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Identification and Quantitative Estimation of Niacinamide and Neolone 950 in an Oil/Water Cream by HPTLC Method

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Abstract: Niacinamide, a derivative of niacin, also known as vitamin B3 ($C_6H_6N_2O$) has ability to treat some skin conditions including aging. It is also beneficial for treating different inflammatory skin conditions such as psoriasis and rosacea. Neolone 950 is a broad spectrum bactericide, compatible with a variety of fungicides and bactericides. It is widely used as a preservative in different cosmetic preparations, and exhibits outstanding antimicrobial activity, inhibiting against a wide variety of Gram-positive and negative bacteria. The present study involves separation and quantitative estimation of Niacinamide and Neolone 950 by High performance thin layer chromatography using two different solvent systems, to illustrate, acetic acid: acetone: methanol: benzene (5:5:20:70) for Niacinamide and was chloroform: methanol (90:10) for Neolone 950. Retention factor for Niacinamide and Neolone were found to be 0.52 and 0.72 respectively. The chromatograms were analysed by scanning through densitometer for quantitative estimation of samples. Linearity of the analytical method was supported by R^2 values greater than 0.9 in each case. Percentage recovery results were found to be more than 90 %, both according to area and height of the standard curves. Statistical parameters like root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) were less than one, therefore, indicated good precision of the method.

Keywords: HPTLC, Niacinamide, Neolone 950, Precision, Statistical Parameters, Quantitative Estimation.

INTRODUCTION

Niacinamide, a derivative of niacin, also known as vitamin B3 ($C_6H_6N_2O$) has the ability to treat some skin conditions including aging of skin. There are some evidences that it stimulates cells in the dermis to produce more collagen, a protein that supports skin and gives it its youthful firmness. That is a good thing

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since collagen levels decline with age, and loss of collagen is a major cause of wrinkles and saggy skin. Moreover, it has anti-inflammatory properties, which makes it effective for treating acne. It is also beneficial for treating other inflammatory skin conditions such as psoriasis and rosacea. Studies show that 4% niacinamide gel can improve the symptoms of acne [1]. Niacinamide was more effective for preventing moisture loss and dryness. It increases the production of ceramides, lipids in the outer layer of the epidermis that shield skin against moisture loss and protect it from bacteria and the environment [2]. Niacinamide helps to lighten areas of pigmentation and age spots [3]. Neolone 950 is a broad spectrum bactericide, particularly suitable as replacements for formaldehyde donors [4]. The active ingredient of Neolone preservative is an isothiazolinone identified by the Chemical Abstract and IUPAC system of nomenclatures as 2-Methyl-4-isothiazolin-3-one and 2-Methyl-3(2H) isothiazolinone [5]. It has several applications, such as, easy to dose and highly water soluble, effective at low levels, excellent stability in a variety of matrices over a wide range of pH (2 to 12) and temperatures, compatible with a variety of fungicides and bactericides [6]. Neolone 950 preservative exhibits outstanding antimicrobial activity, inhibiting a wide variety of Gram-positive and Gram-negative bacteria. The recommended level for Neolone 950 is 0.05% - 0.1% (48 - 95 ppm of active ingredient) [7].

High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements [8]. The modern HPTLC technique, combined with automated sample application and densitometry scanning is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis. HPTLC is a valuable tool for reliable identification, because it can provide chromatographic finger prints that can be visualized and stored as electronic images. Special advantages of HPTLC include high sample throughput and low cost per analysis; multiple samples and standards can be separated simultaneously, and sample preparation requirements are often minimal because the stationary phase is disposable [9]. In cosmetics preparations, nicotinamide can be separated by TLC on silica gel, aluminum oxide, and ion-exchange resins. Detection of the spots can be made by examination of the plates under UV light or by specific detection reagents. In the present investigation, an attempt has been made to estimate the amount of Niacinamide and Neolone 950 in an oil/water cream by HPTLC method. The study involves sample preparations and running the samples in two different solvent systems for identifying and estimating the amount of Niacinamide and Neolone 950 in its cream. Method validation by determination of linearity, accuracy and precision by statistical parameters are also placed in the study.

EXPERIMENTAL

Materials

Niacinamide vitamin standard and Neolone 950 were obtained as gift samples from Emami Ltd., Kolkata, India. HPLC grade acetic acid, acetone, methanol, benzene, water and petroleum benzene were purchased from Merck, India for the estimation of vitamin niacinamide.

HPTLC Method

High performance thin layer chromatographic system (Camag ATS 4.0, HPTLC system) used in the entire study was equipped with a Linomet 5 applicator (syringe size 100 ul), TLC aluminium plated precoated with Silica gel 60 F 254 (10/10cm), Camag HPTLC system with Camag TLC scanner 4 and Win cats planar chromatography manager software was used for the separation of vitamins and preservatives. Mobile phases used for the separation of niacinamide was acetic acid: acetone: methanol: benzene (5:5:20:70) and that for Neolone 950 was chloroform: methanol (90:10). The wave length of detection was 254 nm and 275 nm respectively. The whole chromatography was performed at ambient temperature. The chromatograms were analysed by scanning through densitometer for quantitative estimation of samples.

Preparation of Standard Curve for Niacinamide

0.02 g of niacinamide standard was weighed accurately in a 10 ml volumetric flask, dissolved in methanol: water (1:3) and the volume was made up to the mark with same solvent mixture. The solution was filtered by a Millipore filter paper (pores size 0.45 μm). Then 1 μl, 2 μl, 4 μl and 6 μl of the above solution were applied on the Silica gel 60 F 254 plate (10/10cm) by using a Camag Linomet 5 applicator (syringe size 100 μl). After drying the TLC plate was put into the mobile phase, allowed to run for 10 minutes and scanned using a Camag TLC scanner 4 at 254 nm. In this method, area and height of the HPTLC chromatogram were measured at different concentrations in order to construct the standard curve.

Preparation of Standard Curve for Neolone 950

0.1 g of Neolone 950 standard was weighed accurately in a 100 ml volumetric flask, (1µg/µl) dissolved in methanol: 0.4% Acetic acid (20:80) and made up to 100 ml with the same solvent mixture. The clear solution was filtered by a 0.45 µm filter paper. From the above solution, 1 µl, 2 µl, 4 µl and 6 µl samples were applied on the Silica gel 60 F 254 TLC plate (10/10cm) by using a Camag Linomet 5 applicator (syringe size 100 µl). After drying, the TLC plate was run for 10 minutes and scanned using a Camag TLC scanner 4 at 275nm. Standard curve was prepared by measuring the area and height and of the HPTLC chromatograms at different concentrations.

Preparation of Sample Solution for Estimation of Niacinamide

5 g O/W cream containing niacinamide (1.5 % w/w) was taken in a separating funnel, 100 ml water and 20 ml petroleum benzene were added to it, the mixture was shaken vigorously and pressure was release frequently. The mixture was allowed to stand still for 5 minutes to ensure complete separation of organic phase, then the water phase was collected and the same procedure was repeated thrice. After complete separation, the aqueous phase was filtered and stored. From the above solution, 3 µl, 5 µl and 6 µl samples were applied, dried, run and scanned at 254 nm in a similar manner as described above.

Preparation of Sample Solution for Estimation of Neolone 950

Accurately weighed sample (2 g O/W cream) was taken in a 25 ml volumetric flask, followed by addition of methanol and 0.4% acetic acid mixture (20:80). The sample was ultrasonicated for 10 min, made up to the mark, well shaken and filtered using a membrane filter (4.5 µm). From the above solution, 3 µl, 5 µl and 6 µl samples were applied, dried, run and scanned at 275 nm in a similar manner as described above.

RESULTS AND DISCUSSION

Retention factor (R_f) of sample and standard for Niacinamide were estimated by spectrum scan of chromatograms as shown in figure 1. R_f of Niacinamide sample is 0.51 and that of standard Niacinamide is 0.52. On the other hand, R_f of Neolone 950 was found to be 0.72 and 0.73 in case of standard and sample chromatograms respectively, which is given in figure 2. Scanned HPTLC plate of Niacinamide and Neolone 950 were given in figure 3.

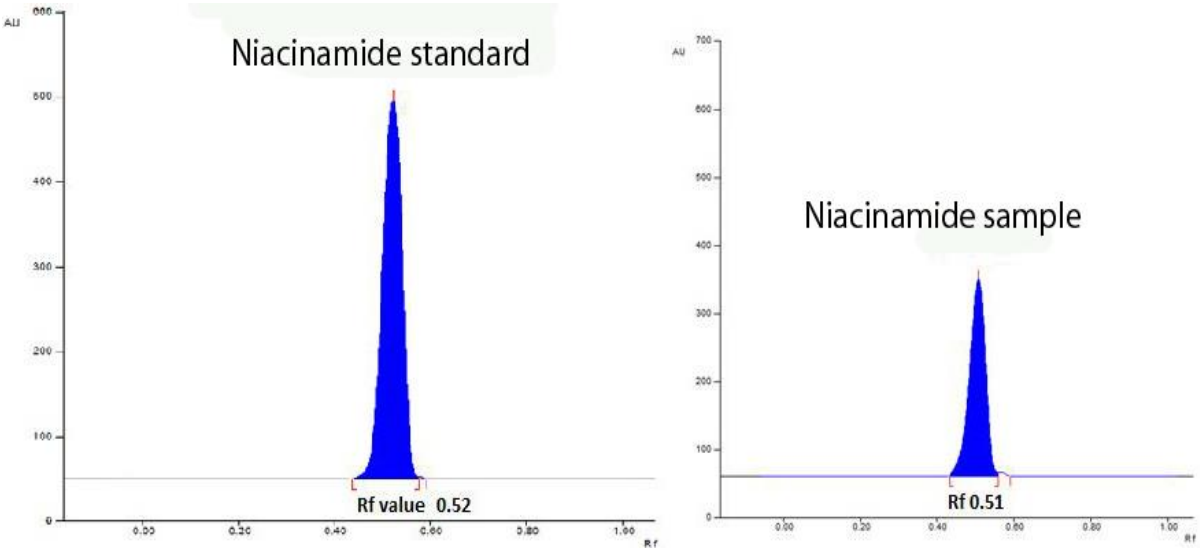

Fig. 1. Standard and sample chromatograms of Niacinamide

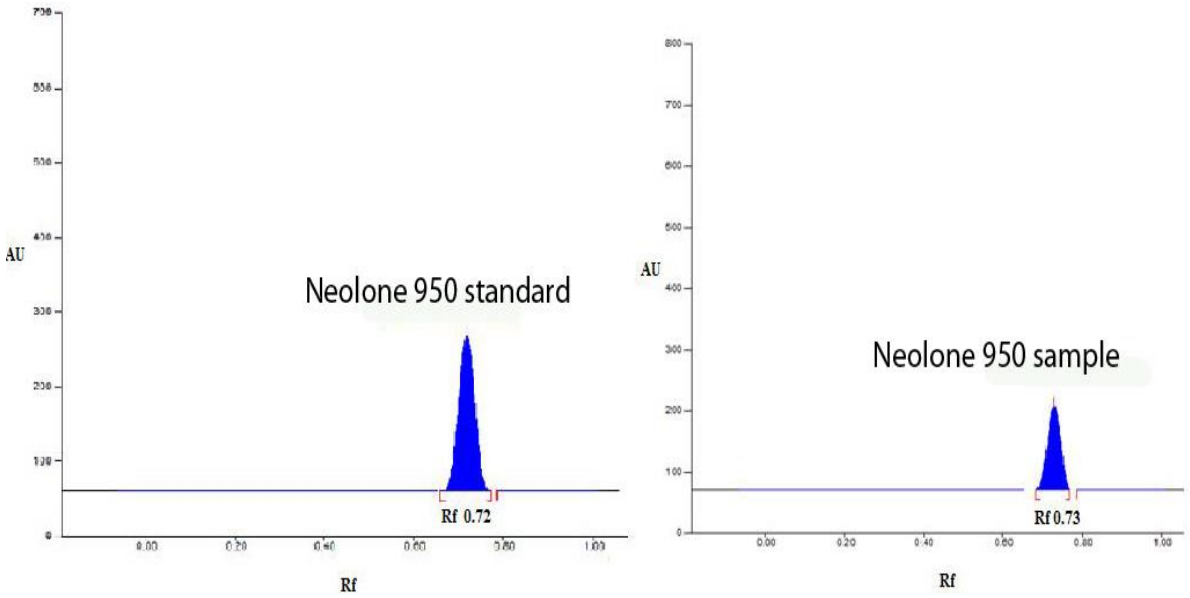

Fig. 2. Standard and sample chromatograms of Neolone 950

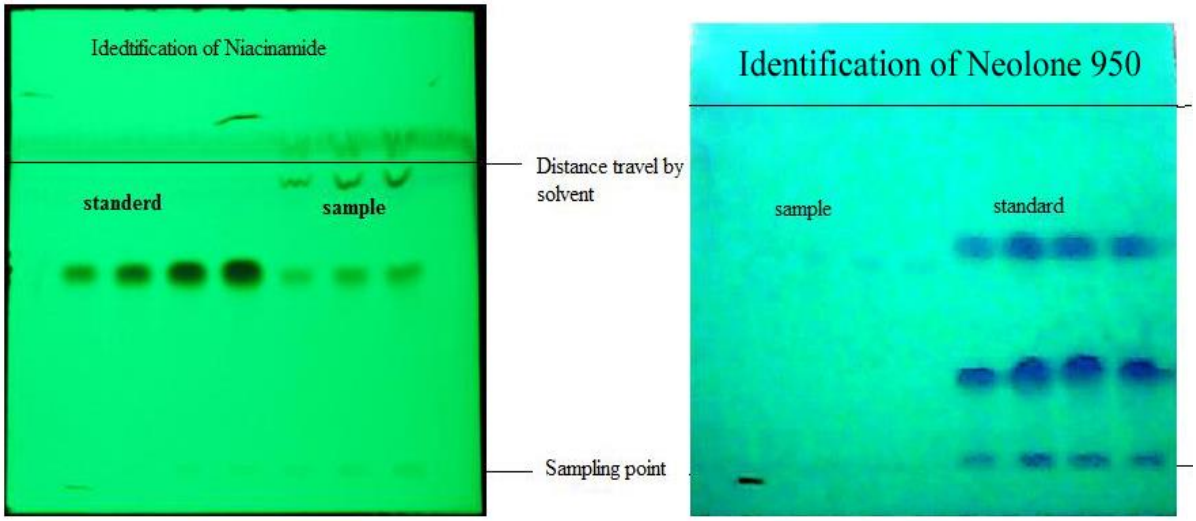

Fig. 3. HPTLC plates of Niacinamide and Neolone 950

Standard Curve of Niacinamide and Neolone 950

The wavelength of absorption maxima (λ max) of Niacinamide and Neolone 950 were found to be 254 nm and 275 nm respectively as determined from U.V. Visible spectrophotometer by scanning through the entire range (190-1100 nm). Table 1 represents the peak height and peak area of the standard chromatogram for Niacinamide and Neolone 950.

Table 1. Peak height and area of standard Niacinamide and Neolone 950

For Niacinamide				For Neolone 950			
R_f value	Amount in µg	Area	Height	R_f value	Amount in µg	Area	Height
0.52	2	12353.18	380.47	0.73	1	2462.25	97.04
0.52	4	15785.92	446.45	0.72	2	3459.33	134.98
0.52	6	19680.35	530.43	0.72	3	4814.21	191.11
0.52	8	22882.25	587.41	0.72	4	6467.49	235.27

Regression equations calculated for Niacinamide standard curves were as follows, having R^2 values 0.9987 and 0.9951 for area and height respectively [10]. Closeness of these values toward unity indicates good correlation between the variables as well as linearity of the analytical method [11].

$$\text{Area} = 1774.08 \times \text{Amount in } \mu g + 8805.01$$

$$\text{Height} = 35.24 \times \text{Amount in } \mu g + 309.99$$

Neolone standard curves were also proven to be having good correlation and linearity (R^2 values were 0.9881 and 0.9950 for area and height respectively). Regression equations were given below.

$$\text{Area} = 1337.06 \times \text{Amount in } \mu g + 958.17$$

$$\text{Height} = 47.08 \times \text{Amount in } \mu g + 46.89$$

Estimation of Niacinamide and Neolone in Samples

Percentage recovery results as compared to label claim were found to be more than 90 % in each case. According to area and height % recovery value for Niacinamide and Neolone 950 were reported in Table 2 as average ± SD, where n = number of observation = 3. Statistical parameters like root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) were calculated to determine the precision of the analytical method [12]. RMSEP values were found to be 0.012 and 0.005 for Niacinamide estimation, whereas those of Neolone were 0.0001 and 0.0009 according to area and height respectively. On the other hand, RSEP values were 0.074 and 0.047 for Niacinamide and 0.057 and 0.086 for Neolone 950 according to area and height respectively. These values were less than one; hence indicate good precision of the method. Figure 4 shows the spectrum scans of Niacinamide standard and sample which indicated a superimposed image and presence of Niacinamide in the sample. Superimposed image scan of Neolone 950 had shown in figure 5.

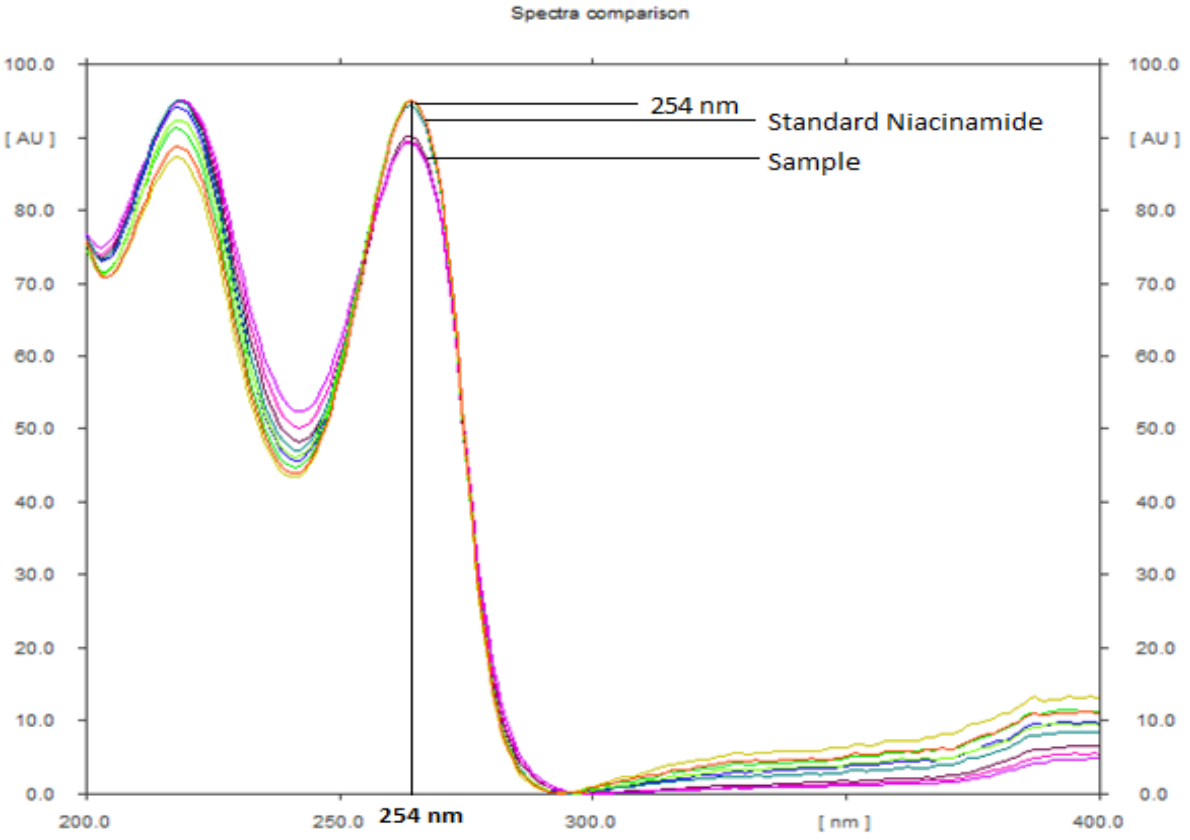

Fig. 4. Spectrum scan of Niacinamide

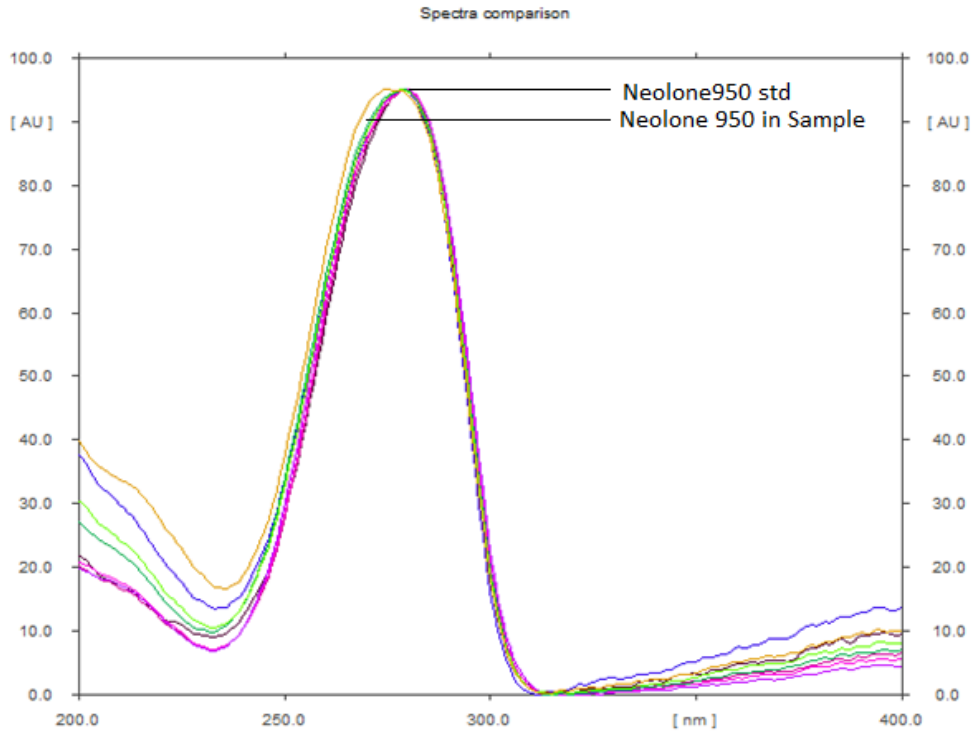

Fig. 5. Spectrum scan of Neolone 950

Table 2. Quantitative Estimation of Niacinamide and Neolone 950

% Recovery (Average ± SD), n = 3	Sample No.	For Niacinamide	For Neolone 950
According to Area	1	97.78 ± 8.80	95.33 ± 5.46
	2	95.33 ± 6.11	97.33 ± 4.16
According to Height	1	99.56 ± 4.91	93.33 ± 6.67
	2	97.33 ± 5.46	95.56 ± 7.70

CONCLUSION

The developed HPTLC method is successfully explored in order to separate the active ingredient Niacinamide and Neolone 950 an oil/water cream. The method is also simple, accurate and precise in order to determine the amount Niacinamide and Neolone 950 by separation through two different mobile phases for the two above said ingredients, which is evidenced from the correlation coefficient, RMSEP and RSEP values. Therefore, this method can be used for quantitative estimation in general research purposes or to accomplish routine quality control processes.

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