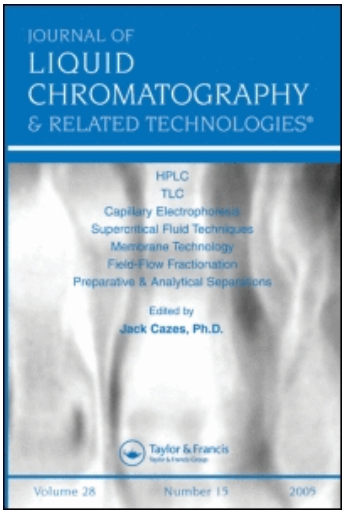

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantification of Niacin and Niacinamide in Vitamin Preparations by Densitometric Thin Layer Chromatography

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To cite this Article Sherma, Joseph and Ervin, Matthew(1986) 'Quantification of Niacin and Niacinamide in Vitamin Preparations by Densitometric Thin Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 9: 16, 3423 — 3431
To link to this Article: DOI: 10.1080/01483918608077792
URL: http://dx.doi.org/10.1080/01483918608077792

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(16), 3423-3431 (1986)

QUANTIFICATION OF NIACIN AND NIACINAMIDE IN VITAMIN PREPARATIONS BY DENSITOMETRIC THIN LAYER CHROMATOGRAPHY

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Abstract

A densitometric TLC method was developed for the determination of the B-3 vitamins niacin and niacinamide in commercial vitamin preparations. The method involves removal of excipients by ethanol precipitation, separation of the vitamins by high performance TLC on phosphor-containing silica gel layers, and in situ scanning of sample and standard zones. The per cent recoveries for niacinamide in vitamin products were 96 to 104% and for niacinamide added to products 99 and 102%. Recoveries of added niacin were 99 to 102%, but recoveries of niacin in products ranged from 0 to 74% of the label values. Additional ingredients did not interfere with the determination.

INTRODUCTION

A method was published recently (1) for identification and quantification of niacin (nicotinic acid) and niacinamide (nicotinamide) (both referred to as vitamin B-3) in pharmaceutical prepar-

 0148-3919/86/0916-3423$3.50/0

ations based on removal of excipients by ethanol precipitation, isolation of the analytes by silica gel thin layer chromatography with water as the mobile phase, scraping and elution of the zones with 0.1 N HCl, and solution spectrometric determination at 262 nm. This paper reports a more rapid, simplified method for this analysis involving direct scanning of the ultraviolet (UV) absorbance of the separated vitamin zones on phosphor-impregnated high performance silica gel layers. Precision and accuracy for the determination of niacinamide in actual and spiked samples were excellent. Assay values of vitamin products for niacin were consistently low, but recoveries of niacin from fortified samples were quantitative.

EXPERIMENTAL

Standard Solutions

Niacin (No. N785-0) and niacinamide (No. 24,020-6) standards were purchased from Aldrich. Standard solutions of each were prepared at a concentration of 1.00 µg/µl in ethanol, using a vortex mixer to hasten dissolving of the solids.

Preparation of Samples

Vitamin samples analyzed were in the form of capsules or tablets. Capsules were emptied and an amount of powder equivalent to ca. 100 mg of niacin or niacinamide according to the label value was accurately weighed and transferred to a 100 ml volumetric flask.

Fifty ml of absolute ethanol was added and the contents were swirled on a Fisher Vortex-Genie mixer at setting 8 for 4 minutes. The solution was diluted to volume with ethanol and the mixing was repeated. Tablets were ground to a smooth powder with a mortar and pestle. Powder equivalent to ca. 100 mg of vitamin was accurately weighed and treated as described above for capsules. Both capsule and tablet solutions had theoretical concentrations of ca. 1.00 µg/µl.

TLC Determination

TLC was carried out on channeled Analtech silica gel G precoated plates with preadsorbent spotting area and fluorescent phosphor that is activated by 254 nm uv light. Layers were purified by pre-development with methylene chloride-methanol (1:1 v/v) and dried in a fume hood before use.

Using a Drummond 10 µl microdispenser, 3, 5, 7, and 10 µg of niacin or niacinamide (3 to 10 µl of the 1.00 µg/µl standard solution) and duplicate 6 µl aliquots of filtered sample (containing 6.00 µg for a theoretical 100% recovery) were applied by streaking on the preadsorbent areas of the center six lanes of the layer. The layer was developed in a paper-lined, vapor-saturated glass tank with benzene-methanol-acetone-glacial acetic acid (7:20:5:5 v/v). Mobile phase was removed by air drying in a hood, and quenched zones of niacinamide and niacin were measured with a Kontes K-495000 densitometer using the short (254 nm) UV source and 5 mm scan head. Areas of recorder peaks were obtained using the

formula height x width at half height, and the amount of analyte in each sample was calculated from the average area of the duplicate sample aliquots and the calibration equation based on the areas of the standard zones.

To evaluate the accuracy of the method, fortification (spiking) experiments were carried out by standard addition of niacin or niacinamide. Two identical portions of a sample were weighed and a known amount of standard was added, as a solid or ethanol solution, to one of the portions. The two samples were analyzed as described above, and the recovery was calculated from the difference between the measured concentrations of the fortified and unfortified samples divided by the concentration of spike added to the sample. Niacin or niacinamide were added to samples in an amount that would double the declared label value when the compound was already present, or at a level equal to the amount of the other compound when none was originally in the formulation.

RESULTS AND DISCUSSION

Development with the mobile phase produced well-separated compact, flat zones of niacin and niacinamide with respective R_F values of 0.61 and 0.43. Thirty minutes were required for development 12.5 cm beyond the silica gel-preadsorbent interface. The vitamins were detected as dark, absorbing zones against a bright, fluorescent background (fluorescence quenching) when the phosphor-impregnated plate was viewed under shortwave UV light. The visual

detection limit was about 100 ng for each compound, but higher amounts were spotted for the analysis in order to achieve more reliable densitometric measurement. No chromogenic detection reagent was required before scanning, such as the use of BrCN and analine to produce yellow spots as reported by Ismaiel et al. (2).

Plots of scan area vs weight spotted had average linearity correlation coefficients (R) of 0.99 and similar slopes for niacin and niacinamide between 3 and 10 micrograms. Sample aliquots were chosen to contain weights of vitamins near the center of this linear range, and standards were always chromatographed in parallel with samples to correct for minor variations in the calibration line from plate to plate. The use of multipoint, in-system calibration curves with multiple samples on a single plate is a principle common in TLC, but not in GC and HPLC, which leads to high accuracy, precision, and sample throughput.

Samples of various brands of commercial vitamin products containing niacin and niacinamide were analyzed by the TLC procedure. For samples containing niacinamide, results ranged from 95.8 to 104% of the declared label values (Table 1). Each analysis was calculated from the average area of duplicate sample aliquots, which always differed by less than 7.0% and usually less than 4.0%. Replicate analyses of samples resulted in reproducibilities (relative standard deviation) of 3.1 to 6.8%. Figure 1 shows the scans obtained for a typical niacinamide determination.

The accuracy of the TLC method was confirmed by analysis of samples fortified with standard niacinamide. An amount of Sample 3

TABLE 1

ANALYSIS OF VITAMIN PREPARATIONS FOR NIACIN AND NIACINAMIDE

Sample	Label Value (mg)		Average % Label Claim from Assay
	Niacinamide	Niacin	
1C[a]	50	-	95.8
2T[a]	50	-	104
3T[b]	100	-	102
4T[c]	100	-	103
5C[a]	50	-	103
6T[d]	1000	500	99.8; 74.4[e]
7T[f]	-	100	76.9
8T[a]	-	25	0

C = capsule
T = tablet

a Also contained vitamins B-1, B-2, B-6, and B-12, folic acid, biotin, choline bitartrate, inositol, PABA, and calcium pantothenate.

b Contained only niacinamide.

c Also contained vitamins E, C, B-1, B-2, B-6, and B-12, folic acid, biotin, and calcium pantothenate.

d This was the only product containing both niacin and niacinamide, plus L-tryptophan and vitamins C and B-6

e Niacinamide recovery is given first, niacin recovery second.

f Contained only niacin.

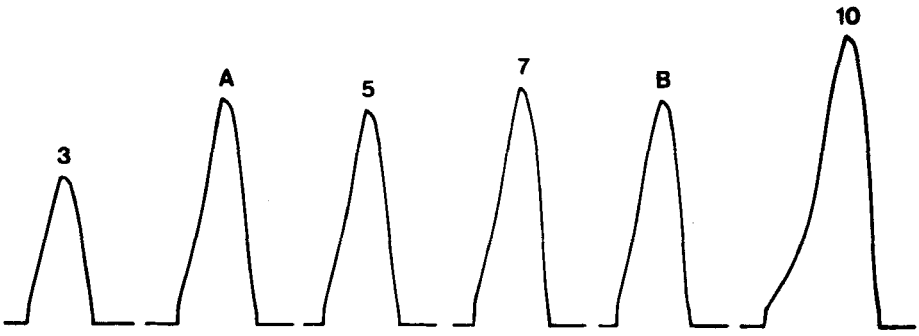

Figure 1. Scans of a chromatogram containing 3, 5, 7, and 10 μg niacinamide standards and duplicate aliquots (A and B) of tablet Sample 3 (Table 1). The standard peaks resulted in a calibration curve with a linearity correlation coefficient of 0.994. The average areas of the sample aliqots represented 98.8% recovery compared to the label value. The attenuation setting of the Kontes scanner was X100.

containing 100 mg of niacinamide (theoretical) was spiked with an additional 100 mg to double the value. Analysis of the sample yielded 98.8% recovery of the spike. The same experiment was carried out with Sample 4, and recovery of added niacinamide was 102%.

Most commercial vitamin preparations contain niacinamide rather than niacin, but three available niacin products were analyzed. Table 1 shows that 0 to 74% of the label value was obtained in these assays. Boiling with ethanol for 10 minutes during sample preparation instead of room temperature shaking did not increase the recovery of niacin for Samples 6 and 8. For Sample 7, the pure niacin capsule, recovery became quantitative with this more vigorous extraction procedure.

Weighed amounts of Samples 3 and 4 containing 100 mg of niacinamide were fortified with 100 mg of niacin, and recoveries

were 101% and 102%, respectively. These results are similar to the quantitative recoveries for added niacin reported by Sarangi et al., who did not analyze any multivitamin preparations formulated with niacin. Samples 6, 7, and 8 were fortified with standard niacin to double their label values, and analyses of fortified and unfortified portions were done. Recoveries of the added niacin were 99.2, 99.9, and 100%, respectively. The reasons for quantitative recovery of added niacin but incomplete recovery of niacin present in preparations according to label declarations are unknown. Some or all of the niacin may be present in vitamin preparations in a form that is non-extractable or not detected by TLC.

None of the additional ingredients present in the preparations tested, including those listed in the footnotes of Table 1 plus coloring materials, excipients, etc., overlapped with niacin or niacinamide. Extra quenched zones were detected on some of the chromatograms, but R_F values were higher or lower than those of the B-3 vitamins. Because interferences were not a problem, no attempt was made to identify these additional zones. One multivitamin capsule containing vitamins A, D, and E was analyzed by the method, and a petroleum ether extraction of the ethanol solution diluted with water before TLC was added to the procedure to remove the interference of fat soluble vitamins. The value obtained for niacinamide was 99.2% of the 100 mg label declaration.

The authors of the earlier paper (1) involving TLC followed by solution spectrometric determination characterized their method as simple, safe, inexpensive, fast, and selective. The densitometric

method shares all of these attributes but is faster and simpler because scraping and elution of spots are not required. The method allows accurate and precise assay of niacinamide and fortified niacin. Niacin was not recovered quantitatively from most of the commercial preparations analyzed. It is probable that the spots could be measured by scanning at 262 nm on non-fluorescent plates instead of at 254 nm on phosphor-containing plates, but this was not tested.

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