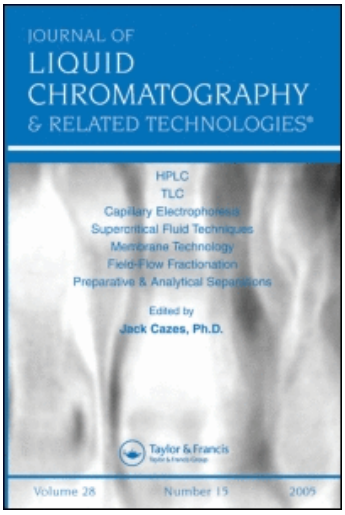

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VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF FELBAMATE IN TABLETS

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ABSTRACT

The validation of an isocratic high performance liquid chromatographic procedure for the determination of felbamate in tablets is reported. The samples were analysed on a Nova-Pak C18 column with a mobile phase composed of acetonitrile–water (1 : 4, v/v) and a UV detection at 210 nm. Phenobarbital was applied as an internal standard.

The method was validated for linearity, precision, accuracy, and limit of detection. The precision of the elaborated method (CV) was less than 1% ($n = 10$). The recovery of felbamate after extraction from the formulation, using the described method, was $99.97 \pm 0.48\%$ (mean $\pm$ SD, $n = 10$).

Key Words: Felbamate; RP-HPLC; Analysis in tablets

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INTRODUCTION

Felbamate (2-phenyl-1,3-propanediol dicarbamate, Fig. 1 is a new antiepileptic drug, which is similar in structure to meprobamate. It is indicated for treatment of partial seizures with or without secondary generalization in adults, and for Lennox–Gastaut syndrome in children.

The major advantages of felbamate, compared with the other existing antiepileptic drugs, are that it is the least neurotoxic and has a broad-spectrum antiepileptic activity with low toxicity and a high protective index.[1–4]

Felbamate is a lipophilic, water-insoluble, nonionic compound. It is used in therapy and, therefore, it is necessary to establish a simple and accurate method for its identification and quantitative determination in pharmaceuticals.

In the literature, there are no publications concerning the analysis of felbamate in dosage forms.

In the present study, a new, simple, and selective HPLC method was applied to the determination of felbamate in commercial dosage forms.

EXPERIMENTAL

Reagents

Felbamate was purchased from Sigma (St. Louis MO, USA), the internal standard (I.S), phenobarbital, was obtained from Pharmaceutical Works "Galenus" (Warsaw, Poland).

Taloxa® (400 mg of felbamate) tablets (Schering-Plough, Belgium) were used.

Acetonitrile and methanol LiChrosolv® for chromatography (E. Merck, Darmstadt, Germany) were applied. All the other reagents were of analytical grade. The water needed in the experiments was double distilled.

Apparatus

A Waters HPLC system (Milford, MA, USA) consisting of a Model 515 high-pressure pump and a Model 2487 variable wavelength detector (UV-VIS),

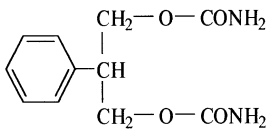

Figure 1. Structure of felbamate.

dual λ absorbance, was used. Manual injections were made using a Rheodyne injection valve (20 μL loop).

The data were collected and analyzed with a Millenium 32 system software on a Pentium MMX 166 MHz computer.

Chromatographic Conditions

Sample volumes of 20 μL were injected into the liquid chromatograph. Analyses were performed at ambient temperature on a Nova-Pak C18 column (150 × 3.9 mm, dp 4 μm) (Waters, Milford, MA, USA).

The mobile phase was a mixture of acetonitrile and water (1 : 4, v/v), filtered and degassed prior to use, and flowing at the rate of 1.2 mL/min. Detection was by UV absorption at 210 nm.

Preparation of the Standard Solutions and Calibration

Standard solutions (1.0 mg/mL) of felbamate and phenobarbital (I.S.) were prepared by dissolving appropriate amounts of these substances in methanol. They were stored at 4°C and were stable for at least 2 months.

Working standard solutions containing 50–800 μg/mL of felbamate and 200 μg/mL of phenobarbital (I.S.) were prepared in methanol. A volume of 20 μL of each sample was injected into the column. All measurements were repeated five times for each concentration. The calibration curve was constructed by plotting the peak area ratios of analyte to I.S. vs. the corresponding drug concentration.

Preparation of the Tablet Solutions and Procedure

Twenty tablets were accurately weighed and the average tablet mass was calculated. The tablets were triturated to a fine powder and amounts equivalent to approximately 100.0 mg of felbamate were extracted with methanol in 25 mL volumetric flasks. Filtered 1.0 mL volumes of the extracts were transferred into 10 mL measuring flasks, 2.0 mL of I.S. solution (1.0 mg/mL) was added and made up to the mark with methanol. A 20 μL volume of the resulting solutions was injected five times into the column.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The chromatographic conditions were optimized to obtain an adequate separation of the eluted substances. The influence of the percentage of acetonitrile in the binary acetonitrile–water mixtures used as mobile phase to separate the compounds in a Nova-Pak C18 column was investigated. A 20 : 80 acetonitrile–water mixture was selected as optimal.

The influence of the flow-rate of the mobile phase was studied in the range 0.5–2.0 mL/min, providing an optimum value at 1.2 mL/min.

Phenobarbital was applied as an internal standard, neutralizing the error inherent in sample injection and eliminating random errors.

The analysed substances were quickly eluted, forming well shaped, symmetrical, single peaks, well separated from the solvent front. The retention times were 3.35 and 5.65 min for felbamate and phenobarbital, respectively.

Validation of the Method

The linearity of the method was maintained over the concentration range 50–800 μg/mL for felbamate. The data were subjected to linear regression analysis in order to obtain the appropriate calibration factors. The regression equation for felbamate was $y = 0.00196\,x - 0.00005$; where y, peak area ratio of felbamate to that of the I.S. and x, concentration of felbamate in μg/mL; the corresponding correlation coefficient was 0.9999.

The precision of the HPLC system was determined using the coefficient of variation of the peak areas for six injections of the standard solutions containing 50 μg/mL and 400 μg/mL of felbamate. The assay in the standard solutions showed a sufficient precision of the HPLC system. The C.V. was less than 1%. The results are presented in Table 1.

The precision of the elaborated method was determined from one lot of the finished product. The assay of content of the drug in formulation was carried out

Table 1. Precision of the HPLC System

Felbamate Concentration (μg/mL)	n	Peak Area (Mean ± SD)	Coefficient of Variation (%)
50	6	1328311 ± 4118	0.31
400	6	10725417 ± 52555	0.49

according to the procedure described in the Experimental section. The precision has been estimated by the means of ten determination of felbamate in powdered tablets. The results are presented in Table 2.

The accuracy of the method was assessed on the basis of determination of felbamate in the model mixtures which were obtained by adding known amounts of felbamate to pharmaceutical preparation. The model mixtures contained 50 (I), 100 (II), and 150% (III) of felbamate in comparing to the labeled tablet amount. For each model mixture, five determination of felbamate were performed. The accuracy of the method is given in Table 3.

The limit of detection was 0.10 μg/mL (C.V. = 4.3%).

Application of the Method

The elaborated method renders a good and rapid separation of felbamate and the internal standard possible.

Methanol was chosen for the extraction from tablets because it is an excellent solvent for both the analyte and the internal standard and it is suitable for the reversed phase mode of chromatography. The recovery of felbamate after extraction was found to be 99.32–100.72% (mean ± SD 99.97 ± 0.48%).

The selectivity of the chosen chromatographic system was also ascertained. All samples were found to meet the requirements for labeled drug content. No

Table 2. Precision of the Elaborated Method

No.	Amount Found (mg in One Tablet)
1	400.02
2	402.89
3	401.63
4	397.42
5	401.12
6	397.28
7	398.64
8	398.92
9	401.88
10	399.16
Mean	399.89
SD	1.9283
C.V. (%)	0.48

Table 3. Recovery Values Obtained for the Determination of Felbamate in Model Mixtures[a]

Model Mixture (%)	Found	Coefficient of Variation (%)
I (50)	100.75	0.95
II (100)	100.40	0.68
III (150)	99.93	1.05

[a]Results are the average of five determinations and are expressed as a percentage of the felbamate added.

interferences were noted from excipients present in the tablets tested. A typical chromatogram of felbamate and the internal standard, after extraction from tablets, is presented in Fig. 2.

Other antiepileptic drugs tested (e.g., vigabatrin, ethosuximide, primidone, phenytoin, tiagabine) did not interfere with the analysis of felbamate.

In summary, a new, simple and selective HPLC assay was elaborated and validated for quantitation of felbamate in tablets. The advantages of the proposed

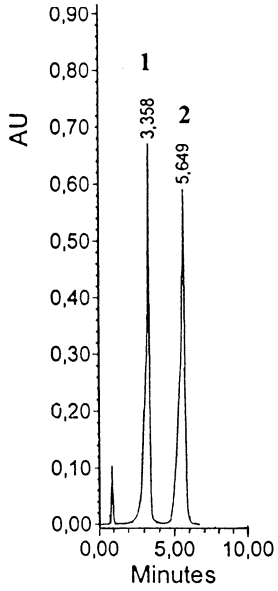

Figure 2. Chromatogram of felbamate (1) and phenobarbital (2) – internal standard after extraction from tablets; HPLC conditions are described in the text.

method are its short analysis time and its simple procedure for sample preparation. The chromatography is performed at room temperature. A simple mobile phase consisting of water and acetonitrile was employed. The method showed sufficient accuracy and precision, and was successfully applied for pharmaceutical analysis.

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