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Development and Validation of UV Spectrometric Method for Estimation of Aminexil (Kopexil) in Bulk

Разработка и валидация УФ-спектрофотометрического метода определения аминексила (копексила) в субстанции

Abstract

Background. Lately Aminexil (Kopexil) is widely added in the hair products to thicken the roots, but there is no reported simple, fast and accurate method to determine quantity of Aminexil just by UV-vis spectrometer.
Objective: to develop method of Aminexil determination by UV spectroscopy and validate it for linearity, range, accuracy, precision, ruggedness and robustness.
Methods. The conditions and concentration of Aminexil were optimized according to the linear correlation with maximum UV absorbencies.
Results. A simple, precise, accurate, reproducible and low cost UV-spectrophotometric method has been developed and validated for the quantification of Aminexil in bulk. The linearity was found for UV absorption maximum at the 284 nm in the concentration ranges of 1.0–1.8 mg/100mL in 0.1 M HCl solution with r^2=0.9987. The LOD was 0.22 mg/100mL, while LQD was 0.65 mg/100mL.
Conclusion: The proposed ready-to-use technique can be successfully used for determination of Aminexil in bulk and for future studies in pharmaceutical formulations.
Keywords: Aminexil, validation, UV-spectrophotometry.

Резюме

Обоснование. В последнее время аминексил (копексил) часто добавляется в средства для волос с целью утолщения корней, но при этом отсутствует простой, быстрый и точный метод определения количества аминексила с помощью УФ-спектрофотометрии.
Цель: разработать методику определения аминексила УФ-спектрофотометрически и валидировать ее по следующим критериям: линейность, диапазон применения, точность, правильность, прецизионность и робастность.
Методы. Экспериментально были подобраны условия определения и концентрация растворов аминексила в 0,1 М HCl в соответствии с корреляцией между концентрацией и значениями максимальной оптической плотности при 284 нм.
Результаты. Простой, точный, воспроизводимый и недорогой УФ-спектрофотометрический метод был разработан и валидирован для количественного определения аминексила в

субстанции. Линейность была обнаружена с r^2=0,9987 в диапазоне концентраций 1,0–1,8 мг/100 мл в 0,1 М HCl. Минимальный предел обнаружения составляет 0,22 мг/100 мл, а минимальный предел для количественного определения – 0,65 мг/100 мл.

Выводы. Предложенный метод может быть успешно использован для определения аминексила в субстанции и для его будущих исследований в фармацевтических и косметологических препаратах.

Ключевые слова: аминексил, валидация, УФ-спектрофотометрия.

■ INTRODUCTION

Dermatologists state that hair has a significant contribution to the general appearance of the human body, specially giving feeling of self-confidence to women [1, 2]. Surprisingly, there are still reported only few researches, aimed at new therapies to prevent hair loss and enhance hair growth [3–5].

Among recently used hair enhancers is known Kopexil (trade name Aminexil) – the 4-pyrrolidine-2, 6-diaminopyrimidine-1-oxide or 6-amino-2-iminopyrimidin-1(2H)-ol (its tautomeric form), which is an altered form of widely used Minoxidil (Fig. 1) [6, 7].

Aminexil influences the hair root thickening in growth stage. Fibrosis condition of the hair roots causes blood vessels to constrict and shorten the life span of the hair follicle [8].

The search for quantity estimation studies exposes, that there are no simple Aminexil determination just by UV absorption, because it is the most commonly used simple method for laboratories in Ukraine. Among found ones is a report of the simultaneous determination of Minoxidil and Aminexil by RP-HPLC method in 0.1% orthophosphoric acid and methanol solution in the ratio of 60:40 v/v at a flow rate of 1 mL/min[6] with mentioned absorbance maximum at the 250 nm [9]. Moreover, RP-HPLC separation of Aminexil and Minoxidil was achieved in phosphate buffer and acetonitrile (78:22) with detection wavelength at the 238 nm using photo-diode array detector [7].

Hence, it was decided to developed and validated simple, fast and accurate method to Aminexil quantity determination just UV-vis spectrometer usage.

■ MATERIALS AND METHODS

Instrumentation

Substance was weighted using analytical balances Kern ABT 120-5DM, KERN&Sohn GmbH, Germany. UV spectra were recorded on Analytic Jena UV-vis spectrophotometer Specord 200 (190–400 nm), Germany.

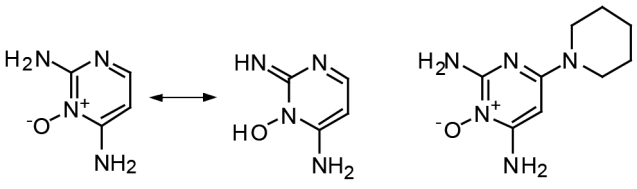

Fig. 1. Aminexil tautomerism and Minoxidil structure

Reagents and solutions

All of the chemicals were of the highest purity available from the LAB-SCAN (Ireland) and were used without any further purification. The distilled water was used throughout the experiments. Working substance of Aminexil was purchased from Changzhou Pharmaceutical Factory (China) (CAS 7438-76-9).

Validation

Calibration curve. Standard solution (0.0016%) was prepared by dissolving 0.0500 g of Aminexil in the 100.0 mL flask with 0.1 M HCl. Stirred for 5 min. Then 0.80 mL of obtained solution was quantitatively transferred into the 25.00 mL flask, added 0.1 M HCl. Stirred for 5 min. The amount of Aminexil was determined by employing UV absorption at the wavelength of the 284 nm.

The working standard solutions (0.001–0.0018%) were made quantitatively in the same way, adding from 0.50 mL to 0.90 mL of 0.0005% Aminexil solution in 0.1 M HCl into the second dilution flask. All solutions were stored at 18–20 °C.

The calibration curve of Aminexil was constructed by a UV-vis spectrophotometer absorbance data monitored 5 times for each sample at the wavelength of maximum absorbance at the 284 nm in comparison to 0.1 M HCl in 3 mL cuvette with 1 cm layer.

The regression equation was obtained by the method of least squares for n=5. Regression equation: Y = slope*x + intercept. Slope, intercept and correlation coefficient were determined from the regression analysis' calculations in Microsoft Excel 2007 [10].

Using this linear equation, correlation coefficient (r^2) and the detection limits were determined. Accuracy: mean ± SD; Linearity (lowest – highest concentration while curve is linear); SE of intercept: √ of $\sum(y–y^2/n)$, where y – standard concentration, y' – found concentration; SD of intercept: SE of intercept*√n.

The limit of detection (LOD): 3.3*(SD of intercept / slope); and the limit of quantitation (LOQ): 10*(SD of intercept / slope). The LOD was defined by the concentration with a signal-to-noise ratio of 3. The analyte peak in the LOQ sample should be identifiable, discrete, and reproducible with a precision of ±20% and accuracy within 80%–120%. The deviation of standards other than LOQ should not be more than ±15% of the nominal concentration.

Precision (repeatability of the method) was evaluated by repeated absorbance detection and the results were expressed as the mean standard deviation (SD) and the percent relative standard deviation RSD (%) = SD / Mean. For intra-day analysis the samples were analyzed six times a day at 09:00 am, 11:00 am, 01:00 pm, 03:00 pm, 05:00 pm, and 07:00 pm, while for inter-days stability was analyzed for 6 consecutive days at 09:00 am.

■ RESULTS AND DISCUSSION

Considering the chemical structure of Aminexil, namely presence of aromatic primary and tertiary Nitrogens (Fig.1), that had basic properties, it was decided to use acid solution as the solvent for investigation. The most safest and cheapest was chosen 0.1 M HCl. When analyzing the most suitable concentration range of Aminexil, it was found, that maximum wavelengths were the 229 and 284 nm in 0.0016% solution in 0.1 M HCl with absorbance about 0.6 for the last peak, just according to the Beer's Law (Fig. 2).

So, the second peak was taken as the priority one for studies, especially because usually in this region there are no absorbencies of the additional substances presented in the pharmaceutical dosage form.

The best linear correlation of maximum UV absorbance with solutions' concentration was achieved in 1.0–1.8 mg/100mL at the 284 nm (Fig. 3).

Validation of the method was prepared in accordance to the analytical methods validation parameters [11].

The appropriate concentrations, calculated accuracy and recovery data are presented in the Table 1.

The linearity was evaluated by linear regression analysis by the least-square regression analysis. The calibration curve of Aminexil had good correlation coefficient 0.9987 (Fig. 3).

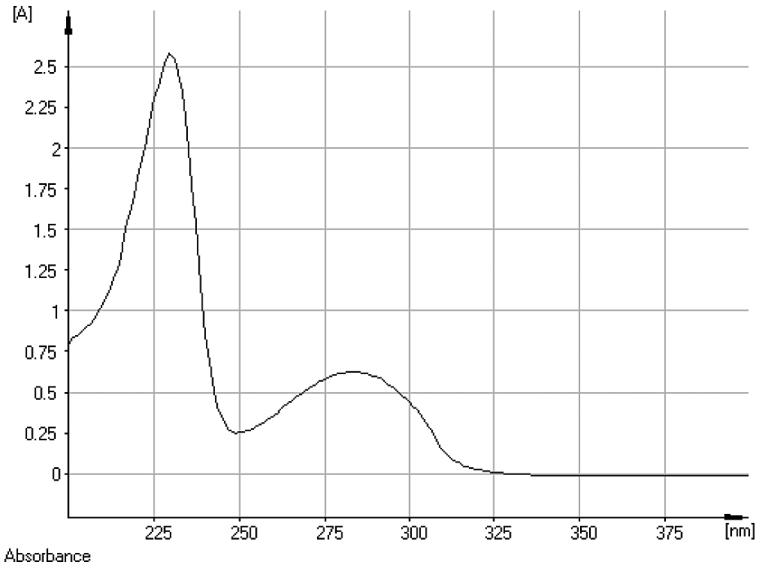

Fig. 2. UV spectrum of 0.0016% Aminexil solution in 0.1 M HCl with Amax at the 219 and 284 nm

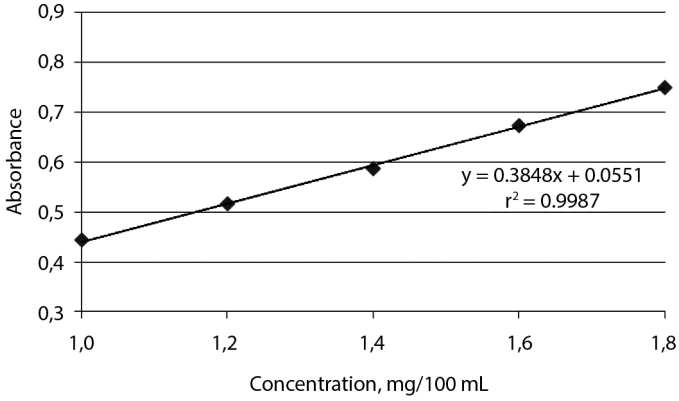

Fig. 3. Calibration curve of 0.001–0.0018% solution of Aminexil in 0.1 M HCl by UV absorbance at the 284 nm

The accuracy of the method was proven by recovery of five different concentrations of the calibration range (100.01±0.84%) with 100.01±0.01% percent of recoveries (Table 1).

Slope, intercept and correlation coefficient were found from the regression analysis' calculations (Table 2).

The limit of detection (LOD) was found to be 0.22 mg/100mL, while limit of quantification (LOQ) was 0.65 mg/100mL, even better than 0.31 mg/100mL and 0.92 mg/100mL, correspondingly, reported earlier by RP-HPLC data [7].

Calculations of the method ruggedness have shown its good reproducibility: during the day RSD was 0.01% and during the week – 0.03% (Table 3).

The robustness of the method was studied through temperature influence, stirring and usage of different 0.1 M HCl solutions. It was found, that usage of the cuvette without glass cap, or even with cap, due to water evaporation or condensation at the cap, slightly resulted the absorption results, when left at least for one hour because of lamp heating. The mechanical stirring for 5 min. of each flasks and temperature of 20–25 °C were the best conditions to obtain accurate data.

Hence, to detect the amount of Aminexil in bulk the next methodic is proposed.

Table 1
Recovery and accuracy of working Aminexil solutions in 0.1 HCl

#	Conc., %	Conc., mg/100 mL	ΔA of 5 meas.	[a] Conc. found, mg/100mL	[b] Recovery
1	0.001	1.0	0.4434	1.0091	100.9096
2	0.0012	1.2	0.5161	1.1980	99.8354
3	0.0014	1.4	0.5866	1.3812	98.6598
4	0.0016	1.6	0.6732	1.6063	100.3931
5	0.0018	1.8	0.7496	1.8048	100.2685
Mean					100.0133
SD					0.8480
% RSD					0.0076
Accuracy, %					100.0133±0.8480
Recovery, %					100.0133±0.0076

[a] Found concentration = (absorbance – intercept)/slope;
[b] Recovery = found concentration / labeled concentration*100.

Table 2
Linearity data, accuracy and precision of Aminexil solutions in 0.1 HCl

Parameters	Results
Slope	0.3848
Intercept	0.0551
Linearity (mg/100 mL)	1.0–1.8
Regression equation	y = 0.3848x – 0.0551
r^2	0.9987
SE of intercept	0.0112
SD of intercept	0.0251
LOD (mg/100 mL)	0.2155
LOQ (mg/100 mL)	0.6529

Table 3
Intra-day and inter-day precision data of 0.0016% Aminexil solutions in 0.1M HCl

#	Amax at the 284 nm	
	Intra-day precision	**Inter-day precision**
1	0.6754	0.6754
2	0.6752	0.6542
3	0.6713	0.6450
4	0.6653	0.6378
5	0.6562	0.6230
6	0.6603	0.6234
Mean	0.6673	0.6431
SD	0.0080	0.0200
% RSD	0.0109	0.0283

Quantitatively place 0.0500–0.0600 g of Aminexil in the 100.0 mL flask, dissolve it with 0.1 M HCl. Stir for 5 min. Quantitatively transfer 0.80 mL of obtained solution into the 25.00 mL flask, fill with 0.1 M HCl. Stir for 5 min. Determine the amount of Aminexil by employing UV absorption at the wavelength of the 284 nm in comparison to 0.1 M HCl in 3 mL cuvette with 1 cm layer.

Calculate the sample concentration in accordance to the obtained calibration equation:

$$\text{C, mg/100 mL} = \frac{A_i - 0.0551}{0.3848},$$

$$\text{C, \% of final solution} = \frac{A_i - 0.0551}{0.3848 \times 1000},$$

where Ai – absorption of the final experimental sample solution;

Or in accordance to calculated Amax of standard solution:

$$\text{C, \% of final solution} = \frac{A_i \times 0.0016}{0.6708},$$

where Ai – absorbance of the final experimental sample solution;

0.0016 – concentration of the standard solution of Aminexil with Amax of 0.6708 in 0.1 M HCl at the 284 nm, %.

Or in accordance with sample weight calculate concentration in bulk:

$$\text{C, \% in bulk} = \frac{A_i \times C_0 \times 100.0 \times 25.0}{A_0 \times p \times 0.80 \times l},$$

where Ai – absorbance of the final experimental sample solution;

C_0 – concentration of the standard solution of Aminexil is 0.0016, %;

100.0, 25.00 – flasks dilutions volume, mL;

A_0 – maximum absorbance of 0.0016% standard solution at the 284 nm is 0.6708,

p – taken sample weight (0.0500–0.0600) to obtain Amax (0.6–0.8) at the 284 nm, g,

0.80 – sample volume taken by pipette, mL;

l – cuvette layer, 1 cm;

■ CONCLUSION

It was found, that Aminexil in 0.1 M HCl could be simply, low-cost, fast and accurately quantitatively determined by UV spectroscopy in bulk by maximum absorption at the 284 nm. Validation of the proposed method showed, that calibration curve had good linearity (r^2=0.9987) in the concentration range between 1–1.8 mg/100mL. The LOD was found to be 0.22 mg/100mL, and LOQ was 0.65 mg/100mL. Such criteria like accuracy, precision, robustness and ruggedness also showed high validity and reproducibility. The proposed simple technique could be successfully used for determination of Aminexil in bulk in analytical laboratories and pharmaceutical factories.

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