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Short Communication

Rapid determination of minoxidil in human plasma using ion-pair HPLC

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Abstract

A rapid, simple and sensitive ion-pair high-performance liquid chromatography (HPLC) method has been developed for quantification of minoxidil in plasma. The assay enables the measurement of minoxidil for therapeutic drug monitoring with a minimum detectable limit of 0.5 ng ml^{-1}. The method involves simple, one-step extraction procedure and analytical recovery was complete. The separation was performed on an analytical 150 × 4.6 mm i.d. μbondapak C_{18} column. The wavelength was set at 281 nm. The mobile phase was a mixture of 0.01 M sodium dihydrogen phosphate buffer and acetonitrile (60:40, v/v) containing 2.5 mM sodium dodecyl sulphate adjusted to pH 3.5 at a flow rate of 1 ml/min. The column temperature was set at 50 °C. The calibration curve was linear over the concentration range 2–100 ng ml^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 8%.

Keywords: Minoxidil; Plasma; HPLC

1. Introduction

Minoxidil is an antihypertensive that acts predominately by causing direct peripheral vasodilation of the arterioles. About 90% an oral dose of minoxidil has been reported to be absorbed from the gastro-intestinal tract. Minoxidil is extensively metabolized by the liver. It is excreted predominantly in the urine, mainly in the form of metabolites [1,2]. For pharmacokinetic studies, a sensitive method that allows an accurate measurement of low concentrations of minoxidil in plasma is required. Methods for the quantitation of this drug in biological fluids include radioimmunoassay (RIA) [3], GLC [4] and high-performance liquid chromatography (HPLC) [5–9]. Procedures involving RIA techniques lack specificity, while GLC methods used a complex derivatization procedure and are time-consuming. Measurement of minoxidil by HPLC is preferred due to its sensitivity and specificity. HPLC methods differ with respect to the mode of detection (electrochemical or ultraviolet) and sample preparation. Most of HPLC methods required liquid–liquid extraction with evaporation of the extract or on-line solid phase extraction and therefore, sample preparation is time-consuming, complex or both. In particular, minoxidil is polar and is therefore, difficult to extract from biological fluids. This paper describes a rapid and sensitive HPLC method which enables the determination of minoxidil with good accuracy at low drug concentrations in plasma, using single-step extraction procedure. The sample preparation only involves protein precipitation and no evaporation step is required. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Experimental

2.1. Chemicals

Minoxidil and propylparaben were supplied by Modava Pharmaceuticals (Tehran, Iran). Minoxidil is available as oral tablet containing 10 mg of minoxidil and the follow-

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Table 1
Assay linearity[a]

Coefficient of the linear regression analysis ($r \pm$ S.D.)		Slope ± S.D.	Intercept ± S.D.
Intra-assay ($n = 6$)	$0.9996 \pm 6.34 \times 10^{-4}$	0.0380 ± 0.0013	0.0220 ± 0.0026
R.S.D. (%)	0.0634	3.42	
Inter-assay ($n = 9$)	$0.9999 \pm 7.91 \times 10^{-4}$	0.0395 ± 0.0014	0.0205 ± 0.0034
R.S.D. (%)	0.0791	3.54	

[a] The applied concentrations are over the range 2–100 ng ml^{-1}.

ing inactive ingredients: Avicel, corn starch, and magnesium stearate. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany).

Water was obtained by double distillation and purified additionally with a Milli-Q system.

2.1.1. Instruments and chromatographic conditions

A Knauer HPLC system (Germany) employed consisted of a model Wellchrom K-1001 pump, a model Rheodyne 7125 injector and a model K 2501 UV detector connected to a model Eurochrom 2000 integrator.

The separation was performed on an analytical 150 mm × 4.6 mm i.d. μbondapak C_{18} (4 μm, particle size) column. The column temperature was kept at 50 °C. The wavelength was set at 281 nm. The mobile phase was a mixture of 0.01 M sodium dihydrogen phosphate buffer and acetonitrile (60:40, v/v) containing 2.5 mM sodium dodecyl sulphate adjusted to pH 3.5 at a flow rate of 1 ml/min. The mobile phase was prepared daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

2.1.2. Standard solutions

Stock solutions (1 mg ml^{-1}) and appropriate dilutions of minoxidil and propyl paraben were prepared in methanol and stored at +4 °C.

2.1.3. Sample preparation

To 500 μl of plasma in a glass-stoppered 15 ml centrifuge tube were added 50 μl of methanolic propyl paraben solution as internal standard (1 μg ml^{-1}), 250 μl of borate buffer (pH = 9.5) and 500 μl of acetonitrile. After mixing (30 s), the mixture centrifuged for 15 min at 6000 rpm. Then 50 μl of supernatant was injected into liquid chromatograph.

2.1.4. Biological samples

Minoxidil was administered in a single dose of 20 mg to healthy volunteers after over night fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at −20 °C until assayed.

2.1.5. Stability

The stability of minoxidil was assessed during all the storage steps and during all steps of the analytical method. No change in stability over the period of 1 month was observed.

2.1.6. Plasma standard curve

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at −20 °C. After thawing, stock solution of minoxidil was added to yield final concentrations ranging from 2–100 ng ml^{-1}. Internal standard solution was added and the samples were then prepared for analysis as described above.

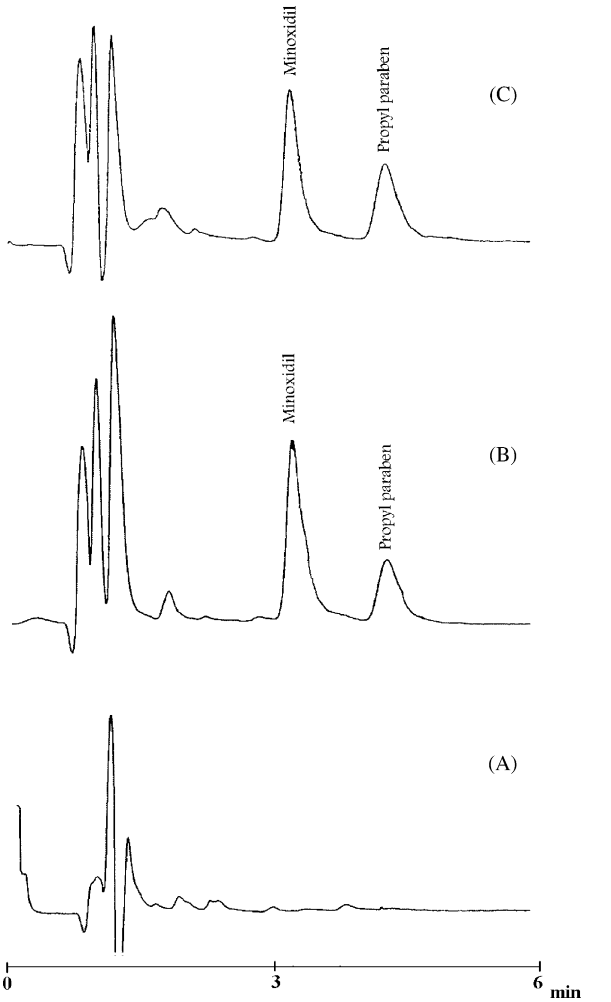

Fig. 1. Chromatograms of (A) blank plasma; (B) blank plasma spiked with 50 ng ml^{-1} minoxidil and 35 ng ml^{-1} propyl paraben (internal standard); (C) plasma sample from a healthy volunteer 1 h after oral administration 20 mg of minoxidil.

Table 2
Repeatability of the analysis of minoxidil in human plasma (n= 6)

Concentration added ($\mu g\,ml^{-1}$)	Concentration measured (mean ± S.E.)	
	Intra-day	Inter-day
5	5.02 ± 0.21 (4.2)	4.98 ± 0.32 (6.4)
25	24.71 ± 0.46 (1.9)	24.81 ± 0..51 (2.1)
60	58.50 ± 1.85 (3.2)	59.10± 2.12 (3.6)

Values in parentheses are coefficients of variation (%).

3. Results and discussion

Under the chromatographic conditions described, minoxidil, and the internal standard peaks were well-resolved. Endogenous plasma components did not give any interfering peaks. Fig. 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. The average retention times of minoxidil and propyl paraben were 3.6 and 4.8 min, respectively. The calibration curve for the determination of minoxidil in plasma was linear over the range 2–100 $ng\,ml^{-1}$. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.999. The relative standard deviation (R.S.D.) values of the slope were equal to or better than 5%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (Table 1). The relative analytical recovery for plasma at three different concentrations of minoxidil was determined. Known amounts of minoxidil were added to drug-free plasma in concentrations ranging from 5 to 60 $ng\,ml^{-1}$. The internal standard was added and the relative recovery of minoxidil was calculated by comparing the peak areas for extracted minoxidil from spiked plasma and a standard solution of minoxidil in methanol, containing internal standard with the same initial concentration. The average recovery was 96.1 ± 1.9% (n = 5). The limit of detection was defined, as the minoxidil concentration that produced a signal-to-noise ratio greater than three. The limit of detection in plasma was 0.5 $ng\,ml^{-1}$ based upon this criterion. At this level, the relative standard deviation (R.S.D.) was lower than 8%. This is sensitive enough for drug monitoring and other purposes, such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of minoxidil. As shown in Table 2, coefficients of variation were less than 6%, which is acceptable for the routine measurement of minoxidil. The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic minoxidil research. This method is well suited for routine application in the clinical laboratory because of the simple extraction procedure and good sensitivity. Over 300 plasma samples were analyzed by this method without problems, thus proving its suitability. In this study, plasma concentrations were determined in 12 healthy volunteers, who received 20 mg of minoxidil each. Fig. 2. Shows the mean plasma concentration–time curve of minoxidil: plasma concentration reached a maximum 1.50 ± 0.20 h after dosing with a level of 56.85 ± 8.20 $ng\,ml^{-1}$. These results are agreement with previous reports [2,10].

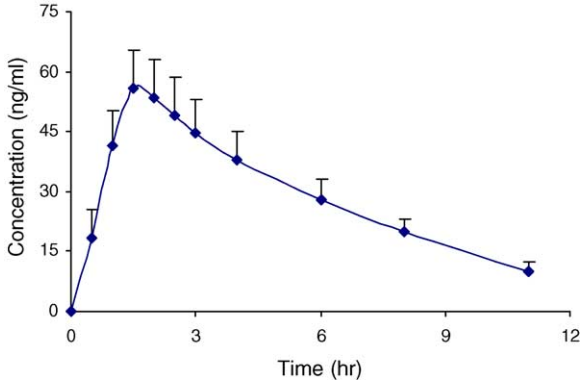

Fig. 2. Mean plasma concentration-time profile of minoxidil in healthy volunteers (n = 12) after a single 20 mg minoxidil.

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