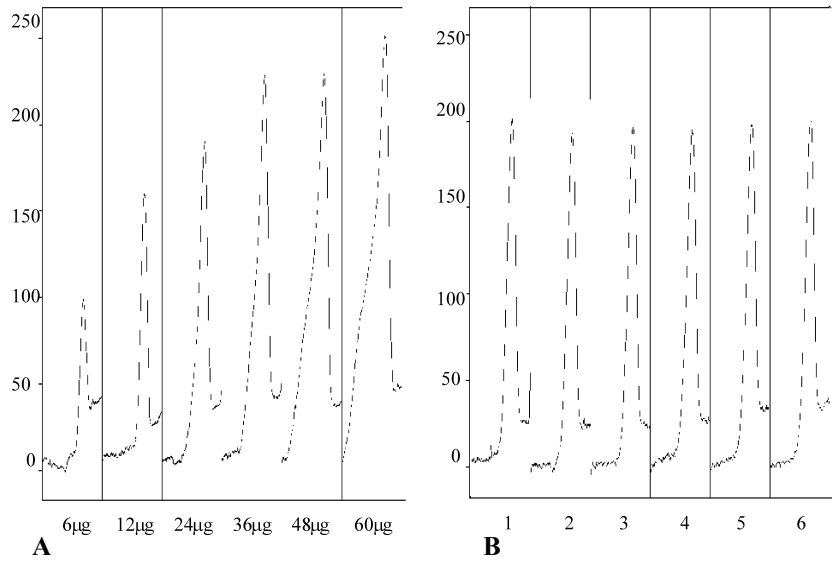

Fig. 3. **A** – Densitogram recorded for standard solutions of felbamate in the calibration range 6.0–60.0 μg per spot. **B** – Densitogram obtained during analysis of Taloxa tablets

The densitometric method was successfully applied for the determination of felbamate in Taloxa tablets. A single spot at $R_F = 0.61$ 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 the from analysis of felbamate in the pharmaceutical product were evaluated statistically; the results are shown in Table 3. Densitogram obtained during analysis of pharmaceutical formulation is shown in Figure 3B.

CONCLUSIONS

The densitometric method described in this paper is accurate, precise, and convenient for routine pharmaceutical analysis. It shows adequate linearity and no matrix effect occurred.

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SUMMARY

A new, simple, rapid, precise and accurate thin-layer chromatography method with densitometric detection was developed for the determination of felbamate in pharmaceutical preparations. The analysis was performed on silica gel 60 F_{254} plates in horizontal chambers with acetone-chloroform-acetic acid (59:40:1, v/v) as mobile phase. Densitometric assay was performed at 205 nm. The active substance was extracted from tablets with methanol. Calibration plot was constructed in the range 6.0–60.0 μg/spot with good correlation coefficient (r=0.9990). Intra- and inter-day precision, calculated as relative standard deviation (RSD), was better than 1.5%. The mean ± SD recovery from commercially available tablets was 100.05 ± 0.36% (n=6). Tablet excipients did not interfere with the chromatography.

STRESZCZENIE

Opracowano prostą, szybką, precyzyjną metodę oznaczania felbamatu w preparatach farmaceutycznych, wykorzystując technikę chromatografii cienkowarstwowej z detekcją densytometryczną. Analizę prowadzono stosując płytki chromatograficzne TLC pokryte żelem krzemionkowym $60F_{254}$. Chromatogramy rozwijano techniką poziomą przy użyciu fazy ruchomej: aceton–chloroform–kwas octowy (59:40:1, v/v) i analizowano densytometrycznie przy długości fali 205 nm. Substancję czynną ekstrahowano z masy tabletkowej metanolem. Oznaczenie felbamatu przeprowadzono w zakresie stężeń 6,0–60,0 μg/plamka, uzyskując dobrą korelację, r=0,9990. Precyzja oznaczeń densytometrycznych w ciągu dnia oraz precyzja międzydniowa cechują się współczynnikiem zmienności < 1,5%. Odzysk felbamatu z tabletek wynosił średnio 100,05 ± 0,36 % (n=6). Substancje pomocnicze wchodzące w skład tabletek Taloxa nie wykazywały pików na chromatogramach w opisanych warunkach analizy.