

COLORIMETRIC DETERMINATION OF VALPROIC ACID AND ITS SALTS

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Abstract: A colorimetric procedure for quantitative determination of valproic acid is described. The method is based on the condensation of valproic chloride with 2,4-dinitrophenylhydrazine in an alkaline medium. The method has been successfully applied for the assay of the drug in sodium valproate and aluminum subvalproate.

Keywords: colorimetry, valproic acid, sodium valproate, aluminium subvalproate.

Valproic acid ($C_8H_{16}O_2 = 144,2$) and its derivatives as semisodium valproate or sodium hydrogen bis (2-propylvalerate), $C_{16}H_{31}NaO_4$, sodium valproate or sodium 2-propylvalerate, $C_8H_{15}NaO_2$, calcium valproate or calcium di(2-propylvalerate), $C_{16}H_{30}CaO_4$, magnesium di(2-propylvalerate), $C_{16}H_{30}MgO_4$, valpromide or 2-propylvaleramide, $C_8H_{17}NO$, valproate pivoxil or hydroxymethyl 2-propylvalerate pivalate, $C_{14}H_{26}O_4$ are antiepileptic agents used in the treatment of various forms of epilepsy (1,2).

Qualitative and quantitative analysis of valproate compounds is carried out by thin-layer chromatography and high-pressure liquid chromatography (3,4), infrared absorption spectrum (5), assay on valproate-selective electrodes (6) or by titration methods (5).

There are no data in the literature concerning colorimetric determination of valproic acid. Therefore, the aim of this research was to elaborate quantitative analytical methods for valproic acid and its magnesium and aluminum salts.

The color reaction based on the acylation reaction of carboxylic acid chlorides and acid anhydrides with secondary aromatic amines have been described (7,8,9,10).

EXPERIMENTAL

Substances, apparatus, reagents, solutions

Valproic acid and magnesium valproate produced by POLFA S.A., Rzeszów, aluminum subvalproate ($C_{16}H_{33}AlO_6 = 348,4$) produced in our Laboratory.

Absorption was recorded on a UV/VIS SPECTROMOM 195 spectrophotometer. Standard solutions of valproic acid with concentrations 150 μg , 200 μg , 250 μg , 300 μg , 400 μg , 500 μg , in 5 ml chloroformic solution, 0.4% (v/v) chloroformic so-

lution, 2,4-dinitrophenylhydrazine (0.16 g/l CHCl_3) reagent, 2 mol/l sodium hydroxide aqueous solution, aqueous 2% (w/v) formamide solution.

Valproic acid determination – general procedure

Transfer 5 ml standard solution of valproic acid and 5 ml thionyl chloride solution to glass ampule with 20 ml in volume. Heat the mixture at 60°C for 3.5 h, cool and add 10 ml dinitrophenylhydrazine solution, mix and evaporate the solvent completely on steam bath with the aid of a current of air.

Dissolve the residue in 10.0 ml 2 mol/l sodium hydroxide solution and add 1.0 ml of aqueous 2% (w/v) formamide solution mix, let it stand for about 70 minutes, and measure absorbance at $\lambda = 500$ nm against a concurrently prepared blank.

Color stability and calibration curve

According to the general procedure a color solution was prepared with final concentration of valproic acid 45.45 $\mu\text{g/ml}$ and blank solution, and absorbance was measured in 160 min.

Using the same general procedure, colored solutions were prepared with concentrations as follow: 13.64, 18.18, 22.72, 27.27, 36.36 and 45.45 $\mu\text{g/l}$, and absorbance was measured at $\lambda = 500$ nm against the blank solution.

Determination of active ingredient in preparation

Weigh accurately a sample of valproic acid, dissolve it in chloroform to obtain a solution having a concentration about 450 μg per 5 ml, and proceed as in the general procedure.

Assay of valproic acid in magnesium salt

The magnesium salt contains 92.18% valproate anions. Therefore, weigh accurately about 542 mg of magnesium valproate, dissolve and

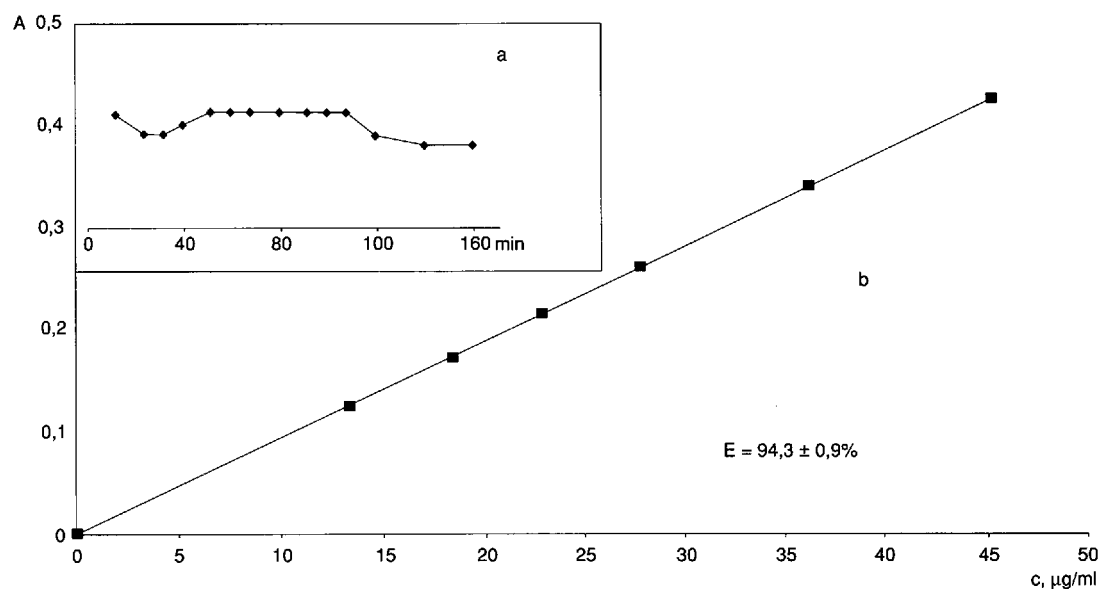


Figure 1. Time related absorbance curve at 500 nm (a), and calibration curve (b).

Table 1. Results of determination of valproic acid free and in its magnesium and aluminium salt

Substance	Sample Weight mg	Quantities acid anion mg		Racovery %	Standard deviation, %
		declared	assayed		
Valproic acid	462.1	462.1	455.9	98.7	100 \pm 4.5
	472.2	472.2	472.2	101.1	
	424.0	524.0	545.4	104.1	
	446.0	546.0	524.2	96.0	
	483.5	483.5	455.9	94.3	
	450.6	450.6	477.2	105.9	
Magnesium valproate	542.0	499.6	498.4	99.8	99.2 \pm 3.4
	515.1	475.1	487.8	102.7	
	545.7	503.0	519.6	103.3	
	509.7	469.8	445.4	94.8	
	537.4	495.4	477.2	96.3	
	539.4	497.2	487.8	98.1	
Aluminium subvalproate	690.1	490.9	477.2	97.2	99.3 \pm 4.2
	620.4	441.3	424.1	96.1	
	652.4	464.1	445.4	96.0	
	642.0	456.7	477.2	104.5	
	653.7	465.0	487.8	104.9	
	661.4	470.5	455.9	96.9	

dilute with CHCl_3 to obtain a solution with concentration about 250 μg per 5 ml, and proceed as above.

Assay of valproic acid in aluminum subvalproate

Aluminum subvalproate contains 71.13% of valproate anions. Weigh accurately a sample of the substance (about 703 mg) dissolve in 100 ml of methanol and add 1 ml of 36.6% hydrochloric acid (w/w), mix, and allow to stand for 15 min to precipitate AlCl_3 , filter into a 100 ml volumetric flask and evaporate methanol in water bath. Dissolve the residue in CHCl_3 added to 100.0 ml, and the concentration of anions is 50 $\mu\text{g}/\text{ml}$, and proceed as in the general procedure.

RESULTS AND DISCUSSION

Average values, A (n = 3), as time-related absorbance curve, and absorbances A (n = 6) in relation to concentration are present in Fig. 1.a and 1.b. Valproic acid contents (M) in assayed samples were calculated from:

$$M = \frac{A}{a_{1\text{cm}}^{1\%}} \times 100\,000 \text{ (mg)}$$

The data are in Table 1.

Previous experiments indicated that valproic chloride reacts with 2,4-dinitrophenylhydrazine in anhydrous chloroform. This acylation product dissolved in 2 mol/l aqueous sodium hydroxide solution and in presence of formamide gave a reproducible purple coloured solution. The optimum intensity of color (at 500 nm) was produced in 50 min (Fig. 1a) and was stable for about 60 min. The regression graph shows, that there is a linear dependence of absorbance on concentration over the Beer's Law ranging from 13.65 $\mu\text{g}/\text{ml}$ to 45.45 $\mu\text{g}/\text{ml}$ (Fig. 1.b.).

The method proposed has been applied to assay the valproic acid and its salts. The determination shows that the recoveries of free acid from valproic acid, magnesium valproate and alu-

minum subvalproate were 100%, 99.2%, 99.3% with standard deviations of 4.5%, 3.4%, 4.2%, respectively.

The procedures proposed can be applied in any quality control laboratory.

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