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QUANTITATIVE ANALYSIS OF WATER-SOLUBLE VITAMINS IN MULTICOMPONENT PHARMACEUTICAL FORMS*

DETERMINATION OF TABLETS AND GRANULES

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SUMMARY

A method has been developed for the individual determination of water-soluble vitamins in compound pharmaceutical forms after thin-layer chromatographic separation. Procedures for tablets and granules containing thiamine hydrochloride, riboflavine, pyridoxine hydrochloride, nicotinamide and *p*-aminobenzoic acid are proposed. The quantitative separation of vitamins was achieved on silica gel plates, using the solvent mixture glacial acetic acid–acetone–methanol–benzene as the mobile phase. Thiamine hydrochloride, pyridoxine hydrochloride and nicotinamide were determined after extraction from the sorbent by UV spectrophotometry, while riboflavine was determined by fluorimetry and *p*-aminobenzoic acid by colorimetry after diazotation. Satisfactory standard deviations were obtained for all the vitamins examined.

INTRODUCTION

Every analyst who has been faced with the problem of analysing vitamins in multicomponent pharmaceutical forms is well aware of the many inconveniences that such an assay may involve.

The number of multivitamin preparations commercially available increases constantly. This fact, as well as the individuality of the contents of the vitamin mixtures, leads to a variety of analytical methods for the quantitation of these compound drugs.

There are several possible approaches to the assay of vitamin mixtures and the problem of devising an accurate method of analysis has become classical in the literature of pharmaceutical analysis.

Besides the classical funnel–funnel extraction, procedures with binary solvent

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systems followed by colorimetric or fluorimetric assay of the separated vitamins, direct differential[1], indirect spectrophotometric[2] and polarographic[3] methods have been proposed. Although rapid, the indirect spectrophotometric method has the disadvantage that the ingredients of the mixture may interfere in the UV spectrum if the vitamins have not been first isolated. The direct differential method requires the use of special apparatus and includes elaborate instrumentation and calculations.

Although thin-layer chromatography (TLC) is distinguished by its simplicity and efficiency, relatively few publications have appeared so far that deal only with the qualitative analysis of water-soluble vitamins in multicomponent pharmaceutical forms. GÄNSHIRT AND MALZACHER[4] separated several water-soluble vitamins by one-dimensional TLC on layers prepared according to STAHL from silica gel using the solvent mixture glacial acetic acid–methanol–benzene–acetone. Optimal separations for combinations of six vitamins of the B-group (1–10 μg) were obtained. BOLLIGER[5] proposed a very simple partial separation, adequate for qualitative purposes, of the same vitamin mixture on similar silica gel plates with only water being used as the developing solvent.

PETROVIĆ *et al.*[6] separated a group of water-soluble vitamins on thin-layers of rice starch with *n*-propanol–pyridine–acetic acid–water as solvent.

FRODYMA AND VAN LIEU[7] proposed the UV reflectance technique for the non-destructive analysis of B-vitamins resolved on silica chromatoplates.

This study was undertaken in order to develop an efficient method for the quantitative analysis of vitamin mixtures containing thiamine hydrochloride, riboflavine, pyridoxine hydrochloride, nicotinamide and *p*-aminobenzoic acid. The method was adapted to the quantitation of these vitamins in tablet and granule products commercially available in Yugoslavia.

EXPERIMENTAL

Reagents and materials

U.S.P. reference standards were used as standard substances. The reagents were of p.a. grade (Merck) and the solvents used were of chromatographic grade (Merck). The citrate buffer solution (pH 2.0) was prepared by mixing 30.9 ml of citrate solution (21.0 g of citric acid was dissolved in 200 ml of 1 *N* NaOH and diluted with water to 1000 ml) with 69.1 ml of 0.1 *N* HCl.

Apparatus

Thin-layer plates were prepared with the TLC outfit supplied by Desaga. The vitamins were located by viewing the plates under a low wavelength UV lamp (Hanau). For fluorimetric measurements, the Kipp fluorimeter with filter B_2 was adapted and spectrophotometric measurements were performed with a Zeiss PQ II spectrophotometer.

Preparation of plates

Thin-layer plates, 20 × 20 cm with a 0.50-mm thick layer of Kieselgel HF_{254} (Merck, No. 7739), were prepared. The plates were activated for 5 h at 130°. The plates were stored at room temperature.

Procedures

An amount of the tablet powder or granules corresponding to approximately 4 mg of thiamine hydrochloride, 5 mg of riboflavine, 2 mg of pyridoxine hydrochloride, 25 mg of nicotinamide and 20 mg of *p*-aminobenzoic acid is dissolved in a 10-ml calibrated flask in 50% methanol, with heating on a water-bath for 10 min. After cooling to room temperature, the solution is diluted with the solvent up to the mark. Two 100-μl volumes of the sample solution and 100 μl of the standard mixture solution are applied by means of a micropipette as 4-cm horizontal bands along the starting line. Along the right-hand side of the plate, a zone of 5 × 20 cm is left free for the adsorbent blanks. The chromatogram is run in a previously saturated (*ca.* 3 h) chromatographic chamber until the solvent front reaches the upper edge of the plate. The plate is air-dried and the separated vitamins are located by viewing under a low wavelength UV lamp. Thiamine, nicotinamide and *p*-aminobenzoic acid appear as dark absorption areas while riboflavine appears as a yellow area and pyridoxine as a pale blue area (Fig. 1). Each vitamin-containing area is scraped off the

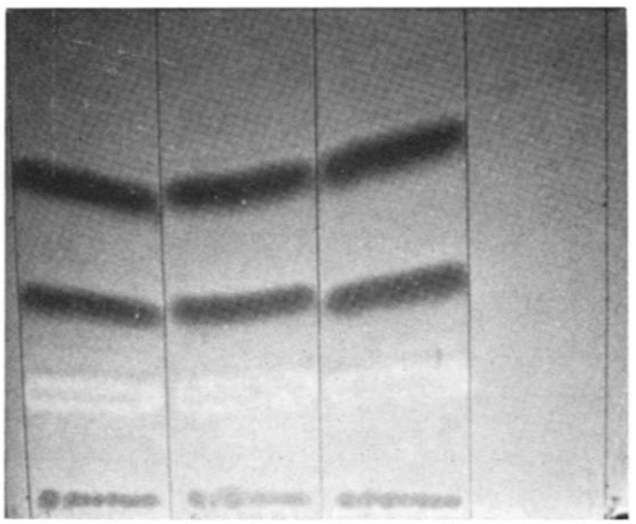

Fig. 1. Chromatogram of sample and standard solution.

plate individually (except for riboflavine) into a 50-ml flask. Simultaneously, blanks are obtained by scraping off adsorbent areas for each vitamin separately at the same level as the chromatograms of the respective sample and standard solutions.

Thiamine hydrochloride

To each adsorbent zone scraped off the plate containing thiamine hydrochloride from the sample solution, for both the standard and the adsorbent blank, 5.0 ml of 0.1 *N* HCl are added, the contents of the flasks are shaken with the aid of a mechanical shaker for 30 min and the silica gel is removed by centrifugation. The absorbances of the clear supernatant solutions are measured at 246 nm against 0.1 *N* HCl as blank.

Riboflavine

To each adsorbent zone containing riboflavine from the sample solution, the respective standard solution and the blank adsorbent zone scraped off the plate into 100-ml calibrated flasks, about 50 ml of water are added and the mixture is shaken with the aid of a mechanical shaker for 1 h. The contents of each flask are diluted with water up to the mark and, after centrifugation, the fluorescent intensity is measured against the riboflavine fluorescent standard using a suitable filter. The fluorescent standard is prepared by dissolving 2 mg of riboflavine in 10 ml of 50% methanol and diluting 100 μl of this solution to 50 ml with water.

Pyridoxine hydrochloride

To each adsorbent zone containing pyridoxine hydrochloride from the sample solution, the respective standard solution and the adsorbent blank, 5.0 ml of the buffer solution is added. The contents of the flasks are shaken with the aid of a mechanical shaker for 30 min. After removing the adsorbent by centrifugation, the absorbances of the clear supernatant solutions are measured at 291 nm against the buffer solution blank.

Nicotinamide

To each adsorbent zone containing nicotinamide from the sample solution, the respective standard solution and the adsorbent blank, 10.0 ml of 96% ethanol is added. After shaking for 15 min and centrifugation, the absorbances of the clear supernatant solutions are measured at 262 nm against 96% ethanol as blank.

p-Aminobenzoic acid

To each adsorbent zone containing *p*-aminobenzoic acid from the sample solution and the respective standard solution, 10.0 ml of 10% hydrochloric acid is added and the flasks are shaken with the aid of a mechanical shaker for 30 min. After centrifugation, 2 ml of the supernatant of both samples and of the standard solution are pipetted into 50-ml calibrated flasks. Then 1 ml of 16% sulphuric acid and 1 ml of a freshly prepared 0.1% solution of sodium nitrite are added and the contents mixed gently. After 3 min, to each flask is added 1 ml of 0.5% ammonium sulphamate solution and after a further 3 min 1 ml of 0.1% N-naphthylethylendiamine dihydrochloride solution. The mixture is made up to the mark with water and after 1 h the absorbances are measured at 545 nm against the blank (2 ml of 10% hydrochloric acid are treated in the same way as the sample and the standard solution).

RESULTS AND DISCUSSION

For the quantitative analysis of the chromatographically separated vitamins, spectrophotometry in the ultraviolet and visible regions as well as fluorimetry were used.

As the vitamins, after separation by TLC, were at the same time purified, UV spectrophotometry with its obvious simplicity, speed and sensitivity seemed to be the most appropriate method for the quantitation of thiamine hydrochloride, pyridoxine hydrochloride[8] and nicotinamide[9]. After the extraction of the vitamins from the sorbent with appropriate solvents and at optimum pH, the absorbances were

determined at the respective maxima. The absence of any possible "irrelevant" absorption owing to the presence of interfering substances that might give rise to erroneous results was checked by isosbestic point determinations.

From all the methods available for the assay of riboflavine, the fluorimetric method seemed to be the most favourable, because of its high sensitivity, for the determination of riboflavine separated on thin layers. Although the bright yellow–green fluorescence of riboflavine is known to be affected by pH changes according to the official method of U.S.P. XVIII, we found that determinations carried out in water only proved to be sufficiently reproducible.

Methods for the determination of *p*-aminobenzoic acid in low-dosage pharmaceutical forms are generally based on two principles: the reaction with *p*-dimethylaminobenzaldehyde or the formation of the azo dye. Although both methods proved to be equally reliable and accurate for the determination of *p*-aminobenzoic acid extracted from the sorbent, the diazo method modified by BRATTON AND MARSHALL[10] seemed to be the most appropriate.

Certain additional experimental results were found to be of significance. Owing to the photosensitivity of vitamins, especially riboflavine and pyridoxine, light-protecting precautions were taken and all operations were carried out under subdued light or in amber-coloured glassware.

The initial extraction of vitamins from the sample, as well as the dissolution of the standard mixture of vitamins at room temperature, were found to be not only lengthy but also unreliable owing to the poor solubility of riboflavine in methanol. Extraction and dissolution were therefore carried out at about 80° and the results obtained by both methods were compared. The results obtained at elevated temperature were not only more reproducible but were also significantly more accurate.

Calculations for all of the vitamins were carried out relative to the respective standard run parallel on the same plate, taking into account the extinctions of the adsorbent blanks. Only for *p*-aminobenzoic acid, which was determined by colorimetry, was the reagent blank taken into account.

To study the precision and reproducibility of the proposed method, analyses were carried out with standard vitamin mixtures prepared in our laboratory and containing exactly known amounts of vitamins in the same concentrations as are present in commercial tablets.

The relative standard deviations were calculated from the equation

$$s = \sqrt{\frac{\Sigma (x-\bar{x})^2}{N-1}}$$

and used as an index of reproducibility. Good reproducibility with satisfactory standard deviations and limits of error were obtained for all of the vitamins (Table I). For very small amounts of pyridoxine (2 μg) applied to the plate the limits of error may be as high as 9.1%, while for all of the other vitamins they were below 5%. Experiments were carried out on nine plates by two analysts.

The results for the determination of vitamins in commercial samples used in Yugoslavia are given in Table II. The results obtained are in good agreement with the manufacturers' specifications.

In Table III, results of analyses carried out by the proposed method are com-

TABLE I

RECOVERY OF VITAMINS FROM THE STANDARD MIXTURE

Vitamin	*Amount of vitamin applied on plate (μg)*	*Recovery*		*Relative standard deviation*	*Confidence limits (%) (P=0.05)*
		μg	*%*		
Thiamine hydrochloride	40.0	41.8	104.5	0.25	4.8
Riboflavine	50.0	49.8	99.6	0.20	3.6
Pyridoxine hydrochloride	20.0	20.9	104.5	0.21	9.1
p-Aminobenzoic acid	200.0	196.8	98.4	0.63	2.6
Nicotinamide	250.0	251.7	100.6	0.54	0.75

pared with those obtained by a standard method based on liquid–liquid extraction followed by colorimetric assay of individual vitamins. As expected, the results obtained by the standard method were lower than those obtained by the proposed method, very probably because of the multiple operations used.

TABLE II

ANALYSIS OF COMMERCIAL MULTIVITAMIN PREPARATIONS

Vitamin	*Recovery (% of claim)*	
	Coated tablets (2 samples)	*Granules (2 samples)*
Thiamine hydrochloride	98.7; 99.5	105.7; 102.5
Riboflavine	98.8; 101.0	99.0; 105.2
Pyridoxine hydrochloride	91.0; 94.0	97.0; 90.5
p-Aminobenzoic acid	97.2; 99.2	91.7; 90.6
Nicotinamide	101.2; 98.4	95.1; 98.6

In concluding, besides good reproducibility and precision, attention should be drawn to the economies in both time and effort effected by the proposed method, which permits the complete analysis of five-component vitamin tablets to be made in not more than 6–7 h, while permitting the determination of low levels of vitamins.

TABLE III

COMPARATIVE ANALYSIS OF A STANDARD VITAMIN MIXTURE BY THE STANDARD METHOD AND THE PROPOSED METHOD

Vitamin	*Recovery (%)*	
	Standard method	*Proposed method*
Thiamine hydrochloride	100.7	100.5
Riboflavine	95.4	99.2
Pyridoxine hydrochloride	90.0	96.5
p-Aminobenzoic acid	98.0	98.5
Nicotinamide	94.4	100.8

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