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The Determination of Individual Components in »B Complex« Dragées

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A method for determination of individual B vitamins in B complex dragées is described. Aneurine and riboflavine are determined photocolorimetrically. Nicotinamide is extracted with chloroform, and titrated with 0.1*N* $HClO_4$ in dioxane. Ca-pantothenate is determined complexometrically. *p*-Aminobenzoic acid is extracted with ether, and diazotated with 0.01*N* $NaNO_2$. Pyridoxine is determined by potentiometric titration of total chlorine (Aneurine·HCl+Pyridoxine·HCl), after which the chlorine bound to aneurine is subtracted and pyridoxine · HCl calculated.

The determination of B vitamins is frequently done by biological methods. Such methods are expensive and time consuming. This is why recently physico-chemical methods, worked out for the determination of B vitamins individualy, have been gaining popularity. As all B vitamins are soluble in water, it was necessary to find either a selective solvent or a specific reaction for each individual B vitamin. In the literature a method for the determination of a mixture of 5 B vitamin has been described, in which nearly all vitamins are determined spectrophotometrically[1]. We applied for the determination of B complex mixture of 6 vitamins the procedures which are quicker and simpler (non- aqueous titration, complexometry, photocolorimetry, diazotation).

The mixture of the B complex had the following composition: Aneurini hydrochloridum 0.005 g., Riboflavinum 0.005 g., Nicotinamidum 0.025 g., Piridoxinum 0.002 g., Ca-panthotenas 0.005 g., Cyanocobalamidum 0.000001 g., Acid. *p*-amino benzoicum 0.002 g.

Aneurine

Aneurine gives with diazotated *p*-aminoacetophenon in alkalic medium a red coloration. This reaction, applied by H. J. Prebluda[2] and later on developed by Sherman[3], is very sensitive (2 μg), the coloration obtained is stable, and the intensity of coloration follows Lambert-Beer's law. Other B vitamins show the same reaction with the difference that using xylol only the coloured product of aneurine was extracted.

Riboflavine

The majority of the chemical methods described in literature are based on measuring the yellow-green fluorescence which is, however, unstable. We chose the Badran Barakat's photocolorimetric method with Denigè-reagent. The colour obtained with the mentioned reagent is sensitive (2 μg) and stable

for 24 hours[4]. This reaction is specific for riboflavine and is not disturbed by other components of B complex.

Nicotinamide

From all components present in the mixture only nicotinamide is soluble in chloroform and it was very simple to extract it with chloroform and to determine it by titration with 0.1 *N* perchloric acid.

Pyridoxine

The synthetical product occurs in the form of hydrochloride, and the methods are based on the determination of chlorine or hydrochloride. As the mixture to be determined contains aneurine · HCl and pyridoxine · HCl, both were determined by means of the potentiometric titration with 0.05 *N* $AgNO_3$ after which the chloride bound to aneurine was subtracted and pyridoxine HCl calculated.

Ca-Pantothenate

Panthothenic acid is most stable in form of salt of calcium or sodium and it usually occurs in this form. We determined it complexometrically, because it is the only metal in the mixture and it can be titrated by complexon III without difficulty.

p-*Aminobenzoic acid*

Being a vitamin H, it does not realy belong to the group of B vitamins, but it is a constituent part of the molecule of folic acid Bc. Because of its specific activity it often occurs in the mixture with B vitamins. The most frequently applied chemical methods are diazotation, photometry and spectrophotometry. In the mixture of B vitamins we determined *p*-aminobenzoic acid very easily by diazotation after extraction with ether.

EXPERIMENTAL AND RESULTS

The individual B vitamins were first determined separately and later on the procedure for the determination in B complex dragées was worked out.

Aneurine, riboflavine, nicotinamide, Ca-pantothenate, pyridoxine and p-aminobenzoic acid were determined. Cyanocobalamin was not examined, because the quantity of solution II was added under further cooling and shaking for another

Reagents and solutions. Reagents were of analytical grade or very pure.

Diazo-reagent. Solution I. 0.159 g. of *p*-aminoacetophenon was dissolved in 2.25 ml. of conc. HCl (spec. gr. 1.19) and diluted with water to 25 ml. The solution was kept in darkness and was stable for 6 months.

Solution II. 2.25 g. of $NaNO_2$ are dissolved in 50 ml. of water. The solution was kept in darkness and is stable for one month. The solutions I and II were mixed in ratio 1:1, by adding dropwise $NaNO_2$ solution to the solution of acetophenone, shaking well during 10 minutes, and cooling in ice. To this solution four times the quantity of solution II was added under further cooling and shaking for another 20 minutes.

Alcalic solution. 5 g. of NaOH were dissolved in 150 ml. of water, 7.2 g. of $NaHCO_3$ were added and the solution was diluted to 250 ml.

Denigè-reagent. 5 g. of yellow HgO were dissolved in 120 ml. of hot sulfuric acid (20 ml. of conc. H_2SO_4 in 100 ml. of water). It was filtered if necessary.

A mixture of previously examined components of B complex and prescribed ingredients was prepared. The method was worked out on the laboratory mixture and later on applied for dragées.

For the separation of the components from the mixture it was necessary to examine their solubility in various solvents. This is shown in Table I.

TABLE I

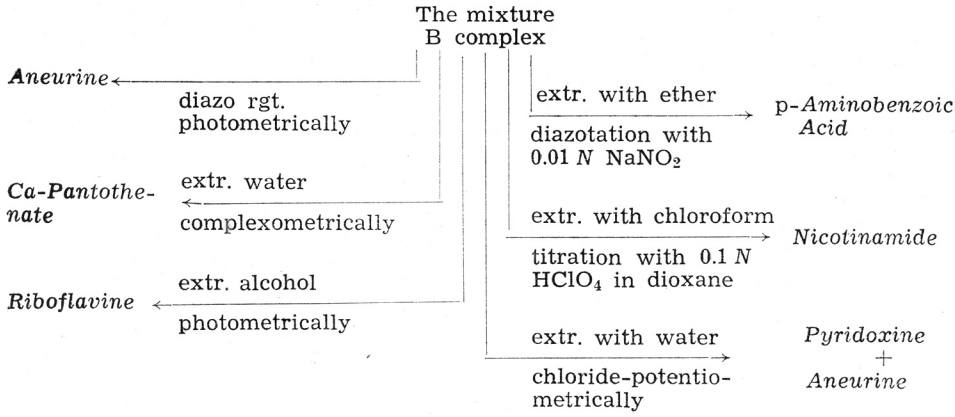
Table of solubility (20°C)

Component	Water	Dil. acids	Dil. alc.	Ethyl alcohol	Methyl alcohol	Ether	Chloroform	Dioxane	Benzene	DMF*	Pyridine	Glycerine	Cyclohexane
Aneurine-HCl	+	+	+	± 100 ml	+	—	—	—	—	—	—	+	—
Riboflavine	+	± warm	+	—	—	—	—	—	—	+ warm	—	—	—
Nicotinamide	+	+	—	+	+	∓	+	+	—	+	+	+	—
Ca-Pantothenate	+	+	∓	—	+	—	—	—	—	+ warm	—	+	—
Pyridoxine	—	+	+	∓ 100 ml	+	—	—	—	—	+	+	+	—
Cyanocobalamine	±	+	+	+	+	—	—	—	—	+	+	+	—
p-Aminobenzoic acid	+ hot	+	+ warm	+	+	+	∓	+	∓	+	+	+	—

* Dimethylformamide

Legend: + soluble
— insoluble
± sparingly soluble

The scheme for determination of individual components is as follows:



The determination of B-complex dragees

Aneurine hydrochloride. 0.20 g. of pulverized dragées were dissolved in water in a volumetric flask, acidified with 1%HCl (cca 20 ml.) and diluted with water to 250 ml. To 1 ml. of this solution, 3 ml. of 0.3% solution of phenol (in 96% alcohol), 0.5 ml. of the diazo reagent and 2 ml. of the alcalic solution (see reagents) were added. The two reagents were mixed in a glass-tube, first adding 0.5 ml. of the diazo reagent into the tube under cooling in ice and a little later 2 ml. of alcalic solution. After 1—2 minutes the pink colour disappeared, and the reagent was immediately added to the solution under examination. The solution was left standing on ice for 20 minutes under repeated shaking. 7 ml. of xylene were then added and

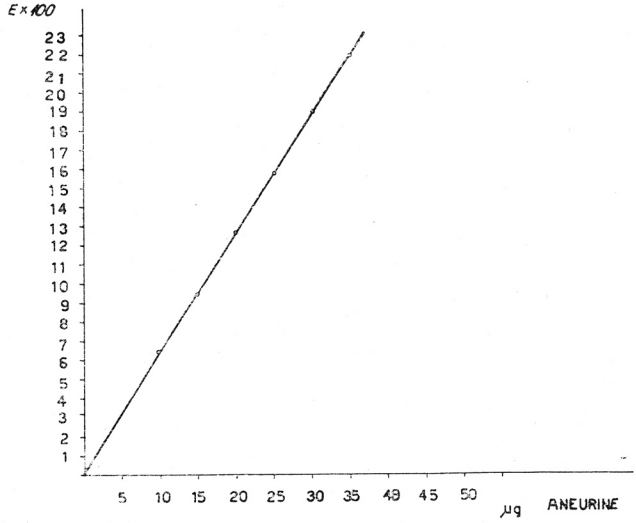


Fig. 1. Standard curve; Fischer electrophotometer, green filter 525 mμ.

the solution was well shaken for ½ minute. The mixture was left standing for 1½—2 hours, so that the layers could separate. The pink xylene layer was carefully separated and the intensity of the colour was measured by Fischer electrophotometer (green filter 525 mμ), using pure xylene as a blank. From the measured extinction the correspondent concentration of aneurine in μg. was determined from the standard curve.

The preparation of the standard curve. Standard solution of aneurine was prepared by dissolving 0.125 g. of pure aneurine hydrochloride in water to 100 ml.

Into a volumetric flask x ml. of the standard solution was introduced, then acidified with 1% hydrochloric acid (cca 20 ml.) and diluted with water to 250 ml. To 1 ml. of this solution (representing concentrations of 10, 15, 20, 25, 30 35, 40, 45 and 50 μg. of aneurine hydrochloride), 3 ml 0.3% solution of phenol, 0.5 ml. of diazo reagent and 2 ml. of the alcalic solution were added. Then was preceeded as directed by aneurine HCl in dragees, beginning with »The two reagents were mixed.«

The standard curve showing the dependence of extinction on the concentration of aneurin is given in Fig. 1.

p-*Aminobenzoic acid.* 1.00 g. of pulverized dragees was accurately weighed in a 50 ml. beaker, transferred on a G-3 glassfilter, extracted with 30, 25, 15, 10 ml. portions of warm ether and filtered under suction. The extracts were collected in a 250 ml. erlenmayer flask and evaporated on a water bath. The dry residue was dissolved in 15 ml. of conc. hydrochloric acid and 15 ml. of water, cooled on ice and

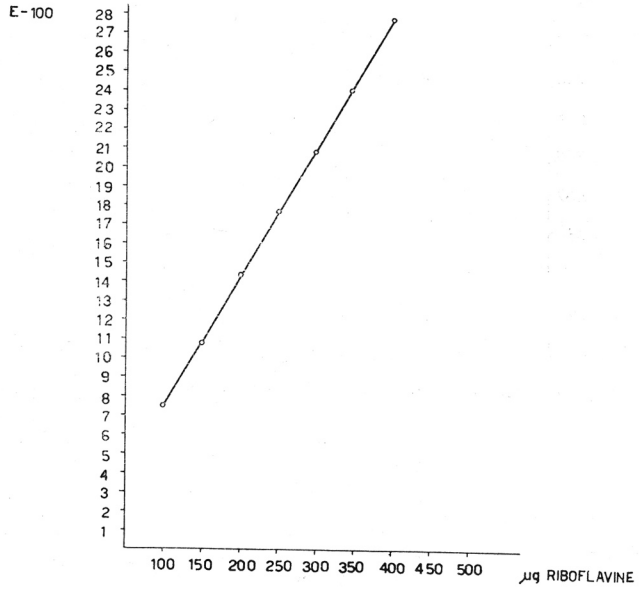


Fig. 2. Standard curve; Fischer electrophotometer, green filter 525 mμ.

slowly titrated with 0.1 *M* sodium nitrite, stirring vigorously, until a blue color was produced immediately when a glass rode dipped into the titrated solution was streaked on a smear of starch iodide paste. When the titration is complete, the end point is reproducible after the mixture has been allowed to stand for 1 minute.

Nicotinamide. 1.0 g. (accurately weighed) pulverized dragees were extracted on a G-3 glassfilter with 20, 20, 10, 10, 10 ml. portions of hot chloroform. The collected chloroform extracts were titrated with 0.1 *N* perchloric acid in dioxane using methyl red (3 drops) until formation of the red-violet colour.

Ca-pantothenate. 3.00 g. (accurately weighed) of pulverized dragees were extracted on a G-3 glassfilter with 30, 25, 20, 10, 10 ml. portions of water. To the solution 2—2.5 ml. normal sodium hydroxide was added and titrated with 0.1 *M* complexon and murexid indicator, until appearance of the dark red colour.

Riboflavine. 0.1500 g. of pulverized dragees was dissolved under vigorous shaking and carefull heating in 50% alcohol in a volumetric flask and diluted to 100 ml. The solution was centrifuged or allowed to settle. To 5 ml. of this solution 3 ml. of Denigè-reagent were added and the solution well shaken. After 3 minutes the obtained colour was measured by Fischer electrophotometer (green filter 525 mμ),

the water being used as a blank. From the measured extinction the quantity of riboflavine was read on the standard curve.

The preparation of the standard curve. The solution of pure riboflavine in 50% alcohol was in the same way so that 5 ml. contained 100—500 μg. of riboflavine. The quantites of 50, 100, 150 μg. were taken and extinctions were measured.

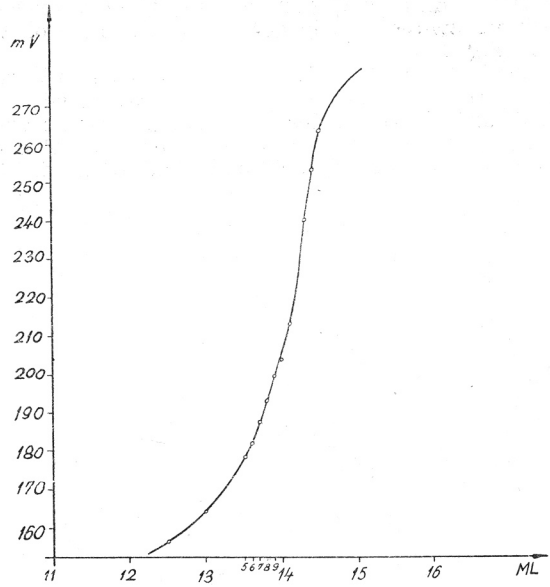


Fig. 3. Potentiometric titration of total chloride in »B complex«.

TABLE II

Results

Component	Quantity in a dragée	Found in a dragée		
		I.	II.	III.
Aneurine hydrochl.	0.005 g.	0.0052	0.0053	0.0049
		0.0053	0.0053	0.0050
p-Aminobenzoic acid	0.002 g.	0.0018	0.0017	0.0018
		0.0018	0.0018	0.0019
Ca-Pantothenate	0.005 g.	0.0052	0.0053	0.0053
		0.0049	0.0052	0.0052
Nicotinamide	0.025 g.	0.023	0.026	0.024
		0.023	0.025	0.024
Riboflavine	0.005 g.	0.0054	0.0050	0.0055
		0.0052	0.0049	0.0052
Pyridoxine	0.002 g.	0.0021	0.0018	0.0022
		0.0018	0.0020	0.0023

The deviations in results are within the limits of Ph. J. II. for tablets.

Pyridoxine. 1.00 g. (accurately weighed) of pulverized dragees was extracted on a G-3 glassfilter with 20, 15, 15, 5, 5, 5 ml. portions of water. The water extract was titrated potentiometrically with 0.05*N* $AgNO_3$.

The quantities of 0.05*N* $AgNO_3$ were plotted on the abcisse and the correspodent mV on the ordinate. The curve was drawn and the equivalent point corresponds to the point in which the curve breaks.

Thereby the total chloride content in aneurine and pyridoxine was determined.

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IZVOD

Određivanje pojedinih komponenata u »B kompleks« dražejama

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Opisana je metoda za određivanje pojedinih B vitamina u smjesi B kompleks. Aneurin i riboflavin određeni su fotokolorimetrijski. Nikotinamid se ekstrahira s kloroformom, a zatim titrira sa 0.1*N* $HClO_4$ u dioksanu. Ca-pantotenat se određuje kompleksometrijski putem kalcija. *p*-Aminobenzojeva kiselina ekstrahira se eterom i diazotira s 0.01*N* $NaNO_2$. Piridoksin se određuje potenciometrijskom titracijom ukupnih klorida (aneurin · HCl + piridoksin · HCl) nakon čega se odbiju kloridi vezani na aneurin, a zatim izračuna sam piridoksin hidroklorid.

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