© 2019 IJSRST | Volume 6 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology
DOI : https://doi.org/10.32628/IJSRST196644

The Development of a Manipulative Spectrophotometric Technique for the Determination of Aminexil in a New Hair Tonic Preparation

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ABSTRACT

A simple and economic ratio difference spectrophotometric method was developed and validated for the determination of Aminexil (AX), which is a new compound patented by L'Oreal, in the presence of niacinamide (NA) and pyridoxine (PD) in a newly introduced cosmetic preparation. The proposed method was found to be suitable for the determination of Aminexil in its laboratory prepared mixture in presence of the two other components; and in the commercial cosmetic preparation where the three components were co-formulated. The calibration curve was constructed with a concentration range from 2-10 μgmL^{-1} showing 100.22± 0.53 accuracy. The LOD and LOQ were found to be 0.53 and 1.6 μgmL^{-1} respectively. The method was validated according to ICH guidelines. The standard addition methods showed satisfactory mean recovery of 99.68±0.56.The obtained results were satisfactory enough for further statistical analysis, in comparison to the reported method. The proposed method had the advantage of being simple since no manipulation or complicated handling of the samples were needed, as well as economic, since there was no use of sophisticated instruments or expensive chemicals.

Keywords : Aminexil, Spectrophotometry, Ratio-Difference, Niacinamide, Pyridoxine

I. INTRODUCTION

Aminexil (AX) is chemically 2, 4-diaminopyrimidine 3-N-oxide **Figure 1a**. It is a new compound invented and patented by L'Oreal [1]. It is used in hair tonic preparations due to its ability to increase the density of human keratin fibers and stimulating their growth [1]. Niacinamide, vitamin B$_3$, (NA), and pyridoxine HCl, vitamin B$_6$, (PD) **Figures 1b-1c,** have a major role in the treatment of hair loss as well as acting as adjunctive aid the Aminexil.

Up to our knowledge, there were no reported methods for the analysis of such an interesting mixture before. However there were some reported methods for the analysis of NA and PD in mixture simultaneously [2-4]. Only one method for the determination of AX was reported[5].

The aim of this work was to develop a rapid, simple and economic method for the determination of Aminexil in presence of the other components found in mixture. The ratio difference spectrophotometric data manipulation can be used for the quantification of AX whose spectra overlap by one another in zero order[6, 7]. Also, all the analytical figures of merits were applied in agreement with ICH guidelines for method validation. In addition, statistical comparison between the obtained results and those of the reported method was conducted to confirm the non-significance hypothesis.

II. METHODS AND MATERIAL

Shimadzu spectrophotometer was used for measuring the spectral absorption of the components of interest. UV probe soft ware (ver. 2.21) was used for the manipulation of the spectra.

Methanol used was AR grade, purchased from sigma Aldrich. Aminexil standard was kindly supplied by L'Oreal Egypt, its potency was found to be 99.87% according to reported method [5]. Pyridoxine and niacinamide working standards were kindly supplied by Pharonia Pharma, Egypt; their potencies were found to be 99.88% and 99.92% according to official methods respectively[8]. Aminexil® L'Oreal hair tonic was purchased from local market.

-Preparation of stock standard solutions:

50 mg of each of the three components were accurately weighed and transferred to three separate 100 mL volumetric flasks, and the volumes were completed to the mark with methanol to produce 0.5 mg mL^{-1} stock standard solutions of each.

-Preparation of laboratory prepared mixtures:

Different aliquots from the stock standard solutions of each of the three components were accurately transferred to a series of 10 mL volumetric flasks. Then, volumes were completed to mark with methanol to prepare laboratory mixtures containing different ratios of AX, NA and PD.

- Construction of the calibration curve:

Variable aliquots from the AX stock standard solution were accurately measured and transferred into a series of 10 mL volumetric flasks. The volumes were completed to mark with methanol, to prepare a concentration levels ranging from 2 to 10 μg mL^{-1}. Zero order spectra were scanned in the range of 200-400 nm and stored to the computer. Then all the stored spectra of AX were divided by 5 μg mL^{-1} PD as a divisor. The difference of peak amplitudes of at 216 and 235 nm (ΔP) was calculated from the obtained ratio spectra of AX. The amplitude differences were related to the corresponding concentrations of AX to construct the calibration curve. The regression equation was then computed and stored for subsequent calculations.

III. RESULTS AND DISCUSSION

Results:

-Analysis of Laboratory Prepared Mixtures

The absorption spectra of the different laboratory-prepared mixtures were measured and recorded, then processed as mentioned under calibration curve construction. The accurate concentration of AX in each mixture was calculated using the computed regression equation.

-Application to Aminexil ® hair tonic

Accurate and exact volume of Aminexil® containing the three components in the ratio of (1.5gm AX, 0.02gm PD and 0.015gm NA) was transferred into 10 mL measuring flask and the volume was made up using methanol as solvent. Further dilution was made to prepare appropriate concentration of AX within its linearity range. The prepared dilution was measured spectrophotometrically and its spectrum was manipulated to estimate the claimed concentration as mentioned under calibration curve construction. Then standard addition technique was applied by spiking small increments to the prepared dilutions of Aminexil® to verify method accuracy.

Discussion:

Spectrophotometry is a simple and economic analytical method for the simultaneous determination of drugs in multicomponent samples. It has the advantage of simple preparation, time saving and it does not require sophisticated software's or instruments. Also, the application of ratio different method permits the quantification of the analyte of interest in presence of its co-formulated compounds in the ratio order without further manipulation steps of derivatization.

Zero order spectra of the three components under analysis showed noticeable overlapping, as appeared in **Figure 2**, thus the clear determination of AX was not applicable and required further manipulation. Ratio difference method was chosen due to its simplicity and accuracy, as only few steps were required and no processing of the samples was needed. Several divisors were tried to achieve the best sensitivity and minimal noise, the best choice was 5 μg mL^{-1} PD. Therefore, zero order spectra of the three components were scanned then they were all divided by 5 μg mL^{-1} PD as a divisor to convert its contribution into constant plateau. The obtained ratio spectra of AX, PD and NA, **Figure 3**, were carefully examined for selecting two different wavelengths whose their peak amplitudes difference corresponds to zero contribution of PD and NA while it corresponds only to the concentration of AX. The amplitude differences were calculated and plotted versus the concentration of AX, to construct the calibration curve.

Method validation:

Method validation is completed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over specified range that an analyte will be analyzed. These common parameters are meant to bridge the differences that often exist between various tools of different labs and different countries. It also provides an assurance of reliability during normal use and is some times referred to as the process of providing documented evidence that the method does what it is intended to do.

Method validation of the proposed method was done in compliance with ICH guideline[9].

Linearity and range was checked by plotting the amplitude difference in absorbance versus the concentration of AX to construct the calibration curve. The linear regression data for the calibration curve showed a good linear relationship over a concentration range of 2-10 μg mL^{-1} as expressed in **Table 1** and the regression equation was computed and found to be:

$$\Delta P=1.4015\ C+1.8259\ (r=0.9999)$$

Where, ΔP: Absorbance difference at the two selected wavelengths, C: Concentration (μg mL^{-1}) and r : Correlation coefficient.

Accuracy and repeatability of the method was confirmed in drug substance by the analysis of three different concentration levels within the linearity range were selected and examined three times each within the same day through specified time intervals. While intermediate precision was assessed by the application of the proposed procedures throughout three successive days using three replicates of three concentration levels within 80%-120%. Data were shown in **Table 1**.

In addition sensitivity of the method was measured in term of LOD and LOQ through the calculation of residual standard deviation of the regression line.

Application to Aminexil ® hair tonic

Calculated volumes from Aminexil® were transferred into a series of 10 mL volumetric flasks. The volume of one volumetric flask was completed to mark with methanol, while the other flasks were spiked with three different volumes of the prepared standard AX stock solution. The spectrum of each dilution was recorded and stored on the computer for further processing. The concentrations were calculated using the regression equation. Data were shown in **Table 2**. Additionally, the obtained results were statistically compared with those obtained from the application of the reported method and it was found that there is no significant difference, which encourages the use of the proposed ratio difference method as a simple alternative for the analysis of AX in quality control labs, **Table 3.**

IV. CONCLUSION

The proposed method was evidenced to be applicable for the routine quality control work of AX quantification. The method was specific and precise to determine AX in its mixture with NA and PD either in the lab prepared mixture or in the cosmetic dosage form. The method was simple and economic, as it did not require any sophisticated instruments or preliminary processing of the samples.

Compliance with Ethical Standards

-Conflicts of Interest

The authors declare that they have no conflict of interest.

-Statement of Human and Animal Rights

This article does not contain any studies with human and animal subjects performed by any of the authors.

V. REFERENCES

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Table 1. Validation and linearity parameters

Parameter	**Aminexil**
Linearity range (μg mL^{-1})	2-10
Slope	1.4015
Intercept	1.8259
Correlation coefficient	0.9999
Accuracy	100.22±0.53
Inter-day precision (%RSD)	±0.76
Intra-day precision (%RSD)	±0.49
LOD (μg mL^{-1})	0.53
LOQ (μg mL^{-1})	1.6

Table 2. Application of the proposed ratio difference method to Aminexil® hair tonic and application of standard addition technique.

Item	**Recovery %± RSD%**
Aminexil® B.N. 44L201	100.01±0.48
Standard addition	99.68±0.56

Table 3. Statistical parameters of the proposed method in comparison to the reported method

Statistical parameters	**Proposed method**	**Reported method**
Mean	100.22	101
SD	0.53	0.13
%RSD	0.53	0.13
N	3	3
F value (19)*	16.62	---
Student T test (2.776)*	2.4757	---
Two tailed P value	0.0685	---

*tabulated F and T values

Figures legends:

Figure 1: chemical structure of a. AX, b. NA and c. PD.

Figure 2 : Zero order spectra of 5μgml^{-1} AX (_ _ _ _ _) , 5μgml^{-1} NA (……) and 5μgml^{-1} PD (______) showing sever overlapping.

Figure 3 : Ratio spectra of 5μgml^{-1} AX (_ _ _ _ _) , 5μgml^{-1} NA (……) and 5μgml^{-1}

PD (______) divided by 5 μg mL^{-1} PD.

Figures

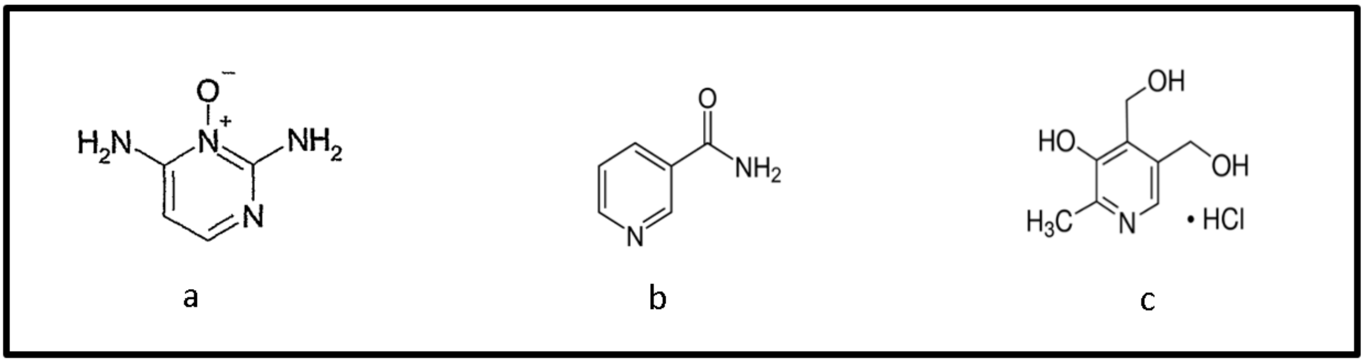
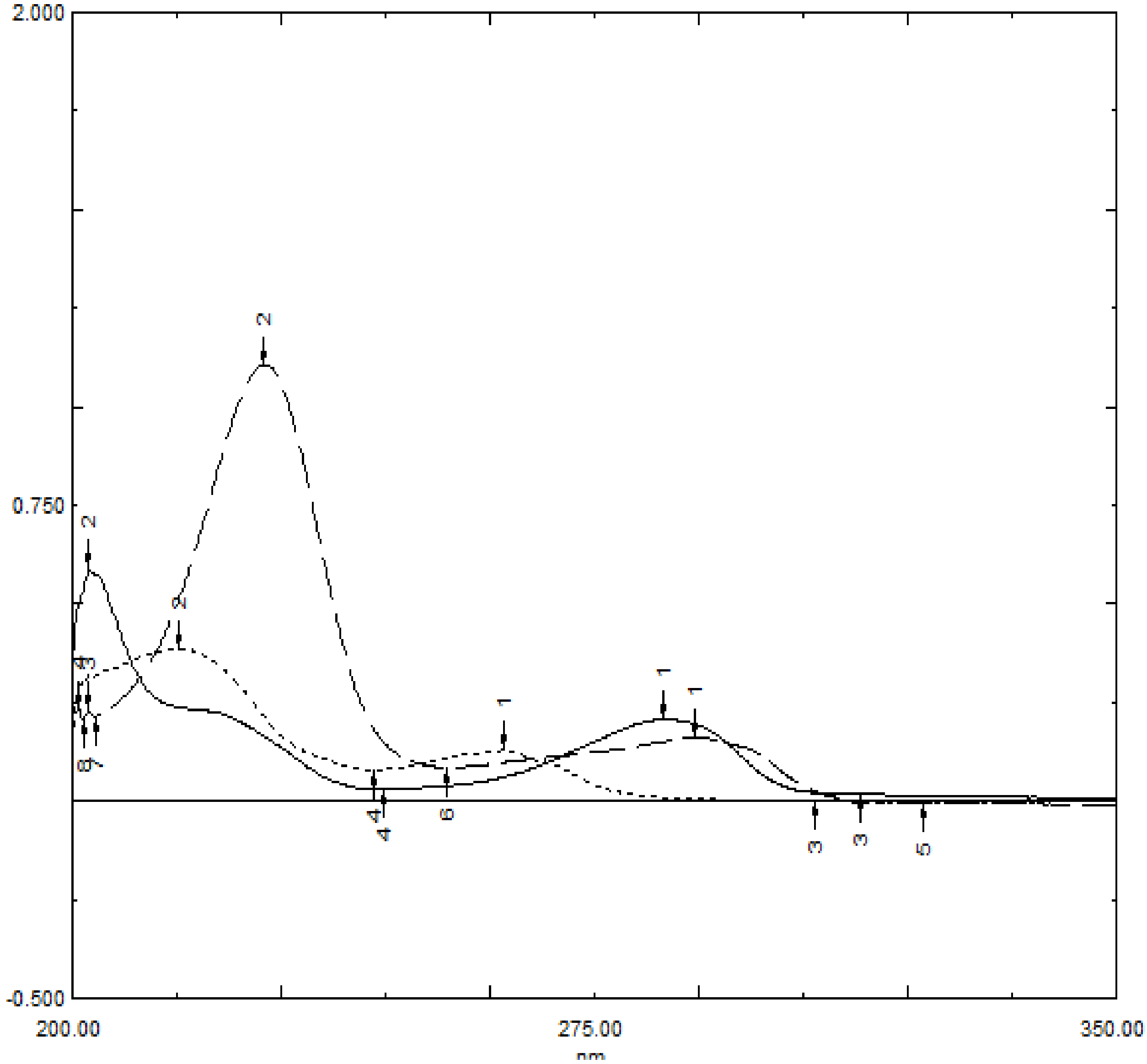


Figure 1 : Chemical structure of a. AX, b. NA and c. PD



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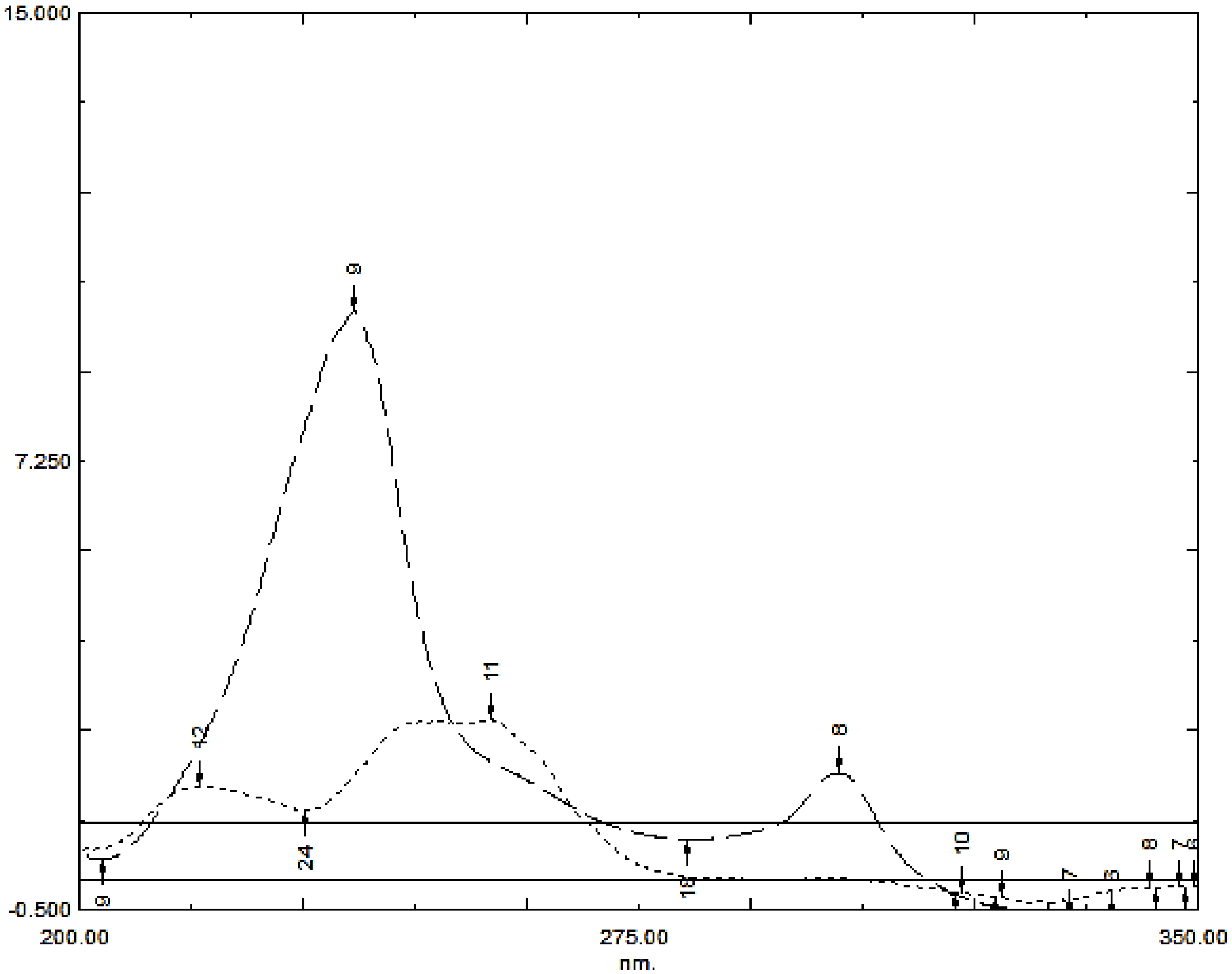

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Cite this article as :

Dina M. Youssif, Mamdouh R. Rezk, Hebatallah M. Essam, "The Development of a Manipulative Spectrophotometric Technique for the Determination of Aminexil in a New Hair Tonic Preparation", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 6 Issue 6, pp. 275-281, November-December 2019. Available at doi : https://doi.org/10.32628/IJSRST196644
Journal URL : http://ijsrst.com/IJSRST196644