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A REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF NIACINAMIDE AND RIBOFLAVIN IN DISSOLUTION SAMPLES OF MULTIVITAMIN-MINERAL COMBINATION CAPSULES.

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ABSTRACT

A reversed-phase HPLC analysis of dissolution media samples for niacinamide and riboflavin has been developed to establish pharmaceutical availability in multivitamin-mineral combination capsules.

INTRODUCTION

Multivitamin-mineral preparations are regarded by most regulatory authorities as nutritional supplements. As these preparations are not regarded as drugs, no standards for purity,

pharmaceutical availability and physical stability exists in either the U.S.P., B.P. or the E.P.

However nutritional supplements are receiving emphasis in the standards-setting and information programme of the U.S.P.[1] Thus the U.S.P. must identify and prepare standards for the articles already used as supplements but not yet included in the U.S.P. Nutritional supplements are formulated into dosage forms, similar to those used for drugs, with the same technology and good manufacturing practices. The performance of these preparations can therefore be tested by using the already established methodologies e.g. dissolution testing.

Using the methodology and apparatus for dissolution testing according to the U.S.P., pharmaceutical availability of multivitamins and minerals from combination products as well as the effect of ageing on the release properties of the dosage form can be established. Although many analytical methods for the assaying of single vitamins are documented, the analytical challenge in the case of multivitamin-mineral combinations arises from the complexity of the combinations and the exipients chosen to formulate these preparations. Further to the quantification problems in terms of dissolution testing is the fact that a fixed volume dissolution medium creates different concentrations for each component to be quantified.

Since economy and speed are valid considerations in developing suitable analytical procedures for dissolution testing, niacinamide and riboflavin were selected as the components in a multivitamin-mineral combination product that possessed good chromatographic properties and gave a quantifiable and reproducible account of the dissolution properties of the dosage form.

In this paper an HPLC method is described whereby niacinamide and riboflavin are quantified in 900 ml of 0.1N HCl dissolution medium in the presence of ascorbic acid, calcium pantothenate, pyridoxine, thiamine and minerals.

EXPERIMENTAL

APPARATUS

A USP dissolution apparatus (Caleva model 6ST) and a sampling apparatus (Hanson Dissoette II with Hanson "snake" sample probe) were used for the dissolution.

A liquid chromatographic system (Waters, Millipore Corporation, 34 Maple Street Milford MA 01757) consisting of a dual piston reciprocating pump (Waters Model 510), a Rheodyne injector 7010 with a 60µl loop, a 2µl pre-column filter (Waters #84560) and a UV/Visible variable wavelength detector (Waters Model 484 Tunable Absorbance Detector) was used. The column used was a NOVA-PAK C_{18} 8 X 100mm, 4 micron (Waters #86342) housed in a Z-MODULE (Waters #86500). Data acquisition was accomplished using an electronic integrator (Waters Model 740). A negative peak lock function was used for the first two minutes of the run.

Reagents and Chemicals

Analytical reagent grade 0.1N HCl (BDH Chemicals Ltd Poole England) was used as the dissolution medium and also for the preparation of the standard solutions. HPLC grade methanol was used in the preparation of the mobile phase (BDH Chemicals Ltd Poole England). The ion-pairing agents used were 1-pentanesulphonic acid sodium in glacial acetic acid (PIC B-5 #85110 Waters/Millipore) and 1-heptanesulphonic acid sodium in glacial acetic acid (PIC B-7 #85103 Waters/Millipore). Analytical reagent grade phosphoric acid (BDH Chemicals Ltd Poole England) was used to adjust the pH of the mobile phase. Water distilled in glass was used to prepare the mobile phase.

Chromatographic conditions

The mobile phase consisted of 17% methanol and 83% distilled water containing 0.01M 1-pentanesulphonic acid sodium and 0.005M 1-heptanesulphonic acid sodium adjusted to a pH of 3.0 with phosphoric acid. A flow rate of 2.5 ml/minute was used, UV detection being carried out at 254nm with a sensitivity of 0.05 AUFS. The mobile phase was filtered through a 0.45 micron Millipore filter prior to use.

Reference standards

Standards of niacinamide (purity 99.72%), riboflavin (purity 99.06%) and pyridoxine (purity 100%) were obtained from the USP through Lederle laboratories, Pearl River, New York. Stock solutions of niacinamide (16.67 µg/ml), riboflavin (5.50 µg/ml) and pyridoxine (0.55 µg/ml) were prepared in 0.1N HCl. Concentrations of niacinamide standards were in the range 8.34 µg/ml to 17.50 µg/ml and riboflavin standards from 2.75 µg/ml to 5.78 µg/ml.

Pharmaceutical dosage form

The dosage form used was a size 00 two piece hard-shell gelatine capsule containing Vitamin A Acetate 5 000 I.U., Vitamin D 400 I.U., Thiamine Mononitrate (B_1) 5mg, Riboflavin (B_2) 5mg, Pyridoxine HCl (B_6) 0.5mg, Vitamin B_{12} 1 µg, Ascorbic Acid (C) 50mg, dl-α- Tocopheryl Acetate (E) 10 U, Niacinamide 15mg,

Calcium Pantothenate 5mg, Calcium (as $CaHPO_4$) 145mg, Phosphorus (as $CaHPO_4$) 112mg, Iron (as Ferrous Fumarate) 10mg, Magnesium (as MgO) 1.5mg, Potassium (as K_2SO_4) 5mg, Iodine (as KI) 0.1mg, Copper (as CuO) 1mg, Manganese (as MnO_2) 1mg, Zinc (as ZnO) 0.5mg, l-Lysine HCl 25mg, Choline Bitartrate 50mg; Inositol 50mg. The Niacinamide and Riboflavin were separated and quantitated from the above active ingredients and other excipients.

Sample preparation

Aliquots drawn from the dissolution bowls were filtered using 0.45μm membrane filters (Millex HV) prior to injection onto the column.

Recovery experiments

(a) Linearity. A series of standard solutions of 8.34, 10.00, 12.50, 14.17, 15.84, 16.67, and 17.50 μg/ml for niacinamide and 2.75, 3.30, 4.13, 4.68, 5.23, 5.50, and 5.78 μg/ml for riboflavin were analyzed in duplicate and a single linear regression analysis of the detector response versus the concentration was performed.

(b) Reproducibility. The same working standard solution was chromatographed twelve times and the average response, standard deviation and percent relative standard deviation were calculated.

(c) Recovery. Known amounts of both niacinamide and riboflavin were placed in empty capsule shells, these were exposed to the test conditions in the dissolution apparatus and aliquots of the solutions were analyzed on the HPLC.

(d) Sample reproducibility. The same sample was assayed six times to check the robustness of the method.

Calculation

The content of niacinamide and riboflavin in the dissolution samples were calculated using the following equation:

$$\frac{PRu \times S.C. \times V \times 100}{PRk \times L.S.} = \text{PERCENT ACTIVE DISSOLVED}$$

where: PRu = Peak response of sample

PRk = Peak response of standard

L.S. = Label strength of active (mg)

S.C. = Standard concentration corrected for purity (mg/ml)

V = Volume of dissolution medium

RESULTS AND DISCUSSION

The initial objective was to develop a method whereby the vitamins of interest could be separated and quantitated with minimal sample preparation and acceptable reproducibility. Preliminary studies using a 300 mm X 4 mm stainless steel ODS column at a flow rate of 1.0 ml/min gave a good separation but

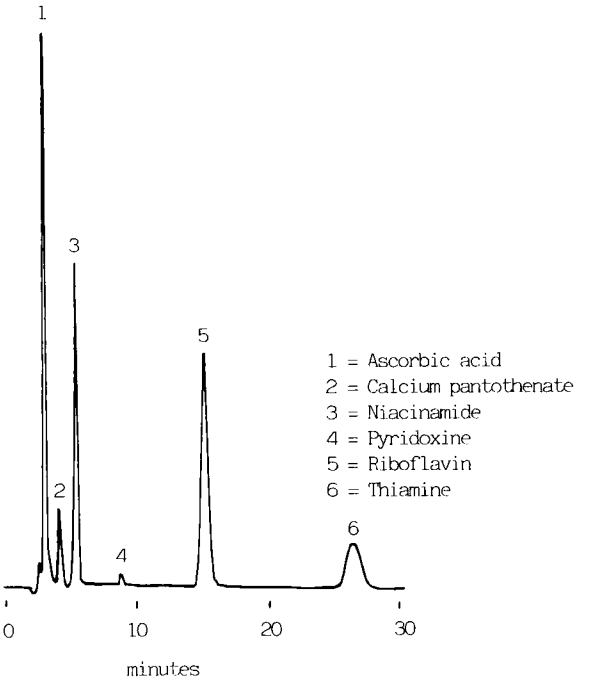
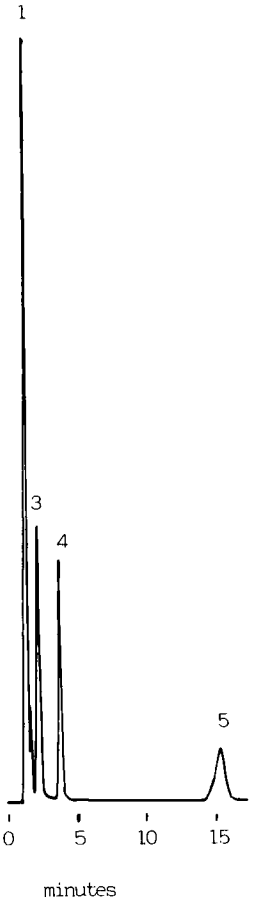


Figure 1

Chromatogram of sample solution

Column: ODS (300 X 4 mm) stainless steel
Flow rate: 1ml/minute
Mobile phase: 17% methanol : 83% water containing 0.01M 1-pentanesulphonic acid sodium and 0.005M 1-heptanesulphonic acid sodium at pH 3.0

involved a 30 minute run time (figure 1).Using a NOVA-PAK 100 mm X 8 mm ODS cartridge at a flow rate of 2.5 ml/min reduced the run time to 16 minutes (figure 2) but then pyridoxine eluted at 3.8 minutes and riboflavin at 3.9 minutes. This problem was overcome by adding a known amount of pyridoxine standard to the working standard solution and by setting the minimum area recorded by the integrator to 100 000 area counts (pyridoxine gave typically 11 000 area counts).



1 = Ascorbic acid
2 = Calcium pantothenate
3 = Niacinamide
4 = Riboflavin
5 = Thiamine

Figure 2

Chromatogram of sample solution.

Column: ODS (100 X 8 mm NOVA-PAK) cartridge
Flow rate: 2.5ml/minute
Mobile phase: 17% methanol : 83% water containing 0.01M 1-pentanesulphonic acid sodium and 0.005M 1-heptanesulphonic acid sodium at pH 3.0

Resolution and tailing factors were determined according to the system suitability test in the U.S.P., the resolution factor being 4.25 between niacinamide and riboflavin. Tailing factors of 0.95 and 0.85 were obtained for niacinamide and riboflavin respectively.

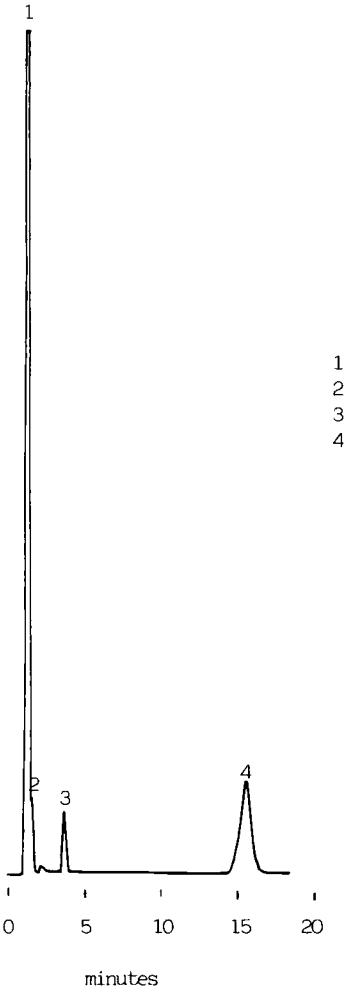


Figure 3

Chromatogram of 'other' ingredients

Column: ODS (100 X 8 mm NOVA-PAK) cartridge
Flow rate: 2.5 ml/minute
Mobile phase: 17% methanol : 83% water containing 0.01M 1-pentanesulphonic acid sodium and 0.005M 1-heptanesulphonic acid sodium at pH 3.0

TABLE 1

Standards Reproducibility Study (peak areas)

	NIACINAMIDE	RIBOFLAVIN
	892212	588789
	883617	565817
	886626	589122
	876489	570481
	871457	571752
	877524	579713
	873867	583591
	877658	569411
	873275	570004
	879091	570666
	879306	572609
	872910	571737
Average	878669	575307
Std dev.	5889	7588
% RSD	0.67	1.32

TABLE 2

Reproducibility results for niacinamide and riboflavin.

NIACINAMIDE (peak areas)

	Standard	Sample
	867137	931030
	875825	935108
	869382	934241
	874332	933592
	862782	931581
	861078	931769
Average	868422	932886
Std Dev.	5447	1267
% RSD	0.63	0.136

RIBOFLAVIN (peak areas)

	Standard	Sample
	568144	627433
	585535	634020
	569247	628511
	587731	634157
	575914	622739
	578986	632929
Average	577592	629964
Std Dev.	7411	4001
% RSD	1.28	0.66

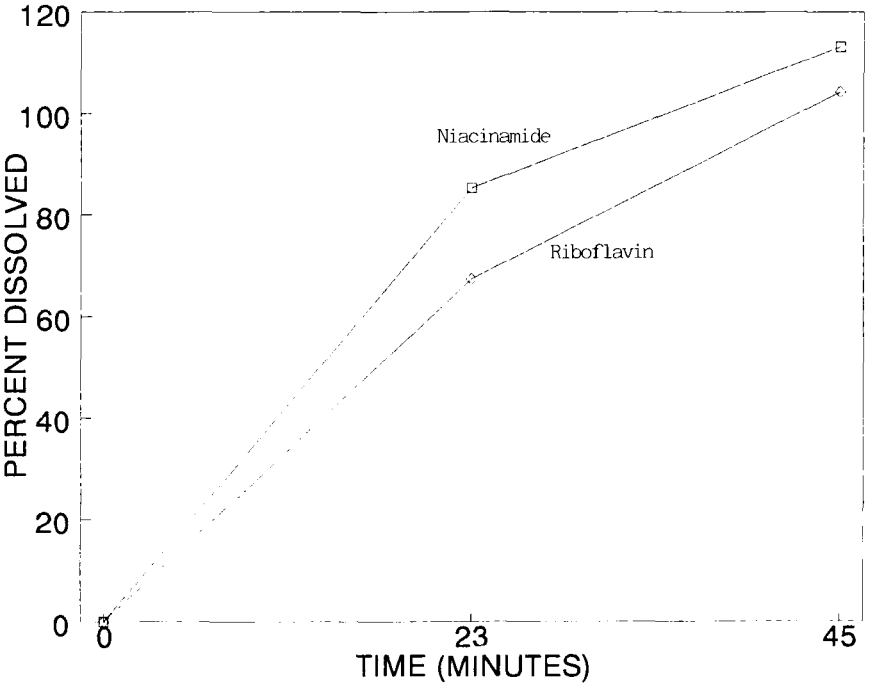


Figure 4

Dissolution profile of Niacinamide and Riboflavin

Note that the percent dissolved is calculated on the basis of percent label claim.

Detector response was found to be linear with correlation coefficients of 0.996 and 0.995 for niacinamide and riboflavin respectively. The standards reproducibility results are contained in Table 1.

Recovery of niacinamide and riboflavin after 45 minutes in the dissolution medium produced 99.9% for niacinamide and 91.1% for riboflavin. The sample reproducibility results are contained in Table 2.

Figure 4 shows a dissolution profile of a stability batch of capsules stored at ambient temperature for 30 months, samples

of the dissolution medium were drawn at 23 minutes and at 45 minutes.

CONCLUSION

The method developed, using the dosage form capsules, is straightforward, direct and can be adopted for use in routine quality control analysis.

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