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Short communication

Determination of nicotinamide and 4-aminobenzoic acid in pharmaceutical preparation by LC

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Abstract

A rapid, precise and time saving high performance liquid chromatographic method has been developed and validated for the simultaneous determination of nicotinamide and 4-aminobenzoic acid in pharmaceutical preparation. The method involves isocratic elution of mobile phase through column in a reverse phase chromatography with UV detection at 254 nm. The ranges of quantification for nicotinamide and 4-aminobenzoic acid were 11–34 μg ml^{-1} and 37–113 μg ml^{-1}, respectively. Investigating specificity, linearity, precision, accuracy, robustness and ruggedness performed the validation of the method.

Keywords: Reverse phase chromatography; Isocratic elution; Nicotinamide; 4-Aminobenzoic acid

1. Introduction

Nicotinamide is a water soluble vitamin-B substance. It is isolated from biological sources and used in the treatment and prevention of pellagra in humans [1]. 4-Aminobenzoic acid is used topically as sunscreen agents in different pharmaceutical preparation. It absorbs UV light of wavelength in the region of 260–313 nm and therefore has the highest protection index of current sunscreen agents [2,3]. It is widely distributed in nature as a B complex factor and therefore sometimes included as a member of the vitamin-B group [4,5]. The pharmaceutical dosage forms may contain nicotinamide alone or in combination with 4-aminobenzoic acid. A high performance liquid chromatographic method has been reported for the determination of nicotinamide in a tablet formulation and 4-aminobenzoic acid in a gel formulation separately [6].

A non-aqueous and aqueous titration method has been described for the analysis of raw material of nicotinamide and 4-aminobenzoic acid, respectively. A spectrophotometric method has been used for the determination of nicotinamide in different pharmaceutical preparations [7,8]. So far, no HPLC method has been reported for the simultaneous determination of nicotinamide and 4-aminobenzoic acid, therefore, it was considered necessary to develop HPLC method for the analy-

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sis of these compounds in different pharmaceutical formulation. Details of the method and its validation are now reported.

2. Materials

Potassium dihydrogen phosphate (BDH, AnalaR), orthophosphoric acid (BDH, AnalaR), acetic acid (BDH, AnalaR) and methanol (BDH, AnalaR) were used. Standard solutions of nicotinamide and 4-aminobenzoic acid were freshly prepared.

Table 1
Regression analysis of the calibration data ($n = 3$)

Drug	Slope	Intercept	Correlation co-efficient (r^2)
Nicotinamide	29 563	−12 186	0.9998
4-Aminobenzoic acid	156 732	−139 780	0.9999

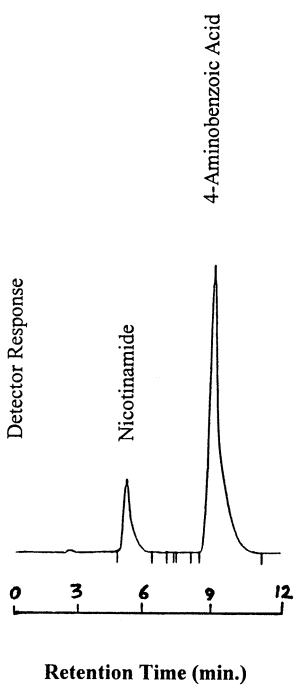

Fig. 1. Chromatogram of authentic mixture of 22 mg ml^{-1} of nicotinamide and 74 mg ml^{-1} of 4-aminobenzoic acid.

3. HPLC method

The high performance liquid chromatographic system used in this research work was equipped with a solvent delivery 515 HPLC pump (Waters, USA), tunable absorbance detector 486 (Waters), an autosampler 717 (Waters) and a data processing unit compaq V50 (Hungary). HPLC column μ Bondapack C_{18}, 300 × 4.6 mm^2 was used for separation of compounds. A mixture of methanol and buffer (75:925) of 0.05 M potassium dihydrogen phosphate having pH 3.6 ± 0.1, adjusted with orthophosphoric (85%) was used as mobile phase. The flow rate was maintained at 1.5 ml min^{-1}. Wavelength of detection was 254 nm. An injection volume of 50 μl was chromatographed. The whole chromatography was performed at ambient temperature.

3.1. Preparation of standard solution

The standard samples nicotinamide 11 and 37 mg of 4-aminobenzoic acid were accurately weighed and transferred into two 100-ml volumetric flasks separately. Initially about 2 ml of acetic acid and 50 ml of methanol were added to each flask, the contents were dissolved by sonication for 10 min and allowed to cool to ambient temperature. The contents were diluted to volume with water and mixed. Aliquots of 10 ml of each solution were transferred into a 50-ml volumetric flask and diluted to volume with water and mixed. This solution was reference working standard solution. Before injecting into the liquid chromatograph, the solution was filtered through 0.45 μm membrane filter.

3.2. Preparation of sample solution

A portion of sample containing nicotinamide and 4-aminobenzoic acid equivalent to 11 and 37 mg were accurately weighed and transferred into a 100-ml volumetric flask. Initially about 4 ml of acetic acid was added and the contents were dispersed by shaking for 2 min and 50 ml of methanol was added and the flask was placed on a mechanical shaker for 10 min. Finally about 40 ml of water was added and the contents were

Table 2
Recovery experiment for nicotinamide and 4-aminobenzoic acid by proposed HPLC method ($n = 6$)

Nicotinamide[a]			4-Aminobenzoic acid[b]		
Amount added ($mg\ g^{-1}$)	Amount found ($mg\ g^{-1}$)	Recovery (%)	Amount added ($mg\ g^{-1}$)	Amount found ($mg\ g^{-1}$)	Recovery (%)
7.9934	8.1596	102.08	27.3878	27.9909	102.20
7.9934	8.0771	101.05	26.5987	27.1691	102.14
12.5443	12.6678	100.98	42.4929	42.7497	100.60
12.4073	12.3934	99.89	42.8860	42.5525	99.22
14.9413	15.1171	101.18	50.5603	50.3630	99.61
14.9533	14.8991	99.64	50.4673	50.4508	99.97
17.9042	17.9416	100.21	60.5900	61.9069	102.17
17.6842	17.5653	99.33	61.2632	61.5303	100.44
21.7110	21.3807	98.48	73.9209	73.5873	99.55
22.0617	22.0133	99.78	74.1840	74.3243	100.19

[a] Mean = 100.3; standard deviation = 1.06; % RSD = 1.06.
[b] Mean = 100.6; standard deviation = 1.15; % RSD = 1.15.

sonicated for 10 min, allowed to cool to ambient temperature, diluted to volume with water and mixed. The solution was filtered through Whatman No. 42, discarding the first 10 ml of filtrate. An aliquot of 10 ml of filtrate was transferred into a 50-ml volumetric flask and diluted to volume with water. The solution was filtered through 0.45 μm membrane filter before injection into LC system.

4. Results and discussions

Standard solutions containing nicotinamide and 4-aminobenzoic acid equal to 20, 50, 80, 100, 120, 150, and 180% of the nominal assay concentration (22 and 74 $\mu g\ ml^{-1}$) were prepared and examined by the assay procedure.

The peak area responses, measured for nicotinamide and 4-aminobenzoic acid, were plotted versus concentration and a linear response was obtained over the range of concentration studied for both ingredients. The slope of calibration curve and proximity of all points to the calibration curve demonstrates that the method has adequate sensitivity to the concentration of nicotinamide and 4-aminobenzoic acid.

Regression analysis of the calibration data ($n = 3$) for each molecule was done and value of the slope, intercept and correlation co-efficient were calculated (Table 1). A typical chromatogram of authentic mixture of nicotinamide and 4-aminobenzoic acids is shown in Fig. 1.

The accuracy of the assay procedure was determined by carrying out recovery experiments. Amounts of nicotinamide and 4-aminobenzoic acid equivalent to 50, 80, 100, 120 and 150% of the theoretical assay concentration were added to the formula amount of an inert preparation and the mixtures were subjected to the assay procedure. The results so obtained are summarized in Table 2. The recovery experiment shows that the

Table 3
Precision under repeatability conditions

Determination	Nicotinamide (% L.S)	4-Aminobenzoic acid (% L.S)
01	98.11	101.28
02	98.86	101.73
03	97.91	102.00
04	97.77	101.41
05	98.49	102.05
06	98.93	101.71
Mean	98.35	101.70
Standard deviation	0.49	0.31
% RSD	0.50	0.30

L.S, label strength.

Table 4
Intermediate precision ($n = 4$)

Instrument	Analyst	Nicotinamide (% L.S)	4-Aminobenzoic acid (% L.S)
1	A	100.07	100.17
	B	99.33	100.41
2	A	99.92	101.69
	B	99.08	99.40
Mean		99.6	100.42
Standard deviation		0.47	0.95
% RSD		0.47	0.95

Table 5
Effect of sample solvent and extraction time

Statistical tests	Sample solvent ($n = 10$)		Extraction time ($n = 6$)	
	Nicotinamide (% L.S)	4-Aminobenzoic acid	Nicotinamide (% L.S)	4-Aminobenzoic acid
Mean	99.93	100.26	99.65	101.52
Standard deviation	1.36	1.19	0.78	0.77
% RSD	1.36	1.19	1.00	0.76

Table 6
Stability indicating results

Time period (h)	Peak area of standard solution (a.u.)		Peak area of test solution (a.u.)	
	Nicotinamide	4-Aminobenzoic acid	Nicotinamide	4-Aminobenzoic acid
0	2 849 497	15 795 446	2 832 944	16 071 382
8	2 835 692	15 947 992	2 802 306	16 091 425
16	2 818 339	1 589 959	2 823 106	15 970 966
24	2 826 909	15 948 742	2 816 942	15 658 568

method is sufficiently accurate and there is no significant interaction between the active components and excipients.

The precision of assay method was determined under repeatability condition by an experiment in which six preparations were made from the same batch of formulation and were analyzed by one operator on a single occasion. The results are presented in Table 3. The intermediate precision was assessed by another experiment in which two analysts on two different instruments with four independent determinations assayed the same batch of formulation. The results are statistically valid as shown in Table 4.

The ruggedness of the assay method was assessed with respect to alternations in sample solvent composition, (± 2 ml for acetic acid and ± 10 ml for methanol), sample extraction time including sonication and shaking (± 5 min each), as well as stability of working standard and test solutions stored in amber glass at ambient temperature. The results show that the proposed HPLC method is rugged to small changes in sample solvent composition and extraction time as shown from mean result, standard deviation and % RSD in Table 5, and the solutions exhibited a good degree of stability as revealed from Table 6. The LOD for nicotinamide and 4-aminobenzoic acid were found to 2 and 7 $\mu g\ ml^{-1}$, respectively.

An inert preparation omitting nicotinamide and 4-aminobenzoic was examined by the assay proce-

dure. No peak due to excipients in the formulation was observed at the typical retention times for nicotinamide and 4-aminobenzoic acid. Therefore, it is concluded that the assay method is specific for both active ingredient in the presence of excipients of pharmaceutical preparation. The suitability of the system was defined by determining the value of column efficiency, tailing factor and resolution factor using the method in USP. Column efficiency was greater than 1000 per column, tailing factor was not more than 2 and resolution factor is greater than 3 for both nicotinamide and 4-aminobenzoic acid.

The dependence of retention time on pH of mobile phase has also been observed and it was found that a decrease in pH decreases the retention time of nicotinamide while there was no significant influence on 4-aminobenzoic acid peak. This behavior clearly demonstrates that at lower pH level the two nitrogen atom in nicotinamide molecule are protonated and hence, the resultant ionic form does not retain on the non-polar surface of the reverse phase column [9]. The HPLC method has been found to be time saving with a high degree of precision and accuracy. The method presented here has been considered to be appropriate for the similar type of composition of product in Quality Assurance and R&D Laboratories.

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