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PHENOBARBITAL IN PHARMACEUTICAL TABLETS BY MODIFIED CONDUCTIMETRICAL ANALYSIS

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Abstract

A simple and reliable assay for Phenobarbital in tablets, by modified conductimetric titration was developed. The use of ammonium hydroxide addition enhanced the acidity of Phenobarbital and allowed its indirect determination, with NaOH as titrant. Optimal Work conditions were established to perform assays with tablets containing 100 mg of phenobarbital provided by the Medical Plant of Corrientes (PLAMECOR) Argentina. The proposed method was found to be highly precise, having a relative standard deviation (CV) below 2.0% in repeatability and intermediate precision studies. Using this approach the calibration curve showed $r^2 = 0.9996$ with a linearity range from 50 to 150 mg. Accuracy based on the average recovery of known amounts of drug in placebo was 100.15% ± 1.31, value included inside the limits established by USP. Trivial signal in titration of placebo demonstrated the high specificity of this method. Measurements were compared with determinations by the reference method (HPLC), using two tails Student`s statistical t-test. It

was established with a 95% confidence level, that there are no significant differences among results obtained with both methods. The modified conductimetric titration method is preferred due to its simplicity, ease, low cost and absence of pre-treatment procedures of the samples

Keywords: conductimetric titration, phenobarbital, pharmaceutical quality control

Resumen

Se desarrolló un método simple y confiable para la cuantificación de fenobarbital en comprimidos, por titulación conductimétrica modificada. La adición de amoníaco exalta la acidez del fenobarbital y permite su determinación indirecta, utilizando hidróxido de sodio como titulante. Se hallaron las condiciones óptimas de trabajo para efectuar el ensayo con tabletas de 100 mg de fenobarbital provistas por la Planta de Medicamentos de Corrientes, Argentina (PLAMECOR). El método propuesto es preciso, con valores del coeficiente de variación (CV) menores al 2,0% tanto en estudios de repetibilidad como de precisión intermedia. El rango lineal está comprendido entre 50 a 150 mg (r^2= 0,9996). La exactitud, basada en la recuperación promedio de cantidades conocidas de la droga pura sobre placebo de 100,15 % ± 1,31 está incluida dentro de los límites establecidos por la Farmacopea de los Estados Unidos (USP). La señal despreciable obtenida en la titulación del placebo demuestra la especificidad del ensayo. Se efectuaron comparaciones con el método de referencia (HPLC), usando el test t de Student de dos colas. Se estableció, con el 95% de confianza, que no hay diferencia significativa entre los resultados obtenidos por ambos métodos. El método de titulación conductimétrica modificado es simple, de bajo costo y no son necesarios procedimientos de tratamiento previo de las muestras.

Palabras clave: titulación conductimétrica, fenobarbital, control de calidad farmacéutico

Introduction

Phenobarbital ($C_{12}H_{12}N_2O_3$; PM 232.23) is the most used anticonvulsant. It is effective in partial clonic tonic crisis, and has some sedative action. It causes tolerance effects in long time use [1]. The chemical structure is shown in figure 1.

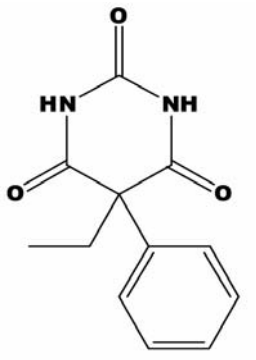

Figure 1. Chemical Structure of Phenobarbital.

Several methods for Phenobarbital determination in pure form and in tablets have been used [2]. USA Pharmacopoeia recommends its determination by liquid chromatography

methods [3]. The main research interests regarding phenobarbital determination have focused on techniques such as: capillary electrophoresis method [4]; ion-selective electrode potentiometric method [5], and micellar liquid chromatography used for the determination of this drug in blood serum samples [6].

The conductimetrical technique avoids extraction and further treatment of the samples and its application is suitable for systems as inert matrices (water-insoluble excipients) containing water-soluble drugs, like Phenobarbital. In this conductimetrical technique the increase in conductivity due to the dissolution of the drug can be related to the amount of drug dissolved. Some of the advantages of this technique are the short time of analysis, around ten minutes, and the low cost of equipment required. An additional advantage is the possibility of automation [7].

Several drugs were determined by the conductimetrical technique such as oxytetracycline in veterinary drugs [8], levofloxacin in pharmaceutical preparations [9], naltrexone and morphine in tablets [10], ascorbic acid in vitamin C tablet by Flow Injection conductimetric determination [11], and the antibiotics ampicillin, amoxycillin and rifampin, in non- aqueous media [12].

Conductimetric titration is a useful technique for determination of weak acids and basis. The acid dissociation constant of phenobarbital is in the order of 10^{-8} [13]. The addition of ammonium hydroxide in excess increases the value of the constant and improves the results (Eq 1):

$$C_{12}H_{12}N_2O_3 + NH_3 \leftrightarrow C_{12}H_{11}N_2O_3^- + NH_4^+ \quad \text{(Eq 1)}$$

Then, the NH_4^+ liberated is titrated with a standard solution of NaOH.

This paper introduces a simple, versatile, cost-efficient and fast quantitative Phenobarbital determination by conductimetric titration and its application to pharmaceutical preparations.

Experimental Section

Samples

Phenobarbital in pure form lot 20002071(powder), origin Denmark, (99,99 % purity, determined by HPLC method) and Phenobarbital 100 mg tablets lot Nº 003/07 and lot 007/07 from PLAMECOR were used.

Placebo used in specificity and accuracy determinations, was provided by Plamecor and its composition is the same present in the tablet.

Reagents

All chemicals were of analytical grade. NaOH purchased from Cicarelli (Argentina) and NH_4OH (26%) purchased from Tejon (Argentina).

Equipment

Conductivity measurements were carried out with a conductivity meter (Parsec, Argentina), rank: $0.1\text{-}2.10^5$ μS, temperature rank: 0 to 80 °C using a glass titration cell of 150 mL useful volume with thermostatic water jacket.

A LKB- HPLC instrument with UV detector was used in the reference method.

Conductimetric Titration

Standard Phenobarbital was weighed, transferred to titration cell; 100 mL of distilled water were added and magnetically stirred for 20 min. After stirring 0.35 mL of ammonium were added and the solution was titrated with 0.5 M NaOH. In the conductivity meter calibration 0.01 M KCl was used. The electrode was submerged into the titration solution, and conductivity was recorded at the same time of the addition of each titrant volume.

Tablets Analysis

Twenty tablets were weighed and an average weight was calculated before being ground into fine powder in a mortar. A portion of the powder, equivalent to the average weight of one tablet (250 mg) was weighed and transferred to the titration glass cell; 100 mL of distilled water, measured in volumetric flask, were added and magnetically stirred for 20 min. After stirring, 0.35 mL of ammonium were added and the solution was titrated with 0.5 M NaOH. Titrations were repeated five times and average values calculated. Considering the volume change, the observed values were corrected by a dilution factor. The end point in conductimetric titrations was obtained by extrapolation of the two linear branches of the plot [14].

A solution of Phenobarbital 1 mg/ml was used to determine the optimal parameters for the analytical procedure. To investigate the volume of NH_4OH, it was varied between 0.25 and 3.00 mL, which correspond to an ammonium hydroxide excess over 100%. It was found that the optimum volume of NH_4OH, considering Phenobarbital recovery, was 0.35 mL.

The specificity of the method developed was assessed by conductimetric titration of placebo, with 0.05 M NaOH. The conductimetric titration curves of: a) Phenobarbital in pure form; b) Phenobarbital 100 mg tablets manufactured by Plamecor in the investigated range of concentration and c) conductimetric titration curves of placebo, all with 0.05 M NaOH, are presented in Fig 2.

For the study of linearity a calibration curve was constructed in a mass range of 50 – 150 mg, including five different masses (50, 80, 100, 120 and 150 mg). The analysis was done by triplicate.

The study of repeatability was carried out with a homogeneous sample of 100 mg of Phenobarbital, according to the procedure descript above. Twelve (12) aliquots were analyzed at the same day, by the same analyst, with the same equipment. Results are shown in Table 1.

For intermediate precision study, homogeneous samples of 50; 100 and 150% of the claimed Phenobarbital content, were analyzed by two different analysts on two different days, by triplicate.

The accuracy was based on the recovery of known amounts of analyte in placebo. Spiked samples with different levels of Phenobarbital (50; 80; 100; 120 and 150 mg) were prepared. The analysis was done by triplicate.

Chromatographic Method

The HPLC analysis of Phenobarbital was performed with RP-18 column (4.2 x 125 mm), Reodhyne injector (10 μL), mobile phase buffer phosphate pH 3.5: methanol (1:1), flow rate 0.6 mL/minute and UV detector (210 nm). Standard solutions of 15.0; 22.5; 30.0; 37.5 and 45.0 μg /mL were prepared by using pure drug dissolved in mobile phase and a calibration curve was built. Samples were prepared from a pool of twenty tablets and an equivalent weight to one tablet was dissolved in 10 mL of methanol, filtrated across

membrane filter (0,5 µm). The solution was further diluted with the mobile phase to 30 µg /mL.

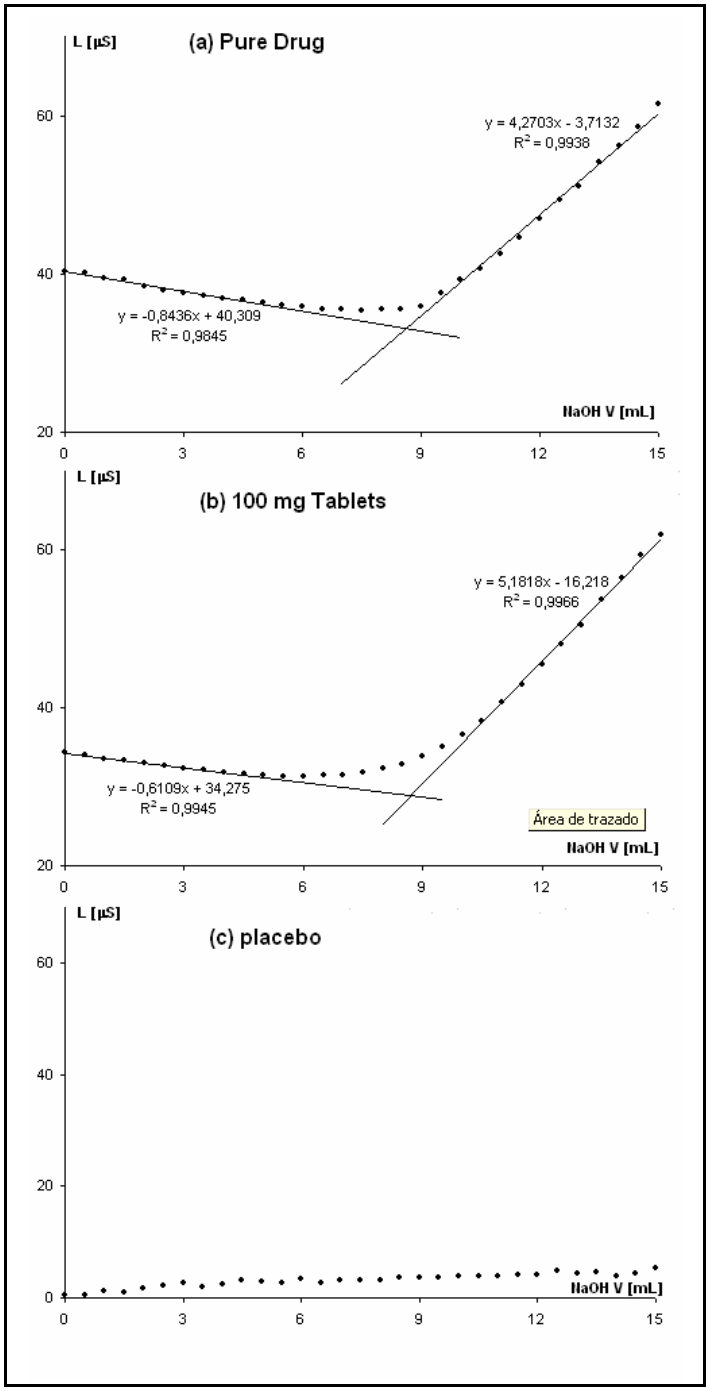

Figure 2: a) Conductimetric Titration curve of pure Phenobarbital b) Conductimetric Titration curve of Phenobarbital 100 mg tablets c) Conductimetric Titration curve of placebo, a) b) and c) with 0,05 M NaOH

Discussion

The shapes of titration curves of pure Phenobarbital and Phenobarbital tablets with NaOH were similar (Fig 2), while titration curve of placebo is a straight line with near zero slope. This fact demonstrates the chemical inertia of Phenobarbital tablets excipients to the conductimetric acid – base titration, and therefore the specificity of the method.

A good linearity has been found from the regression analysis of the calibration curve of recovery mass vs. added mass [$y = (1.0113 \pm 0.0094)\ x + (-0.5157 \pm 0.8630)$] with $r^2 = 0.9996$ as correlation coefficient ($n = 20$) and $F = 50728$ as Snedecor ratio ($P = 0.05$).

Precision measured as repeatability (Table 1) was less than 2%. Global variation coefficient for intermediate precision, for the three concentration levels, was always minor to the double of CV% of repeatability (Table 2), which indicates sufficient precision for this type of analyses. Reproducibility, which refers to the use of the analytical procedure in different laboratories, was beyond the scope of the present study.

Table 1: Repeatability of Conductimetric Determination of 100 mg Phenobarbital tablet.

DETERMINATION Nº	RECOVERY MASS [mg]
1	102.53
2	101.53
3	101.70
4	100.53
5	102.53
6	101.30
7	98.75
8	100.16
9	100.60
10	98.19
11	100.40
12	101.97
X_{mean}	100.85
SD	1.37
CV %	1.36

Table 2: Intermediate Precision for the Conductimetric Determination of Phenobarbital

	ANALYST A		**ANALYST B**		**REPRODUCIBILITY**	
Mfen [mg]	**Day 1**	**Day 2**	**Day 1**	**Day 2**		
50	52.37	52.72	51.76	51.75		
	53.82	53.52	51.50	51.66	**n =**	**12**
	52.94	53.72	50.90	50.95	**M =**	**52.30**
M	53.04	53.32	51.39	51.45	**SD =**	**1.04**
CV%	1.38	0.99	0.86	0.85	**CV%**	**1.98**
100	98.75	98.19	101.09	98.64		
	100.16	100.40	103.53	99.23	**n =**	**12**
	100.60	101.97	103.22	99.82	**M =**	**100.47**
M	99.84	100.19	102.61	99.23	**SD =**	**1.74**
CV%	0.97	1.90	1.29	0.59	**CV%**	**1.73**
200	198.86	200.80	199.16	201.16		
	196.12	200.80	198.20	198.20	**n =**	**12**
	202.92	202.58	201.09	200.09	**M =**	**200.00**
M	199.30	201.39	199.48	199.82	**SD =**	**1.97**
CV%	1.72	0.51	0.74	0.75	**CV%**	**0.98**

The recovery found in the samples of placebo enriched with pure drug (100.15% ± 1,31) (Table 3) turned out to be satisfactory since they are within the criterion established for pharmaceutical analysis. These results indicate the good accuracy of the proposed method.

The validity of the conductimetric method was evaluated by a statistical analysis [14] between the results obtained and those of the reference HPLC method using F-test and two tails t-test (Table 4).

Attending at calculated student's t-test and variance ratio F- test, there is no significant differences between proposed and HPLC methods regarding to accuracy and precision.

Nevertheless the chromatographic method requires stages of separation of the excipients before the analysis, an expensive equipment and an experienced operator.

The direct addition of an equivalent mass of a tablet to the conductimetric cell, for its analysis, is another contribution to the simplicity of the proposed method.

Table 3: Accuracy of Conductimetric Method for Phenobarbital

M added [mg]	M recuperated [mg]	% Recovery	Precision	
50.00	49.81	99.62	**M mean =**	49.27
	48.94	97.88	**SD =**	0.47
	49.07	98.14	**CV =**	0.95
80.00	80.17	100.21	**M mean =**	80.16
	80.92	101.15	**SD =**	0.77
	79.39	99.24	**CV =**	0.95
100.00	101.01	101.01	**M mean =**	100.24
	100.90	100.90	**SD =**	1.25
	98.80	98.80	**CV =**	1.24
120.00	118.96	99.13	**M mean =**	120.29
	121.80	101.50	**SD =**	1.43
	120.12	100.10	**CV =**	1.19
150.00	153.49	102.33	**M mean =**	152.25
	152.25	101.50	**SD =**	1.24
	151.01	100.67	**CV =**	0.81
	Mean Recovery =	100.15		
	CV =	1.31		

Table 4: Statistical Comparison between Proposed Method and Reference Method.

Parameter	Conductimetric Method	HPLC Method
Mean ± SD	103.22± 0.74	102.63±0.72
CV%	0.73	0.70
S^2	0.56	0.53
F-test	1.103 (7.146)*	
t-test	0.789 (2.23)*	

***The values between brackets are the tabulated F-and t-values at P= 0.05.

Conclusions

The conductimetrical method has been confirmed to be useful for the quality control of Phenobarbital dosage forms. This method was found to be simple, rapid, specific, linear, reliable and robust, allowing the determination without preliminary extraction procedures.

Acknowledgments

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