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SPECTRODENSITOMETRIC DETERMINATION OF NICOTINAMIDE IN SOME MULTIVITAMIN PREPARATIONS

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ABSTRACT

A sensitive, highly selective and stability-indicating procedure for the determination of nicotinamide in some multi-vitamin preparations is described. The procedure is based on thin-layer chromatographic separation of nicotinamide from other constituents of the dosage form, the reaction with aniline and cyanogen bromide vapours, and spectrodensitometric determination at 468 nm. The method is suitable for content uniformity studies.

INTRODUCTION

The problems inherent in the analysis of nicotinamide in multivitamin preparations have been previously reported[1,2]. However, this separation has been successfully accomplished by the use of thin-layer chromatography of nicotinamide from other constituents of the multivitamin preparations, followed by removal of the portion of the thin-layer chromatographic media containing nicotinamide. The quantity of nicotinamide

in the eluted fraction is subsequently determined spectrophotometrically. While this procedure is selective for nicotinamide[2], it is lengthy and requires substantial quantity (15-40 mg) of nicotinamide and thus is not readily amenable to many single dosage forms.

A spectrodensitometric determination of the chromatogram after its development would simplify the determination and result in an increased sensitivity, such a procedure has therefor been developed and evaluated.

EXPERIMENTAL

Apparatus:

A scanning densitometer type camag Z-Scanner (Camag Assocites, New York) was used with the subsequent absorption measurement at 468nm.

Materials:

Nicotinamide reference standard (U.S.P. Reference Standard, Rockville, Md. 20852,U.S.A.).

0.3 mm Thin-layer chromatographic plates(20 cm x 20 cm) of silica gel G. (E. Merck, Germany) were prepared and activated at 105 °C for one hour prior to use[3].

Developing Solvent mixture: Chloroform- ethanol (60:25 v/v[1]).

Visualising reagents: Aniline (E.Merck,Germany) and cyanogen bromide solution-4% cyanogen bromide aqueous freshly prepared solution.

Samples:

The samples used to study the method were obtained from commercial suppliers or prepared (using the nicotinamide reference standard) according to the formulations mentioned in the method of Ismaiel and Yassa[5].

Procedure:

(i)- Calibration Curve: Nicotinamide standard solutions were

prepared to contain 1.0,1.5.1.75,1.9,2.0,and 2.4 mg of the nicotinamide reference standard per mL of water. An aliquot (25 µL) of this solution was spotted, and the chromatogram was developed until the solvent front was within 6cm of the top. The chromatogram was removed, residual solvent was volatalised in a stream of nitrogen and the plate was placed in a closed chamber jar with 2 beakers, the first containing bromide solution and the other aniline. After about 30 minutes, the plate was removed and scanned. Areas of the peaks were determined by triangulation. The calibration curve was obtained by plotting the area under the peak vs. concentration of nicotinamide.

(ii)- Preparation of Samples:

The samples solutions were prepared according to the method of Ismaiel and Yassa[4], Table(1), to contain 2.0 mg per mL of nicotinamide, based on the label claim. The prepared solutions were treated as mentioned above for the calibration curve starting from "An aliquot (25 uL) and the results were determined by reference to the calibration curve and expressed as per cent of the label claim.

RESULTS AND DISCUSSION

Figure (1) represents the data obtained in an investigation of development time and fading characteristics of the visualising reaction. It will be noted that, in each case maximun absorbance devlopment occurs after about 20 minutes and the colour is stable for a minimum of one hour. This determination was carried out numerous times and the charactristies are reproducible under the stated conditions.

Accuracy and precision of the proposed procedure were investigated by the analysis of laboratory prepared samples prepared according to the mentioned method and formulas[5] and the results are presented in Table (2). Recoveries are in good agreement with the expected values and the standard deviation of results is low in each case. It is worthy to note that reults for sample VII, which contained an equal quantity of nicotinic acid in addition to other ingredients, are both accurate and precise.This indicates that the procedure is capable of the selective determination of nicotinamide in the presence of nicotinic acid (may be formed by the partial hydrolysis of nicotinamide content of the injectable solutions during sterilisation by heating and/or ageing), an important advantage over previous spectrophotometric methods [1,2].

Results of the proposed method and those of the thin -layer chromatographic method[4] for the determination of nicotinamide in samples of commercial preparations are presented in Table(3). These results are comparable with the exception of sample IV which is an aged sample containing a crude liver extract. This substantial difference was initially surprising since the T.L.C. conditions of the proposed and the other[4] methods for developing the chromatogram were the same. However, upon examination of the chromatograms of some of the commercial samples it was noted that a fluorescent substance was incompletely resolved from nicotinamide in the T.L.C. method[4], while it was well resolved from nicotinamide in those of the proposed method. Apparently, the smaller application in case of the proposed method prevents overloading of the plate with a consequent gain in efficiency. Thus, the use of spectrodensitometry in this case not only increases the sensitivity but also increases the selectivity in case of the old samples containing decomposition products of liver extract and thiamine.

Spectrodensitometric Determination of Nicotinamide in Some Multivitamin Preparations.

The proposed method therefore appears to be more sensitive, selective and precise than previous methods used[1,2,4,6] for analysis of nicotinamide in mulivitamin preparations.

It is also interesting to note that the results of the ammonia distillation method of the British pharmacopoeia[6] were very high in spite of the dropping concentration of nicotinamide in the old samples IV an V, Table (3), due to the partial decomposition of nicotinmide to nicotinic acid and ammonium salts as well as to the partial decomposition of the other constituents of the two samples.

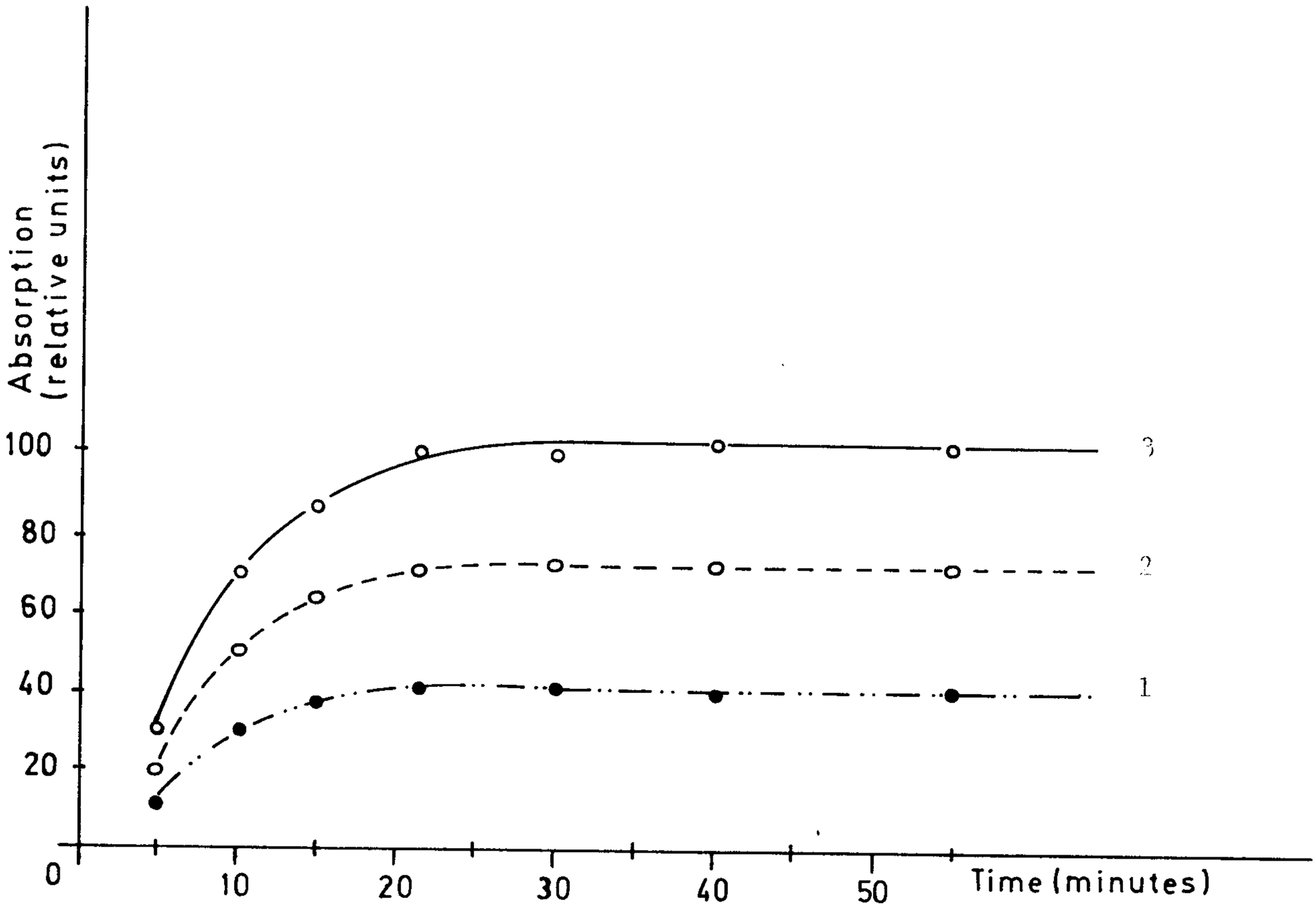

Fig. 1 - Relation Between Reaction Time and Colour Development.
1- 20 µg of nicotinamide,
2- 35 µg of nicotinamide,
3- 50 µg of nicotinamide.

Table (1): Formulations of the Laboratory Prepared Samples

	Laboratory-Prepared Sample							
	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)
	Vial for injection	ampoule	ampoule	ampoule	syrup	syrup	tablets	
Thiamine hydrochloride(mg)	50	100	5	10	100	10	10	50
Riboflavine(mg)	0.5	2	1	0.5	1	1	1	2.5
Nicotinamide (mg)	50	100	5	10	100	10	10	50
Nicotinic acid (mg)	--	--	--	--	--	--	10	--
Pyridoxine hydrochloride(mg)	--	10	1.5	1	5	5	5	5
Calcium pantothenate (mg)	--	5	--	--	5	--	5	4
Sodium pantothenate (mg)	--	--	--	--	--	5	5	--
Dextropanthenol (mg)	--	--		4	--	--	--	--
Cyanocobolamine (µg)	--	100	205	20	--	--	--	--
Biotine (µg)	--	--	5	--	--	--	--	--
Choline chloride (mg)	--	--	--	--	--	--	--	31.4
Ascorbic acid (mg)	--	--	--	--	--	--	--	75
Benzyl alcohol (mg)	20	20	10	20	20	--	--	--
Chlorocresol (mg)	--	2.5	2.5	2.5	2.5	--	--	--
Dry liver extract for parentral use (mg)	--	--	100	100	--	--	--	--
Water for injection to	--	--	1 mL	1 mL	1 mL	--	--	--
Crude liver extract (USP XV) to	1 mL	2 mL	--	--	--	--	--	--
Tetracycline hydrochloride(mg)	--	--	--	--	--	125	--	--
Oxytetracycline hydrochloride (mg)	--	--	--	--	--	--	125	--
Ethinylestradiol (µg)	--	--	--	--	--	--	--	--
Methyltestosterone (mg)	--	--	--	--	--	--	--	2.5
Syrup to	--	--	--	--	--	5 mL	5 mL	--

Table 2 : Results of Determination of Nicotinamide in the Laboratory Prepared Samples by the Proposed Method

Sample (x)	Nicotinamide Stated (mg)	Nicotinamide Added (mg)	Percentage Recovered (xx)
I	50	---	98.6 (2.12)
		25	99.1 (1.74)
		50	100.2 (1.60)
II	100	---	101.0 (2.41)
		50	100.3 (1.21)
		100	98.3 (1.46)
III	5	---	100.2 (2.04)
		2.5	101.9 (2.27)
		5	98.7 (1.39)
IV	10	---	97.4 (2.17)
		5	97.5 (1.98)
		10	98.3 (1.83)
V	100	---	99.5 (1.57)
		50	100.3 (1.69)
		100	100.2 (1.43)
VI	10	---	100.1 (1.05)
		5	99.3 (1.32)
		10	100.4 (1.74)
VII	10	---	99.0 (1.66)
		5	101.2 (1.39)
		10	100.7 (1.96)
VIII	50	---	100.4 (1.51)
		25	98.5 (2.14)
		50	99.7 (2.02)

(x) Formulations are given in Table (1)

(xx) Mean of 7 determinations, values between parentheses are those of the standard deviation.

Table (3): Results of Analysis of Commercial Samples by the Proposed and the T.L.C. Procedures

Pharmaceutical Formulation(x)	Stated (mg)	Nicotinamide(xx) Percentage Recovered		t-value 2.447, at P=0.05
		Proposed method	T.L.C. Method	
1. Beco Forte Tablets	60 / tablet	104.6	105	1.02
2. Polyvit Capsules	10 / capsule	101.6	102.4	0.94
3. Beco Ampoules	200 / ampoule	96.8	96.4	0.66
4. Hepabeco B_{12} Forte Vial for Injection(xxx)	200 / 1 mL	64.3	77.4	1.13
5. Polyvit Ampoules(xxx)	40 / ampoule	84.8	85.6	0.85

(x) Products of Misr Company for Pharmaceutical Industries, El-Mataria, Cairo - Egypt

(xx) Mean of 7 Determinations.

(xxx) Stored over three years and their nicotinamide centents were determined periodically.

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طريقة الكثافة الضوئية لتقدير النيكوتيناميد فى بعض المستحضرات متعددة الفيتامينات

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يصف هذا البحث طريقة حساسة وعالية الاختبارية لتقدير النيكوتيناميد دون غيره فى بعض المستحضرات الطبية . والطريقة بسيطة اذ تعتمد على فصل النيكوتيناميد على شرائح السيلكا الرقيقة من الفيتامينات الاخرى وبعدها يتم اظهار المادة المراد تقديرها عن طريق تعريضها لابخرة الانيلين وبروميد السيانوجين وعندها يمكن تقديرها بواسطة قيمة الكثافة الضوئية عند موجة طولها ٤٦٨ ـ ن٠م٠

والطريقة هنا تصلح كمعيار لقياس النيكوتيناميد بنسبة استرداد مئوى جيدة وكذلك يمكن تقدير المادة الفعالة بحساسية عالية دون تدخل حمض النيكوتينك الذى قد ينتج فى بعض المستحضرات التى يتم تعقيمها بالحرارة كالامبولات المعدة للحقن او قد يكون نتيجة للتحلل الى الحمض المقابل اثناء فترة عرض او تخزين الدواء المحتوى عليه .

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