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An Isocratic Reversed-Phase Separation and Determination Of Phenobarbiton, Methylphenobarbitne And Phenytoin In Tablets By High Performance Liquid Chromatography.

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ABSTRACT

An isocratic, sensitive high-performance liquid chromatographic method has been developed for the determination of phenobarbitone, methylphenobarbitone and phenytoin in tablets. A reversed-phase octadecylsilane column, 10 µm, was utilized with a mobile phase consisting of 45% methanol, 55% water and 0.5 ml glacial acetic acid at a flow rate of 1.8 ml/min. The samples were dissolved in methanol containing diethylbarbituric acid as an internal standard. Quantitation was achieved with UV detection at 240 nm and by the measurment of the peak area ratio.

INTRODUCTION

Phenobarbitone (I), methylphenobarbitone (II) and phenytoin (III) are formulated together in tablets known as Comital-L (containing 50 mg of each drug). The tabelts are very effective in controlling chronic epilepsy, since methylphenobarbitone is demethylated by hepatic microsomal enzymes and accumulated as Phnobarbitone [1].

The majority of the analytical methods, especially liquid chromatography, were directed towards the determination of such drugs in biological fluids for monitoring the anticonvulsant therapy [2-4]. Few analytical procedures have been developed for the quantitative determination of such drugs in pharmaceutical tablets or as a barbiturate mixture [5-7]. For quality control laboratory, it is quite important to find a suitable analytical technique to determine the exact constituents of such mixture. For this purpose, our goal was to develop a simple, isocratic HPLC procedure for the simultaneous determination of (I), (II) and (III) in tablets.

EXPERIMENTAL

Apparatus & Conditions

The following apparatus from Waters Associates (Milford,MA,USA) was used : a Model 501 pump, with

automated gradient controller Model 680 equipped with U6K universal injector and a Model 481 variable wavelength UV detector. The instrument was also equipped with a Waters 740 data module. Separation and quantitation were made on a 30cm X 3.9 mm i.d. column of Waters Bondapak RP-18 (10 um). Detection was made at 240 nm and sensitivity was set at 0.1 a.u.f.s. The samples were injected (15 ul) with a 25 ul Waters analytical syringe (Scientific Glass Engineering,Australia). The chart speed was 0.5 cm/min.

Materials & Reagents

Phenobarbitone (Medexport,USSR), methylphenobarbitone (siegfried Zofingen, Switzerland) and phenytoin (Katwijk Chemie B. V.,Netherland) were kindly supplied by Alexandria Pharmaceutical company,and their purity were certified and analyzed to be 100%. Diethylbarbituric acid was used as received without any further purification (VEB Chemische Werke, Czecheslovacia). The water for HPLC was prepared by double glass distillation and filtration through 0.45 um membrane filter. The methanol used for the chromatographic separation was HPLC grade (Romil Chem,UK) and the acetic acid was analytical grade (Prolabo,France). The commercial product,Comital-L, contains the combination of (I), (II) and (III) was supplied by Alexandria Pharmaceuti-

cal Company, which manufactures the tablets under license from Bayer (Leverkusen, FRG).

Procedures

Preparation of standard solution (pure substance)

Internal standard solution : The internal standard solution was prepared by dissolving 600 mg of the diethylbarbituric acid in 100 ml methanol.

Drug standard solutions : 100 mg of (I), (II) and (III) were dissolved in 100 ml methanol(A). A 10 ml aliquot of these standard solutions, with 5 ml of the internal standard solution, was further diluted to 100 ml with mobile phase.

Standard solutions for linearity : Using the drug standard solutions (A) previously prepared, the following concentrations of (I), (II) and (III), with constant concentration (30 mg%) of the internal standard, were prepared : 5, 7, 9, 10, 12, 14 and 15 mg%.

Preparation of samples (pharmaceutical preparation) : Twenty tablets were weighed and powdered. Accurately weighed portions of the powder (each equivalent to the weight of one tablet) were placed in a 100 ml volumetric flask. Each sample was dissolved in 25 ml of the internal standard solution, then completed to volume

with methanol and stirred for a few minutes. These solutions were filtered and the samples were further diluted with mobile phase to obtain a final dilution of 10 mg% of each of (I), (II) and (III), respectively.

Percent recovery study : This study was performed by spiking the powder obtained from the commercial tablets with a known concentration of the pure active ingredients (standard addition method). The resulting mixture were assayed and the results obtained were compared with the expected results.

Assay method : Equal volumes (15 µl) and approximately equal concentrations of the standard and sample solutions were injected into the HPLC and chromatographed under the conditions described above. The standard and sample solutions contained the same concentration of the internal standard. The quantity of each component injected was always within the linearity range.

Calculations : The results were calculated using the response ratio (RR) relative to the internal standard based on peak areas :

Percent of the label claim found = (RRx / RRs) X 100.

Where

RRx = sample response ratio ; RRs = standard response ratio.

RESULTS AND DISCUSSION

To optimize the assay parameters, the effect of methanol composition and pH on the capacity factor (k') were studied (Figs 1&2). The capacity factor (k') values for the three drugs and the internal standard were substantially affected by the variation of methanol composition in the mobile phase (Fig. 1). At high methanol composition, methylphenobarbitone and phenytoin gave sharp peaks but very close to each other (almost overlap). This means that the resolution was inadequate. Lowering methanol below 45%,the peaks become a little bit broad with longer retention times for (II) and (III) peaks. Therefore, 45% methanol composition was selected since it provided sharper peaks and reasonable elution time.

Variation of pH (Fig. 2) yielded maximum k' values at pH of ca. 3.8, but with long retention times for the (III) peak. Lowering the pH to 3.4,3.3,and 3.2 gave a good resolution with sharp peak and the k values were acceptable with a reasonable analysis time. At pH lower than 3.2 , interference has occurred for the (II) and (III) peaks. It was concluded that working in the range of 3.2-3.4 was acceptable. The best resolution and efficiency was achieved at pH 3.4 which was selected for the analysis.

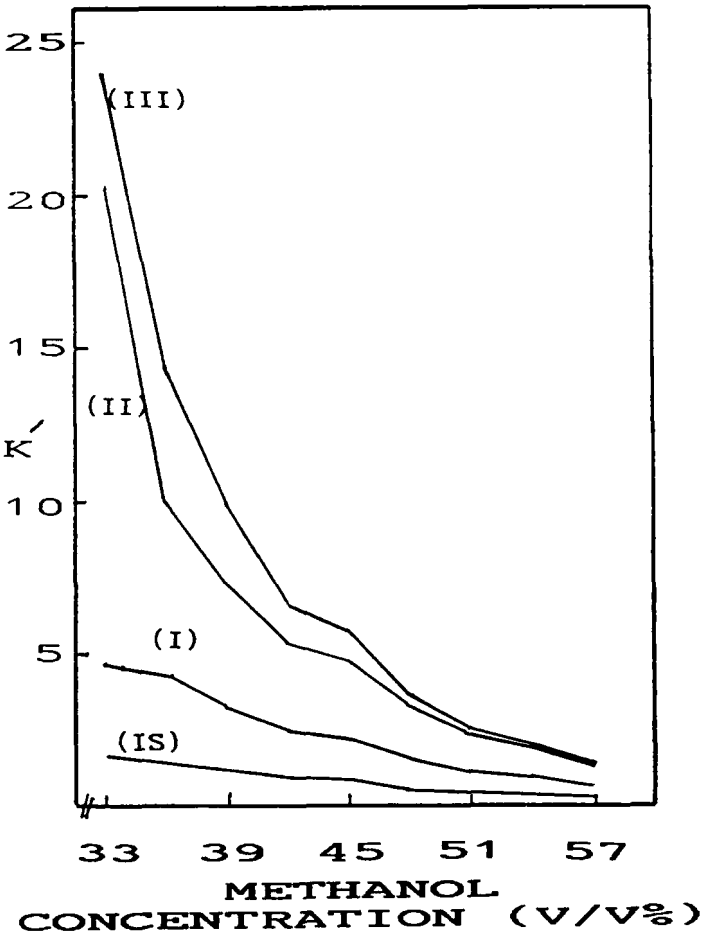

Figure 1 : Plots of the capacity factor versus the methanol composition in the mobile phase.
Key: Phenobarbitone (I), Methylphenobarbitone (II), Phenytoin (III) and Diethylbarbituric acid (IS).

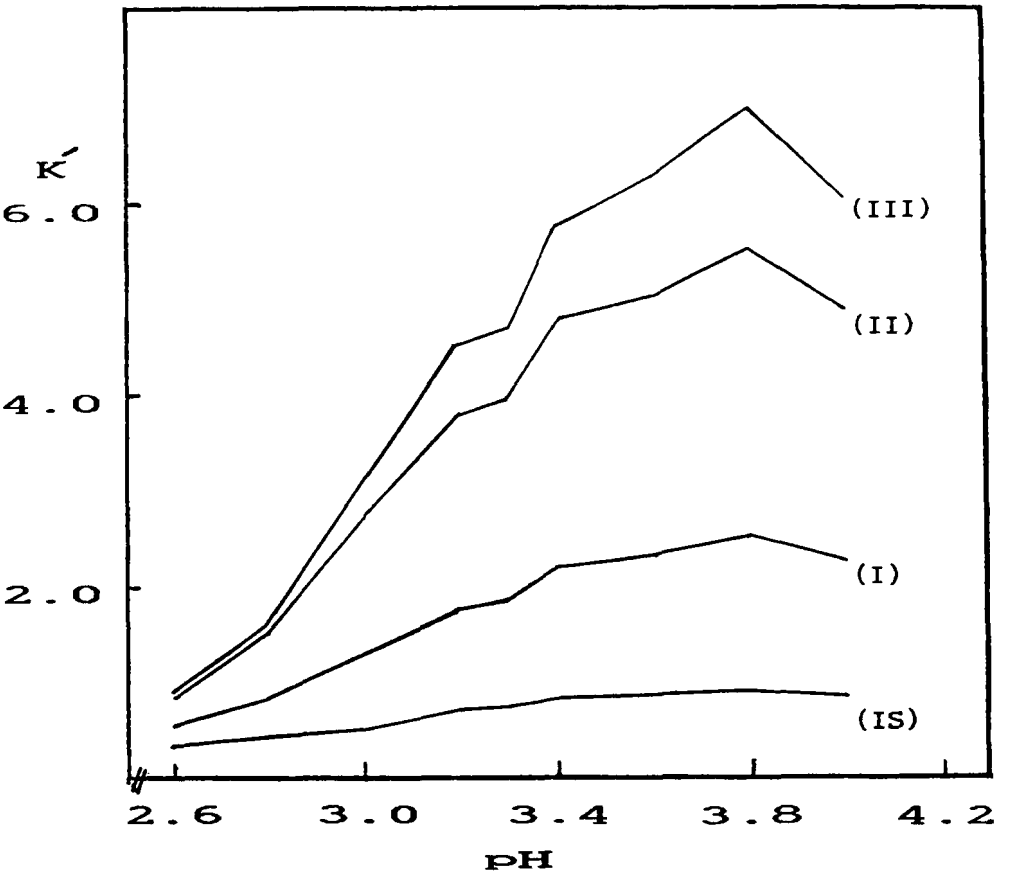

Figure 2 : Plots of the capacity factor versus the pH of the mobile phase.
Key: Phenobarbitone (I), Methylphenobarbitone (II), Phenytoin (III) and Diethylbarbituric acid (IS).

To determine the linearity of the detector respone, calibration standard solutions of (I), (II) and (III) were prepared as described in the text. A plot of peak area ratio versus amount injected was linear in the range of 5-15 mg% of (I),(II) and (III). Regression analysis on the calibration curves relating peak area

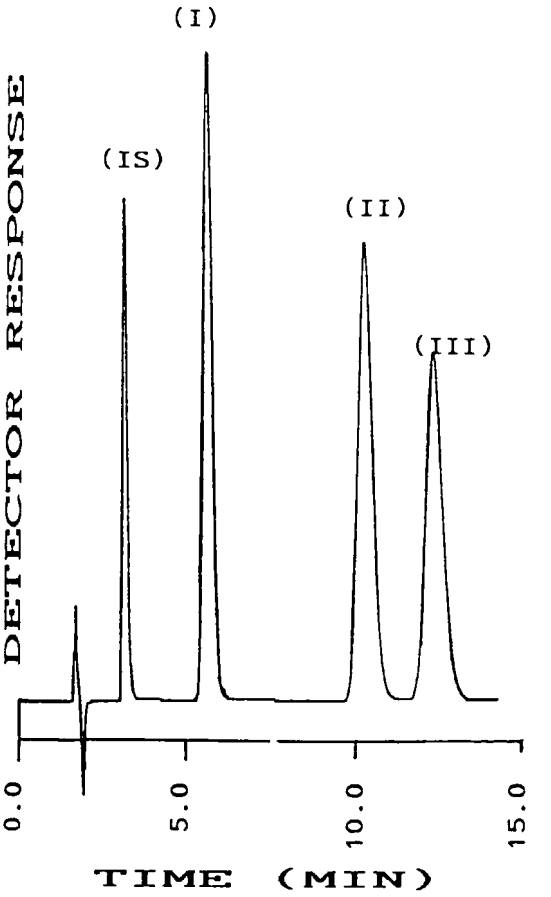

Figure 3 : A typical chromatogram of a 15 µl injection of synthetic mixture of Phenobarbitone (I) , Methylphenobarbitone (II), Phenytoin (III) and diethylbarbituric acid (IS).

(y) and concentration (c in mg%), gave the following equations:

$$y\ (I) = -0.0047 + 0.229\ c$$

$$y\ (II) = 0.0051 + 0.283\ c$$

$$y\ (III) = 0.0460 + 0.255\ c$$

with a correlation coefficients ,r, of 0.9999 for each of them.

The specificity of the method is illustrated in Fig. 3 where a complete separation was noticed for a synthetic mixture of (I). (II), (III) and internal standard. The peaks obtained were sharp symmetrical and have a clear base line separation. The retention times for the internal standard, (I), (II) and (III) are 3.2 ± 0.002 min, 5.6 ± 0.005 min, 10.2 ± 0.009 min and 12.2 ± 0.014 min, respectively, for twelve replicates.

The reproducibility of the proposed analytical method was shown by chromatographing seven solutions of the above mentioned three drugs at various ratio of concentrations in presence of constant concentration of the internal standard (Table 1). In all cases, satisfactory recoveries with small standard deviation was given as indication of the high reproducibility and accuracy.

To measure the analytical recovery of (I), (II) and (III), the three pure active ingredients were added to a commercial powdered tablets to achieve the concentration shown in Table 2. The solutions obtained were analysed by HPLC under the above mentioned conditions. The recovery of the three drugs was calculated by comparing the concentration from the spiked mixtures with the pure active ingredients.

TABLE 1
HPLC Determination of Synthetic Mixtures of Phenobarbitone (I), Methylphenobarbitone (II) and Phenytoin (III) Using Diethylbarbituric Acid as Internal Standard.

Mix.No.	Composition mg%* I	II	III	% Recovery I	II	III
1	5	15	9	99.8	100.1	100.2
2	7	14	5	99.8	100.1	100.7
3	9	12	7	99.7	100.1	99.5
4	10	10	10	99.7	100.0	100.0
5.	12	9	15	99.7	99.8	100.0
6	14	7	12	99.7	100.0	100.0
7	15	5	14	100.0	100.3	100.2
	Mean ± SD			99.8 ± 0.11	100.1 ± 0.15	100.1 ± 0.36

* Each contains 30 mg% Diethybarbituric acid.

TABLE 2
HPLC Determination of a Mixture of Phenobarbitone (I), Methylphenobarbitone (II) and Phenytoin (III) Added to Sample Solutions of Comital-L Tablets.

Exp. No.	mg added of each of I, II&III (mg%)*	% Recovery I	II	III
1	7.0	100.0	100.1	100.1
2	6.0	100.6	100.4	99.9
3	4.0	100.1	100.1	100.0
4	3.0	100.1	100.3	99.6
5	2.0	100.1	99.9	99.9
	Mean	100.2	100.2	99.9
	SD	0.24	0.19	0.19

* Added to a sample solution to give total drugs concentration of 30 mg / 100 mL final solution.

TABLE 3
Determination of Phenobarbitone, Methylphenobarbitone and Phenytoin in Tablets Using the HPLC and UV Methods

	mg found / tablet*								
	Phenobarbitone			Methylphenobarbitone			Phenytoin		
Batch	HPLC	UV	Dev.**	HPLC	UV	Dev.	HPLC	UV	Dev.
A	49.6	50.1	-0.5	49.9	48.6	+1.3	50.6	50.5	+0.1
B	48.7	47.4	+1.3	50.0	50.7	-0.7	48.0	47.6	+0.4
C	49.8	48.6	+1.2	49.2	48.2	+1.0	50.5	49.6	+0.9
D	51.2	50.9	+0.3	49.7	50.5	-0.8	50.3	49.6	+0.7
E	51.6	52.0	-0.4	50.6	50.6	0.0	50.4	49.4	+1.0
Mean	50.18	49.8	+0.38	49.9	49.7	+0.16	50.0	49.3	+0.62
		S.D.	0.85			0.96			0.37
		S.E.	1.38			0.43			0.17
	Conf. Limit#		1.05			1.19			0.47

* Labelled to contain 50 mg of each of the three drugs.
** Deviation = mg found by HPLC method - mg found by UV method.
# 95% double sided confidence limits for Ø = 4 and t = 2.776
= SE X 2.776

The results of the analysis of the commercial tablet (Table 3) indicated that the proposed method can be used for the quantitation of (I), (II) and (III) in commercial samples. The results of the analysis of the commercial tablets (Table 3) and the recovery study (standard addition method) (Table 2) suggested that there is no interference from any excipients which are normally present in tablets.

The results obtained from the proposed HPLC method were compared with the published Δ A spectrophotometric method (5) for the determination of (I) , (II) and (III) in tablets. Statistically (paired comparison) (8), Table 3 shows that the means percentage deviation $\pm$ confidence limits of the results obtained using the chromatographic method from those obtained using Δ A spectrophotometric method were 0.76% $\pm$ 2.10, 0.32% $\pm$ 2.38 and 1.26 $\pm$ 0.94 ($P = 0.05$) for (I), (II) and (III), respectively. The accuracy of both methods (HPLC and Δ A) were supported by the closeness of their results to the label claimed amounts of the three drugs.

The assay presented here has been shown to be applicable to commercially available products, contain any of the combination of the above mentioned drugs. The method is quite simple, accurate, precise and easy to perform. It is experimentally more selective than the Δ A spectrophotometric assay(5).

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