Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(5):344-350
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Spectrofluorimetric method for determination of low concentration of Minoxidil in pharmaceutical Formulations

Hossein Tavallali* and Ehsan Dezfoli

Department of Chemistry, Faculty of science, Islamic Azad University of Omidieh, Omidieh, Iran

---

ABSTRACT

A sensitive method for determination of trace levels of minoxidil, based on the oxidation by cerium(IV) in sulfuric acid media was described. The intensity of fluorescence was followed in excitation wavelength (255nm) and emission wavelength (348 nm). The chemical parameters such as cerium (IV) and sulfuric acid concentration, heating time and temperature were considered. Under the optimum conditions the linea dynamic range is 1-90 ng ml^{-1} with detection limit and relative standard deviation 0.26 ng ml^{-1} and 1.16% respectively. The method was successfully applied to the determination of minoxidil pharmaceutical formulation.

Keywords: Spectrofluorimetry, cerium, monoxidil.

INTRODUCTION

Minoxidil (2,4-diamino-6piperidinopyrimidine-3-oxide, structure in fig. 1) has proven to be efficatious in patients with the most severe and drug-resistant forms of hypertension [1,2]. Minoxidil increases blood flow to the skin, skeletal muscle, gastrointestinal tract and heart more than the central nervous system [1]. Minoxidil has been used as a peripheral vasodilator drug orally administrated, applied in the treatment of refractory hypertension patients [3].

Excessive oral administration of this drug to patient should cause liquid retention and hirsutism [4].

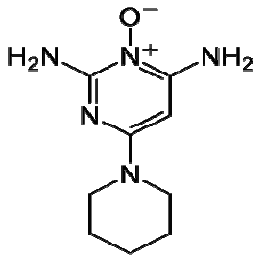

Fig. 1. Molecular formula of minoxidil

Initially described as an antihypertensive drug, minoxidil have also shown new applications in dermatology, especially in the treatment of androgenic alopecia [4,5]. In this case, this drug has been topically applied in order to stimulate hair growth by inducing vasodilatation and increasing the local irrigation and blood flow [5,6].

Commercially available products contain 2% minoxidil (20 mg mL^{-1}), in topic use formulations, containing ethanol and propylene glycol, or their mixture with 2-n-nonyl1-1,3-dixolane as a vehicle [7,8].

The reference method for minoxidil quantification given by the US Pharmacopeias uses liquid chromatography [9]; however, other different methods have been proposed for its determination in pharmaceutical formulas and in human plasma, which include high-performance liquid chromatography (HPLC) with UV detection [10,11], and electrochemical detection [12,13], a solid-phase column with UV detection [4], GC [14], radioimmunoassay [15], differential puls polarography [3], and electrolysis [16].

The suggested spectrofluorimetric method depends simply on the oxidation of minoxidil with ceric ammonium nitrate(Ce(IV)) in presence f sulfuric acid and measuring the fluorescence of the resulting cerium (Ce(III)) at λ_{ex} 255 nm and λ_{em} 348 nm.

In the present work we described a simple and sensitive method for the rapid amperometric quantification of minoxidil in pharmaceutical product any sample pretreatment.

MATERIALS AND METHODS

2.1. Apparatus

Spectrofluorimeter: SFM 23/B, kontron, Switzerland equipped with a recorder and 1×1 cm^2 quartz cell; was used for recording the spectra and carrying out fluorescence measurements. The calibration and linearity of the instrument were checked at frequent intervals with standard quinine sulfate (0.01 μg ml^{-1}). Wavelength calibration was performed by measuring λ excitation and λ emission of the same standard of quinine sulfate at λ_{ex} 275 nm and λ_{em} 430 nm, although no variation in the wavelength was observed. All fluorescence measurements were recorded at the lower set sensitivity.

2.2. Materials and chemical reagents

Analytical grade chemical were used as received. Doubly distilled water was used throughout. Ceric ammonium nitrate (Sigma Chemie GmbH, West Germany): 0.002 M was prepared by dissolving 109.6 mg and transferred into a 100-ml volumetric flask, diluted with double distilled water.

Sulfuric acid: 1 M was prepared by mixing 5.43 ml of concentrated sulfuric acid (Purity 98%, Density 1.84 g cm^{-3}) and transferred into a 100-ml volumetric flask, diluted to vlume with double distilled water.

Minoxidil powder was obtained from Merck (Darmstadt, Germany). Minoxidil(Loniten) tablets (patheon inc, Canada), contain 10 mg/tablet. Minoxidil topical solution(Pak-Darou Pharmaceutical co. Iran-Tehran), each ml 2% minoxidil topical solution contains 20 mg of minoxidil in 1 ml solution. All drugs were used as received and their solutions were prepared freshly every day to be used as working standards.

2.3. Preparation of samples

2.3.1. Minoxidil(Loniten) tablets

Ten tablets (contain 10 mg/tablet) were weighed and finely powdered. A weighed portion equivalent to the weight of one tablet was transferred to a 100-ml volumetric flask, sonicated for 5 min with about 50 ml double distilled water then was completed to volume with the same solvent. The mixture was mixed will, allowed any insoluble matter to settle then filtered. A measured volume of the filtrate was diluted quantitatively with double distilled water. This solution was diluted a hundred times. 1 ml of solution was transferred by pipette into a 25-ml volumetric flask then completed to volume with double distilled water to yield a sample solution having a concentration assumed to be 40 ng ml^{-1} of minoxidil.

2.3.2. minoxidil topical solution

1 g of sample (each ml 2% minoxidil topical solution contains 20 mg of minoxidil in 1 ml solution) weighed by syringe and transferred into a 100-ml volumetric flask, sonicated with 50 ml double distilled water for 3-5 min then completed to volume with the same solvent and filtered. The procedure was then completed as in Section 2.3.1 to yield a sample solution having a concentration assumed to be 80 ng ml^{-1} of minoxidil.

2.4. General procedure

An accurate volume of minoxidil solution (for concentration ranges of: 1-90 ng ml^{-1}) was transferred by a pipette into a 25-ml volumetric flask. A volume of 2.5 ml of ammonium ceric nitrate (0.002 M) was added and 5 ml of sulfuric acid (1 M) was transferred into flask. The solution was mixed well. Measured spectrofluorimetrically(F_1) at λ_{ex} 255 nm and λ_{em} nm 348. The solution was heated in a thermostatic water bath at 90 °C for 90 min. the solution was then cooled (room temperature) and measured (F_2) at the same wavelengths, then ΔF ($F_2 - F_1$) was calculated against a blank experiment treated similarly.

RESULTS AND DISCUSSION

Cerium(IV) has been used as an oxidizing agent for the determination of certain drugs as phenothiazines by monitoring the fluorescence of their sulfoxides or the Ce(III) formed. However, Ce(III) is more than four times fluorescent as the phenthiazines sulfoxides and therefore, measurement of its fluorescence can be used as a very sensitive method for the determination of these drugs [17]. In the present work minoxidil was oxidized by Ce(IV) and relative fluorescence intensities of the induced Ce(III) was monitored at λ_{ex} 255 nm and λ_{em} 348 nm. To avoid interference due to the presence of Ce(III), the fluorescence intensities of Ce(IV) were measured to obtain appropriate blank correction.

3.1. Optimization of reaction conditions:

A series of experiments were conducted to establish the optimum analytical conditions for the oxidation of minoxidil by Ce(IV). The parameters optimized were performed on all the studied minoxidil samples by altering each variable in turn while keeping the others constant.

3.1.1. Effect of Ce(IV) concentration

The effect of Ce(IV) concentrations was investigated using an accurate volume of different concentration of the reagent in the range of 1×10^{-4}- 6×10^{-4} M employing concentration 50 ng ml^{-1} of the minoxidil solutions. Maximum ΔF was obtained with a Ce(IV) concentration of 2×10^{-4} M, above which it remained constant r slightly decreased (fig. 2).

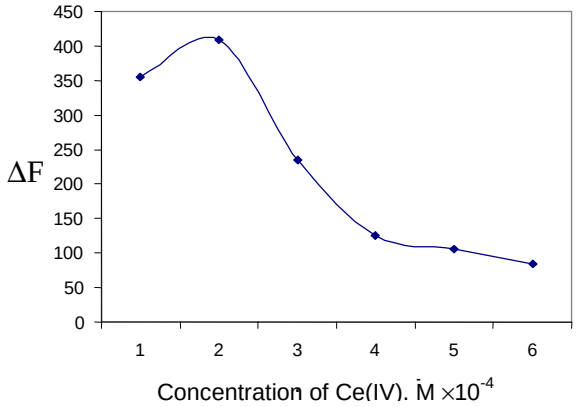

Fig. 2. Effect of Ce(IV) concentration on the ΔF due to Oxidation of 50 ng ml^{-1} minoxidil solution.

3.1.2. Effect of acid concentration

Different acids as HNO_3, HCl, H_2SO_4 were tested t determine the most suitable for optimum reaction development. Either hydrochloric or sulfuric acid could be used as the fluorescence of Ce(III) is high, however sulfuric acid was selected because blank reading was less than in hydrochloric acid medium [18]. The effect of sulfuric acid concentration on the sensitively of the method was studied. ΔF increase when using 0.10-0.20 M sulfuric acid, then decreased upon using higher concentrations. Therefore, 0.20 M sulfuric acid was adopted for this method.

3.1.3. Effect of heating temperature and time

The influence of different heating temperature and incubation time were studied using a thermostatic water bath. Accordingly, best temperature was found to be 90 °C (fig. 3). however complete oxidation was attained a period of 60 min.

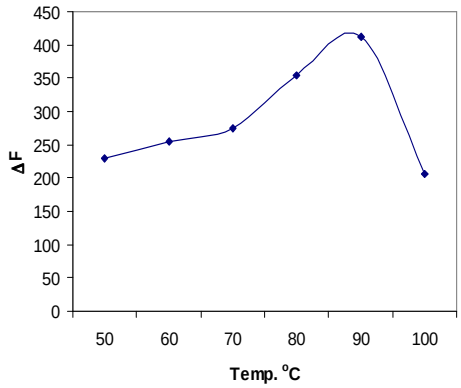

Fig. 3. Effect of heating temperature on the ΔF due to Oxidation of 50 ng ml^{-1} minoxidil solution in a period of 60 min.

3.1.4. Effect of other oxidants

It is well known that same oxidized drugs show excitation and emission maximum in the same spectral region where Ce(III) fluorescence as trimeprazine. In such cases an additional enhancement of the fluorescence readings is achieved [17]. Therefore oxidation of the studied minoxidil samples by other oxidizing agent as hydrogen peroxide, periodate, have been performed and the oxidation products were found not fluorescent. This confirms that the fluorescence induced after oxidation of minoxidil by Ce(IV) is not attributed to their oxidation products but it is mainly due to the formation of Ce(III).

3.2. Validation of the proposed method

3.2.1. Linearity and detection limit

Using the optimal reaction conditions, Beer's law was obeyed. Linear range equation using the least square method listed in table 1 and regression 0.9998. The detection limit (LOD) was calculated as follows: DL= k S.D.$_a$/b where k = 3, S.D.$_a$ is the standard deviation of intercept and b is the slope. Results shown in table 1.

Table 1 Statistical parameters for the proposed method of analysis of minoxidil

Liner range (ng ml^{-1})	*Least square equation ($y = a+bc$)^a*	*S.D.$_a$^{b}*	*LOD^c (ng ml^{-1})*
1-90	Y= 8.2964C+ 44.632	0.73	0.26

[a] *$y = \Delta F$, C = concentration in ng ml^{-1}*
[b] *Average of three determination*
[c] *Ref. [19].*

3.2.2. Repeatability

The mean of the ΔF(mean = 30.10) of seven separate samples solution of the minoxidil (concentration 30 ng ml^{-1}) of the same batch number gave a relative standard deviation of 1.16%. This level of precision is suitable for the routine quality control analysis of pharmaceutical dosage forms.

3.2.3. Recovery

Applying the suggested spectrofluorimetric procedure for the analysis of commercially available dosage forms validated the accuracy of the proposed method. Table 2 present the results obtained for content of minoxidil in two different commercial pharmaceutical samples, in this case standard deviations 2.56 and 2.38% and recoveries 91.00 and 106.25% were observed in the determination of spiked samples of Loniten tablets and minoxidil topical solution, respectively.

Table 2 Assay of minoxidil in pharmaceutical dosage forms by the proposed and reported method

Sample[a]	*Nominal value (mg)*	*Expermental conc.*[b] *(ng ml^{-1})*	*Relative Standard deviation*[b] *(%)*	*Recovery*[b] *(%)*
Loniten tablets	10/tablet	36.40	2.56	91.00
minoxidil topical solution	20/solution	85.00	2.38	106.25

[a] *See experimental part for suppliers and preparations.*
[b] *Average of five determinations.*

CONCLUSION

The proposed method consumes less reagent and time than the others, described in literature can be an alternative to determine minoxidil in commercial samples in a fast, low expensive, easy and generating low residue amounts.

REFERENCES

[1] H. Akama, M. Haramura, A. Tanaka, T. Akimto, N. Hirayama, *Anal. Sci.*, 20, **2004**, 29.
[2] R. A. de Sousa, E. T. G. Cavalheiro, *Ecl. Quim.*, 34, **2004**, 23.
[3] L.Amankwa, L. G. Chatten, S. Pons, *Analyst*, 108, **1983**, 1221.
[4] A. R. Medina, M. L. F. Co′rdova, A. Mplina-Diaz, *Talanta*, 50, **1999**, 277.
[5] J. H. Han, O. S. Kwon, J. H. chung, K. H. Cho, H. C. Eun, K. H. Kim, *J. Dermatol. Sci*., 34, **2004**, 91.
[6] R. A. de Sousa, F. S. Semaan, J. A. F. Baio, E. T. G. Cavalherio, *Ecl. Quim*., 30, **2005**, 79.
[7] W. Z. Zhong, P. E. Sandres, M. J. Hauer, J. J. Vrbanae, *J. Chromatogar*. B, 705, **1998**, 39.
[8] W. Z. Zhong, *J. Pharm. Biomed. Anal*., 16, **1998**, 1179.
[9] V. Pfaffen and P. I. Ortiz, *anal. Sci.*, 22, **2006**, 91.
[10] O. Asmus, J. B. Landis, M. E. Grant, H. A. Havel, *J. Pharm. Sci*., 73, **1984**, 1290.

[11] A. Zarghi, A. Shafaati, S. M. Foroutan, A. Khoddam, *J. Pharm. Biomed. Anal*., 36, **2004**, 377.
[12] G. Carrum, D. R. Abernethy, M. Sadhukhan, C. E. Wrigt III, *J. Chromatogr., B: Biomed. Sci. Appl.*, 381, **1986**, 127.
[13] M. H. Golden, P. H. Zoutendam, *J. Pharm. Biomed. Anal*., 5, **1987**, 543.
[14] T. Kamiewaska, E. Kublin, E. Weclawowicz, B. Kwiatkowska, Pharm. Pol., 43, 1987, 508.
[15] M. E. Royer, H. Cao, T. J. Gillberson, J. M. MacCall, K. T. Jonston, R. Stryd, *J. Pharm. Sci.*, 66, **1977**, 1266.
[16] J. Arcos, B. Garcia, A. Munguia, J. Lo′pez Palacios, *Anal. Lett*., 24, **1991**, 357.
[17] T. Perez-Ruitz, C. Martinez-Lozano, V. Tomas, C. Sidrach de Cardona, *Talanta*, 40, **1993**, 1361.
[18] P. Y. Khashaba, *J. Pharm. Biomed. Anal*, 27, **2002**, 923.
[19] J. N. Miller, *Analyst*, 116, **1991**, 3.