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DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF NICOTINAMIDE IN BULK AND DOSAGE FORMS

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Abstract: Objectives: This study was aimed to develop simple, sensitive and accurate zero (^{0}D), first (^{1}D) and second (^{2}D) order derivative spectrophotometric methods for the analysis of nicotinamide in bulk and dosage forms. **Methods:** The zero-order spectrum of nicotinamide aqueous solution was measured at 262 nm against its blank. This spectrum was differentiated instrumentally to generate the first and second derivative spectra which were measured at 272 nm and 278 nm, respectively. The developed methods were validated as per ICH guidelines. The absorbance ratio of nicotinamide absorbance values at 214 nm and 262 nm was also determined. **Results:** Regression data of the developed methods obeyed Beer's law over the concentration range 10-50 µg/ml with a good correlation coefficient (not less than 0.998). The developed methods demonstrated good inter-day and intra-day precision at the three modes. The obtained recovery percentage (99.2 ± 2.6%, n=3) reflected freedom from interference by the excipients. The absorbance ratio for nicotinamide at 214 nm and 262 nm was found to be in the range between 2.8- 3.2 which can be used as identification test for Nicotinamide (qualitative analysis). **Conclusion:** The statistical validation at 95% confidence level proved the sensitivity, accuracy and precision of the developed methods

Keywords: Nicotinamide, Spectrophotometry, Derivative spectra, Dosage form.

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INTRODUCTION

Nicotinamide (Figure 1), also known as niacinamide, is a derivative of pyridine, with a carboxyl group at the 3-position. Together with niacin it makes the group known as vitamin B_3 complex. Nicotinamide and niacin are convertible to each other with steady world demand [1].

The conjugated p system of nicotinamide heterocyclic aromatic structure allows detection by ultra-violet light. It is soluble in water and generally very stable. It is a well-known precursor in the synthesis of the coenzymes NAD+ and NADP (nicotinamide adenine dinucleotide phosphate) involved in cell metabolism. A deficiency of them causes inflammation of mucus membranes and an illness known as pellagra. (2)

It is used as a dietary supplement, and used as a medication. As a supplement, it is used to prevent and treat pellagra (niacin deficiency). While nicotinic acid (niacin) may be used for this purpose, nicotinamide has the benefit of not causing skin flushing. Nicotinamide has future role in dermatological practice as it can be used of for various dermatological indications, including nonmelanoma cancer prophylaxis, blistering disorders, acne vulgaris and cosmetic indications, and speculate (3)

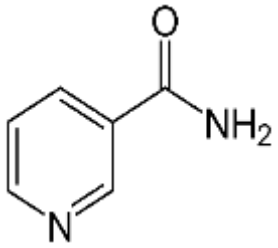

Fig 1: Chemical structure of nicotinamide

Literature review showed that most of the methods used for nicotinamide analysis were HPLC methods [4-9]. There were no previous studies reported for nicotinamide analysis using UV spectrophotometry.

Therefore, the aim of the present work was to develop simple and accurate spectrophotometric methods (^{0}D, ^{1}D, ^{2}D) for the analysis of nicotinamide in bulk and dosage forms.

MATERIALS AND METHODS

Apparatus

UV spectrophotometric studies were carried out on Shimatzu UV- 1800EN240V, double beam, (Kyoto, Japan). The operating conditions were as follows:

- Wavelength range: 240-400 nm.

- Scan speed: Medium, 0.2 nm/s.

Sensitive balance: Kern ALS 120-4, Germany

Reference standard and sample

Nicotinamide reference standard was kindly provided by colleagues in the Central Lab, Riyadh, Saudi Arabia. (Niacinamide Capsules, 500 mg) was obtained from NOW, USA.

Preparation of stock solutions

Standard stock solution

An accurately weighed quantity (0.01g) was dissolved in 20 ml distilled water and transferred to 100 ml volumetric flask. The volume was then completed to mark with the solvent (Solution A; 100µg/ml).

Sample stock solution

An equivalent amount of capsules powder containing 0.01g nicotinamide was accurately weighed, dissolved in 20 ml distilled water and transferred to 100 ml volumetric flask. The volume was then completed to mark with the solvent and filtered (Solution B; 100µg/ml).

Determination of λ_{max}

The standard solution of Nicotinamide was diluted to obtain a concentration of 20µg/ml. the solution was scanned within the range 200-400 nm in ^{0}D, ^{1}D and ^{2}D order derivative modes, respectively.

Absorbance Ratio for identification of Nicotinamide in bulk and dosage forms

Solution A and B were diluted to obtain serial concentrations (3-15µg/ml). The resulted solutions were scanned within the range 200-400 nm.

Method validation

Linearity

Serial dilutions were made from solution A by transferring accurately measured volumes (1-5 ml) into a set of 10 ml volumetric flasks. The volumes were then completed to mark with the solvent and the ^{0}D, ^{1}D and ^{2}D order derivative spectra were recorded over the range 240-400

nm. The procedure was repeated three times. The mean absorbance values were plotted against concentration to construct the calibration curves.

Limits of detection and quantification were determined from the calibration curve using adopted formulae (10):

LOD = 3.3 SB/Slope LOQ = 10SB/Slope

Where SB is the standard deviation $s_{y/x}$ calculated from the regression analysis data.

Content uniformity

The procedure under linearity was repeated using solution B instead of solution A. The content uniformity of the capsules solution was then evaluated by the direct comparison of sample/standard absorbance values.

Precision

Serial dilutions from solution A were done to obtain concentrations of 10µg/ml, 20µg/ml and 30µg/ml. These solutions were scanned using the three modes (^{0}D, ^{1}D and ^{2}D) three times within the same day (inter-day) and at three consecutive days (intra-day). The result obtained were used to evaluate the precision of the developed method in terms of relative standard deviation values (RSD %).

Recovery percentage

The freedom of interference by the capsule excipients was confirmed by results obtained for recovery testing of added amount of authentic nicotinamide to the sample solution in the ratio of 1:1. 2 ml of each solution A and B were transferred to separate stoppered glass tubes. Another 2 ml of solution B was mixed with 2 ml of solution A in a third tube. The above solutions were scanned in the three modes. The recovery percentage was determined using the following equation:

Percent Recovery = $[(A_{mix} - A_{sam})/A_{std}] \times 100$

Where A_{mix} is the absorbance of mixture; A_{sam} is the absorbance of sample, $A_{std}$is the absorbance of the standard.

RESULTS AND DISCUSSION

Absorbance Ratio determination for identification of Nicotinamide in bulk and dosage forms

The zero-order derivative spectrum of nicotinamide shows two sharp peaks at 214nm and 262 nm (fig 2). The ratio between the absorbance at these wavelengths, using the formula below, was found to be in the range between 2.8- 3.2 for all solutions. Thus, this method can be used as an identification test for nicotinamide (qualitative analysis).

Absorbance Ratio= Absorbance at 214nm / Absorbance at 262nm

The results obtained are shown in Table 1.

Table 1. Nicotinamide standard absorbance ratio at 214nm and 262nm (n=3)

Concentration	Absorbance Ratio		
3 µg/ml	2.842	2.907	2.838
6 µg/ml	2.834	2.886	2.888
9 µg/ml	2.886	2.918	2.873
12 µg/ml	2.888	2.901	2.888
15 µg/ml	2.888	2.880	2.893

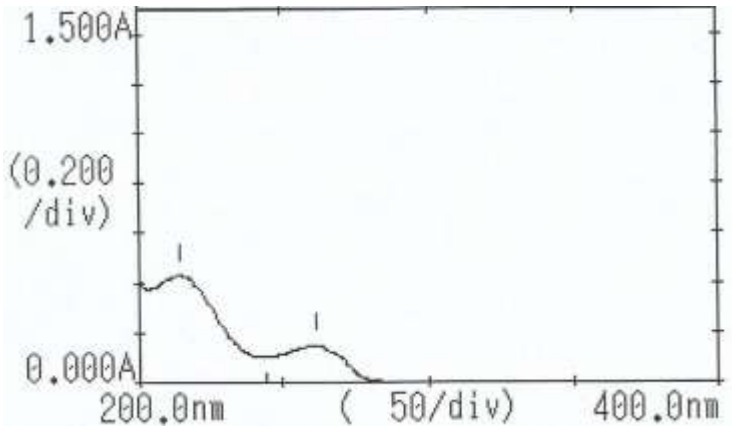

Fig. 2: UV spectrum of Nicotinamide solution (6 µg/ml; 214, 262 nm)

Spectrophotometric analysis

The absorption peak at 262nm in the zero order spectrum (Figure 3) was selected for the quantitative analysis of nicotinamide because it gave more precise and feasible results. Also it was preferred in order to reduce any possible interference which can occur at 214nm. The zero order derivative spectrum of nicotinamide shows absorption maximum at 262 nm (fig. 3). First and second derivatization of the resultant spectrum showed bands at 272 nm and 278 nm respectively. (fig.4 and 5)

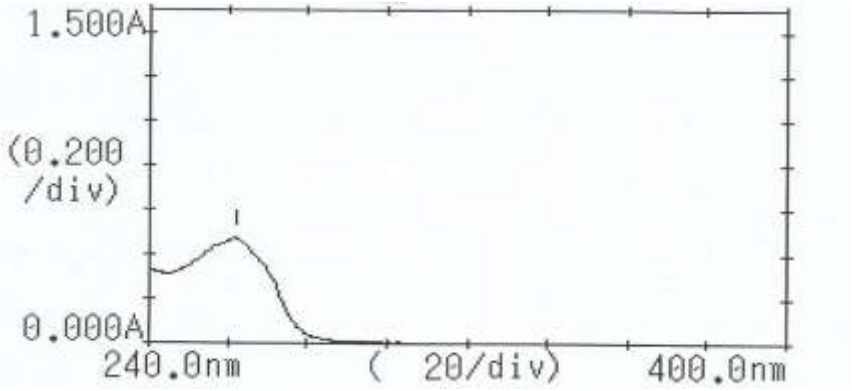

Fig. 3: UV spectrum of Nicotinamide solution (20 µg/ml; 262 nm)

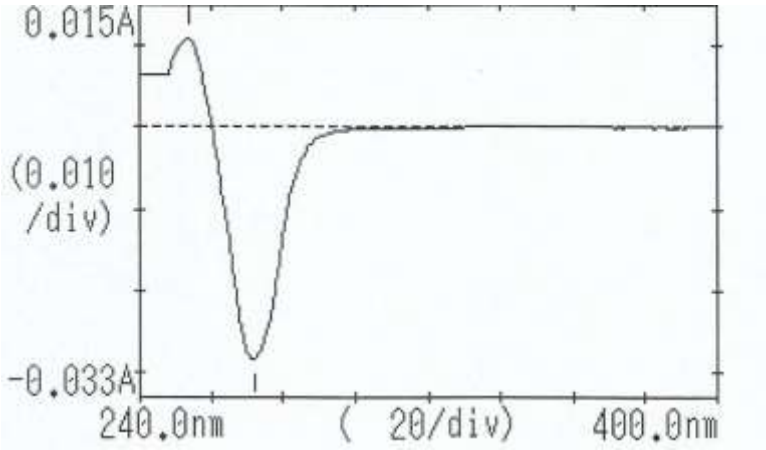

Fig. 4: First derivative spectrum of Nicotinamide solution (20 µg/ml; 272 nm)

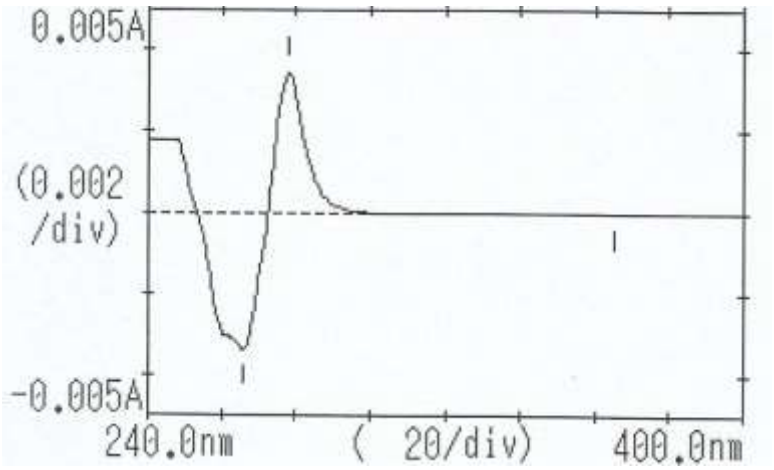

Fig. 5: Second derivative spectrum of Nicotinamide solution (20 µg/ml; 278 nm)

Linearity

The calibration curves, relating nicotinamide concentrations in a range 10- 50 µg/ml to the mean absorbance values, were constructed for the three modes. Linearity was found to obey Beer's law with a good correlation coefficient (not less than 0.998). The regression analysis data was calculated at 95% confidence level for the developed methods using the following formula:

$y = (b \pm ts_b)\, x + (a \pm tsa)$

Where b is the slope, a is the intercep, S_b is the standard deviation of the slope, Sa is the standard deviation of intercept, t is the t-value at 95% confidence level for (n–2) degrees of freedom.

The results obtained for linearity data of the developed methods are summarized in table 2, which reflected the accuracy and consistency of these curves.

Table 2: Linearity data of the developed methods (n =3)

Parameter	^0D	^1D	^2D
λ_{max}	262	272	278
Concentration range	10-50µg/ml	10-50µg/ml	10-50µg/ml
Slopes $\pm ts_b$*	0.0253 ± 0.36	0.00154± 0.032	0.00018± 0.026
Intercept $\pm ts_a$**	-0.00033± 0.012	- 0.0003± 0.0011	0.0004± 0.0008
LOD	0.47µg/ml	0.67µg/ml	0.88µg/ml

LOQ	1.4 µg/ml	2.04 µg/ml	2.65µg/ml
R	0.9999	0.9998	0.998

Assay and validation

The developed methods were applied for the drug uniformity testing in Niacinamide capsules. Good assay results ranged from 100% to 105% n=3 were obtained. The validity of the methods was assessed by statistical evaluation of the results obtained (11). As the calculated t value (1.7) at 95% confidence limit was less than tabulated one (4.3), the developed methods are considered to be accurate.

Precision

The inter-day and intra-day precision was studied for all modes. RSD% values were found to be within 0.00- 1.83% (inter-day) and 0.00-0.956% (intra-day). These results reflect the precision of the developed methods as RSD% values were within the acceptable range (0.00-2%). The results are summarized in table 3.

Table 3: Inter-day and intra-day precision data for °D, ^{1}D and ^{2}D methods

Conc. µg/ml	Inter-day results; RSD%; n=3			Intra-day results; RSD%; n=3		
	^{0}D	^{1}D	^{2}D	^{0}D	^{1}D	^{2}D
10 µg/ml	1.83	0.00	0.00	0.44	0.00	0.00
30 µg/ml	1.31	0.00	0.00	0.92	1.20	0.00
50 µg/ml	0.47	0.00	0.00	0.25	0.76	0.00

Recovery percentage

The accuracy of the developed methods at the three modes and freedom of interference by capsule excipients were confirmed by good results of recovery testing (99.2 ± 2.6 %, n=3).

CONCLUSION

The developed spectrophotometric methods proved to be simple, sensitive, rapid, accurate and precise for the determination of nicotinamide in bulk and dosage forms. In addition, the procedure of the developed methods does not require neither extraction step nor chemicals and thus can be used for routine analysis of the drug.

The results of the zero-order method (^{0}D) reflected good precision. However, its direct application is expected to be limited for samples free from irrelevant absorption; on the other hand ^{1}D and ^{2}D methods are expected to prove their stability-indicating properties that can allow their use in the presence of irrelevant absorption.

The stability-indicating properties of the developed methods are under investigation for the analysis of nicotinamide in the presence of its degradation products (possible amide hydrolysis).

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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