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INVITED ARTICLE

QUANTIFICATION OF 2, 4-DIAMINOPYRIMIDINE 3- OXIDE IN MARKETED HAIR GROWTH FORMULATION USING RP-HPLC

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Abstract

2,4-diaminopyrimidine 3-oxide is widely used as hair growth promoter agent in cosmetics and pharmaceutical preparations. A simple RP-HPLC method was developed for quantification of 2,4-diaminopyrimidine 3-oxide in marketed hair growth formulation. The method was developed using C_{18} column and mobile phase consisted of purified water: methanol (75:25) with flow rate of 0.8 mL/min. The eluent was monitored at 225 nm. The lineraity of the standard 2,4-diaminopyrimidine 3-oxide was performed between concentration 0.01 to 0.1 mg/mL and LOD and LOQ of the method were found. The method was found to be linear. The developed method was successfully applied for quantification of 2,4-diaminopyrimidine 3-oxide in marketed formulation.

Keywords: *RP-HPLC, 2,4-diaminopyrimidine 3-oxide, hair growth formulation*

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Introduction

2,4-diaminopyrimidine 3-oxide (DPO) is a chemical compound similar to minoxidil. DPO is used in hair cosmetics to combat hair loss due to premature exhaustion of the hair root. The compound DPO is generally used for the treatment of hair loss in compositions which can be provided in the form of a lotion, shampoo, gel, foam, emulsion, vesicular dispersion, soap and spray or aerosol foam.

DPO increase the volume of hair in the growth stage by working on the deep structure of the roots. It rejuvenates the hair roots so that healthy hair growth can persist. The perifollicular fibrosis is a condition in which collagen around the roots becomes rigid, tightens and pushes the hair out. DPO help in softening of the collagen and also inhibits lysyl hydroxylase, an enzyme participating in the maturation and hardening of the collagen structure of the hair follicle. Clinical Trials have demonstrated that DPO preserves and strengthens hair fibers. So DPO is widely used as a hair growth promoter. So its quantification is very important as far as effectiveness and quality of the formulation is concerned. A method had been reported for determination of N-oxide metabolites of 2,4-diaminopyrimidines with ion-pair HPLC [1]. Few analytical methods have been reported for other hair growth promoting agents [2-5]. But, no simple HPLC method is reported hence it was endeavored to develop RP-HPLC method for DPO for its analysis in hair growth formulation.

Materials and Methods

Instrumentation

HPLC

Chromatographic analysis was performed on Agilent chromatographic system (Agilent 1260 infinity, USA) equipped with quaternary pump and variable wavelength detector. Samples were injected through a rheodyne 1260 manual injector with 20 µL loop. Method was developed with a reversed- phase, Agilent Eclipse XDB C_{18} column (4.6 X 150 mm, 5µm). Eleuent from the column was monitored at 225 nm. Data acquisition and integration was performed using Agilent Chemstation software.

Materials and Reagents

Standard DPO was purchased from local supplier with % purity of 99.4% w/w. A hair growth formulation was purchased from local market. Methanol and water used for analysis were of HPLC grade (Finar Chemicals Ltd., Mumbai). Nylon syringe filter (0.22 µm) (Prama Instruments Pvt Ltd., Mumbai) were used throughout study for filtration of solutions.

Experimental conditions

HPLC

The mobile phase was consists of water and methanol (HPLC grade) in the ratio of 75:25 (% v/v). The mobile phase was degassed by sonication for 5 minutes in an ultrasonic bath. The flow rate of mobile

phase was kept 0.8 mL/min. The volume was solution injected in chromatographic system was 20 µL. Quantitation based on peak area was achieved with UV detection at 225 nm. All determinations were performed at ambient temperature.

Standard solutions and calibrations

Standard stock solution of DPO was prepared by dissolving 10.6 mg of standard DPO in 10 mL volumetric flask. 5 mL mobile phase (water: methanol, 75:25 v/v) as a diluent was added to the 10 mL volumetric flask. Flask was sonicated for 2 minutes to ensure complete solubilization of standard DPO. Volume was made up to the mark with mobile phase. The final concentration of stock solution was 1.06 mg/mL of DPO.

Preparation of calibration curve for standard 2,4-diaminopyrimidine 3-oxide by HPLC .

From the stock solution of standard DPO, aliquots of 0.1 mL, 0.3 mL, 0.7 mL and 1 mL were transferred to different 10 mL volumetric flasks. 5 mL of mobile phase was added to each flask. Flasks were sonicated for 2 minutes in ultrasonic bath to ensure degassing and proper mixing.

Table I: Calibration curve for standard 2,4-diaminopyrimidine 3-oxide

Sr. No.	Volume of Stock Solution	Final Volume	Final Conc. (mg/mL) of Working Standards
1	0.1 ml	10	0.0106
2	0.3 ml	10	0.0318
3	0.7 ml	10	0.0742
4	1.0 ml	10	0.106

Then, volume was made up to the mark with mobile phase in each flask. All solutions were filtered through the 0.22 µm syringe filters. Final concentration of working standard solutions is shown in Table I. 20 µL of all working standard solutions were injected in HPLC and chromatograms were obtained under the optimized chromatographic conditions described previously. The calibration graph was constructed by plotting peak area versus concentration of drug and the regression equation was calculated.

Analysis of commercial hair growth product.

Commercially available hair growth formulation was procured and two different weights 533.0 mg and 532.5 mg were accurately taken from the formulation and transferred in a 100 mL volumetric flask. Then 60 mL mobile phase (water: methanol, 75:25 v/v) as a diluent was added in both the 100 mL volumetric flasks followed by sonication for 2 minutes to dissolve the sample. Volume was made

up to the mark with mobile phase. Both solutions were filtered through the 0.22 µm syringe filters. Then 20 µL of both samples were injected in to HPLC system.

Results and Discussion

RP-HPLC

Various experimental trials were carried out to optimize the chromatographic conditions for estimation of DPO. The optimized chromatographic conditions are use of Eclipse XDB C_{18} column and mobile phase consisting of water: methanol (75:25, v/v) at the flow rate of 0.8 mL/min. DPO shows good absorptivity at 225 nm, hence it was selected as the wavelength for detection. Figure 1 shows calibration curve of working standard solutions of DPO by the developed HPLC method. Figure 2 shows chromatogram of standard DPO and Figure 3 shows the chromatogram of DPO in commercially available hair growth formulation.

The above HPLC method was partially validated [6]. The Regression coeffficent for linearity of the method was found to be 0.998 and the regression equation was found to be y = 23764x - 69.29. The system suitability parameters like theoretical plates, tailing factor and resolution were also checked. All the parameters were within the limit.

Limit of detection (LOD) and Limit of quantitation (LOQ)

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

LOD and LOQ was found by following equation

$$LOD = \frac{3.3 \cdot S.D.}{Slope\ of\ calibration\ curve}$$

$$LOQ = \frac{10 \cdot S.D.}{Slope\ of\ calibration\ curve}$$

SD = Standard deviation of intercepts

LOD and LOQ of the method was found to be 0.002377 mg/mL and 0.007204 mg/mL respectively. The marketed formulation of DPO was analyzed in duplicate. The area under the curve (AUC) of both the trials were recorded and percentage of DPO was calculated by following equation.

$$\%\ DPO = \frac{AUC_{Sample}}{AUC_{Std}} \cdot \frac{Con_{Std}}{Con_{Sample}} \cdot \%\ purity\ of\ Std$$

Std = Standard

The percentage assay of DPO was found 0.79 ±0.0051 in the marketed hair growth formulation. So, in both the sample analysis, results were reproducible. Validation parameters are summarized in Table II.

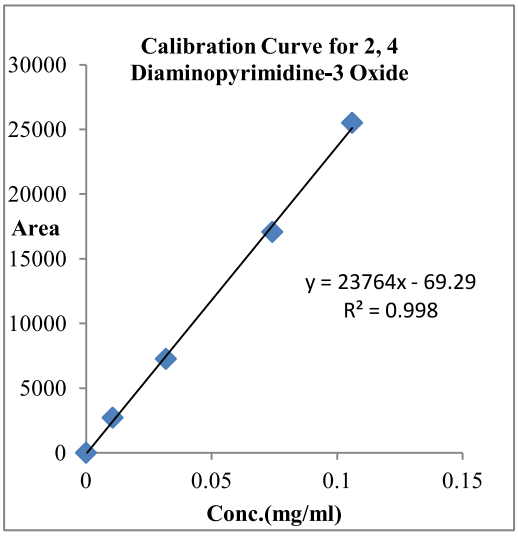

Figure 1: Calibration curve of standard 2, 4-diaminopyrimidine 3-oxide solutions by HPLC

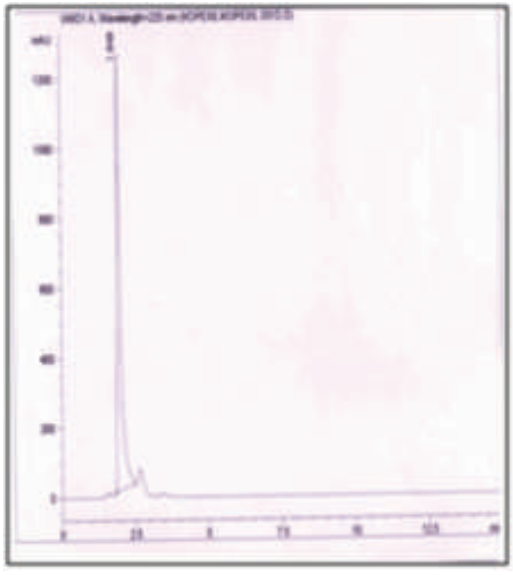

Figure 2: Chromatogram of standard 2, 4-diaminopyrimidine 3- oxide

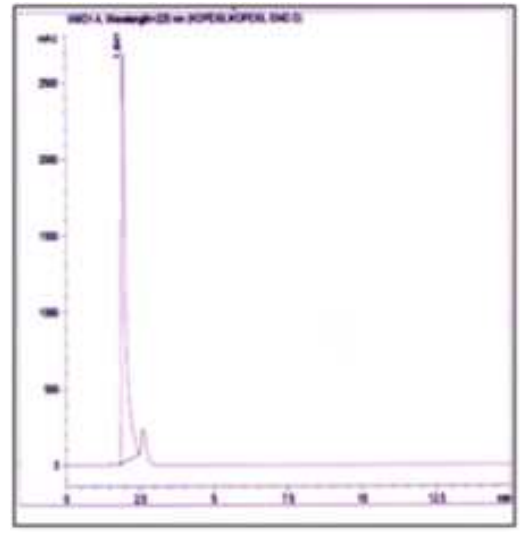

Figure 3: HPLC chromatogram of the formulation

Table II: Summary of the validation parameters

Parameters	Results
Linearity range (mg/mL)	0.0106-0.106
Regression equation: y= mx+c	23764x - 69.29
Regression coefficient value (R^2)	0.998
LOD (mg/mL)	0.002377
LOQ (mg/mL)	0.007204

Conclusion

Various HPLC techniques are generally used for separation and quantification of components in final pharmaceutical preparation and are better with regards to identification and specificity. As the 2,4-diaminopyrimidine 3-oxide is very important in hair growth formulation, its quantification in the product ensures its quality and effectiveness as a formulation. The current RP-HPLC method is very simple, economical, and time saving as the total run time as well as sample and standard preparations are quite easy. So it can be used for routine analysis of 2,4-diaminopyrimidine 3-oxide in commercially available hair growth formulations. However, the method is only partially validated. Further study need to be carried out for impurities of 2,4-diaminopyrimidine 3-oxide and stability of the formulation.

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