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Short communication

Determination of minocycline in human plasma by high-performance liquid chromatography with UV detection after liquid–liquid extraction

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Abstract

A sensitive and specific high-performance liquid chromatographic method was developed and validated for the determination of minocycline in human plasma. The method uses liquid–liquid extraction, reextraction and HPLC with UV detection. The assay shows linearity in the tested range of 28–3533 ng/ml with a limit of quantification of 30 ng/ml. The inter-day precision was found to be ±3.84 to ±6.57% (R.S.D.) in the range of 148 to 2743 ng/ml. The method was successfully applied to pharmacokinetic studies.

Keywords: Minocycline; Antibiotics

1. Introduction

Minocycline has a spectrum of activity and mode of action similar to that of tetracycline hydrochloride, but it is more active against many species. For clinical studies, determination in human plasma is sometimes necessary. There are, however, almost no published papers concerning the determination in plasma or urine. One paper used extraction and HPLC–UV for determining two principal metabolites [1], while another paper used protein precipitation and HPLC–UV [2].

Determination of tetracyclines is difficult. These compounds possess a great number of functional groups and (at least) three dissociation constants in the pH range 1–12. Therefore, extraction and chromatography is rather difficult.

Concerning doxycycline, there are also not many publications about determination in biological fluids. Some used precolumn solid-phase extraction [3,4], others used liquid–liquid extraction [5,6]. All of them, however, had problems with the peak shapes of the chromatographic separation and therefore used specific and complex mixtures of mobile phases.

The following described method used liquid–liquid extraction and reextraction into an acid. Then HPLC was used with a simply composed mobile phase and UV detection.

2. Experimental

2.1. Reagents and chemicals

Minocycline was obtained from Spirig (Switzerland). Methanol was of HPLC grade (Rathburn, UK) and potassium dihydrogenphosphate, perchloric acid (E. Merck, Darmstadt, Germany) and triethylamine (Sigma, St. Louis, MO, USA) were all of analytical–

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reagent grade. Water used for mobile phase was purified by a reversed-osmosis system.

2.2. Chromatography

The HPLC system consisted of a HP-1090M liquid chromatograph (Hewlett–Packard, Waldbronn, Germany) and a variable-wavelength UV detector SpectroMonitor 3100 (Milton Roy, Ireland) set at 350 nm. The analog output from the detector was connected to a PE-Nelson 2600 data system (Perkin–Elmer, USA) via a PE-Nelson Series 900 analog-to-digital interface.

The analytical column was 125×4.0-mm I.D. stainless steel packed with Nucleosil 5-CN (SRD–Pannosch, Vienna, Austria) and was operated at 25°C.

The mobile phase consisted of methanol and 20 m*M* perchloric acid/4 m*M* triethylamine in water (20:80, v/v; pH approx. 2) and was degased with helium prior to use. The flow-rate was set to 1.0 ml/min (pressure approx. 1700 p.s.i.; 1 p.s.i.= 6894.76 Pa). The mixture of perchloric acid (ion pairing reagent) and triethylamine (prevents peak tailing caused by silanol groups) was necessary for a good peak shape.

2.3. Stock solution and standards

A stock solution of approx. 500 μg/ml minocycline was prepared by dissolving minocycline monohydrochloride in methanol. Calibration samples were prepared by diluting the stock solution with drug-free human plasma to obtain the highest calibration sample and successive dilution with blank plasma. A calibration row of nine levels, including a blank, was thus obtained, extending from 28 to 3533 ng/ml. Each level was divided into aliquots of 1.0 ml and immediately frozen at −20°C.

2.4. Sample preparation

Plasma samples were thawed at 25°C for about 10 min. A 1.0-ml volume of sample was transferred into a vial and mixed with 0.4 ml of 0.5 *M* KH_2PO_4 (pH of approx. 5 resulted) and 5.0 ml of ethyl acetate. After shaking for 1 min, the samples were centrifuged (2 min, >2000*g*).

Then 4.0 ml of the upper organic phase were transferred into a conical glass vial and mixed with 0.5 ml of 0.02 *M* HCl. After shaking and centrifuging (2 min, >2000*g*) about 300 μl of the lower water phase were taken by a gas tight syringe and transferred into a plastic HPLC–autosampler vial. After this, 20 μl of the samples had to be injected within 12 h.

3. Results and discussion

The specificity of the method described was investigated by screening five different batches of blank human plasma. The plasma samples were cleaned up as described above. The time interval, where minocycline eluted, was free of interference in all of the blank human plasma tested. Fig. 1 shows a sample of a drug-free plasma, a quality-control sample spiked in plasma at a concentration of 148 ng/ml and plasma samples taken from a volunteer 0.5 h and 48 h after oral intake of 50 mg minocycline (concentration 447 ng/ml and 37 ng/ml, respectively).

The assay showed linearity (calculated by weighted linear regression; weighting factor 1/concentration) over the concentration range of 28–3533 ng/ml. The peak area vs. concentration fitted well to a straight line, with $r^2>0.999$ for all calibration curves. The intra-day precision of the quality-control samples ($n=3$) were between ±1.24 and ±4.26% (concentration 148–2743 ng/ml), the accuracy of the back-calculated concentration values were between +1.59 and +7.59%. Near the lowest calibration sample the limit of quantification was determined at 30 ng/ml (precision ±5.38%, accuracy: −0.36%).

3.1. Precision and accuracy

For determination of the inter-day precision and accuracy, three replicates of three quality control samples were measured in one sequence on each of four days. The inter-day precision determined as the relative standard deviation (±R.S.D.) ranged between ±3.84 and ±6.57% (see Table 1). The accuracy calculated as the individual deviation of the quality control samples was between −0.80 and 7.43%.

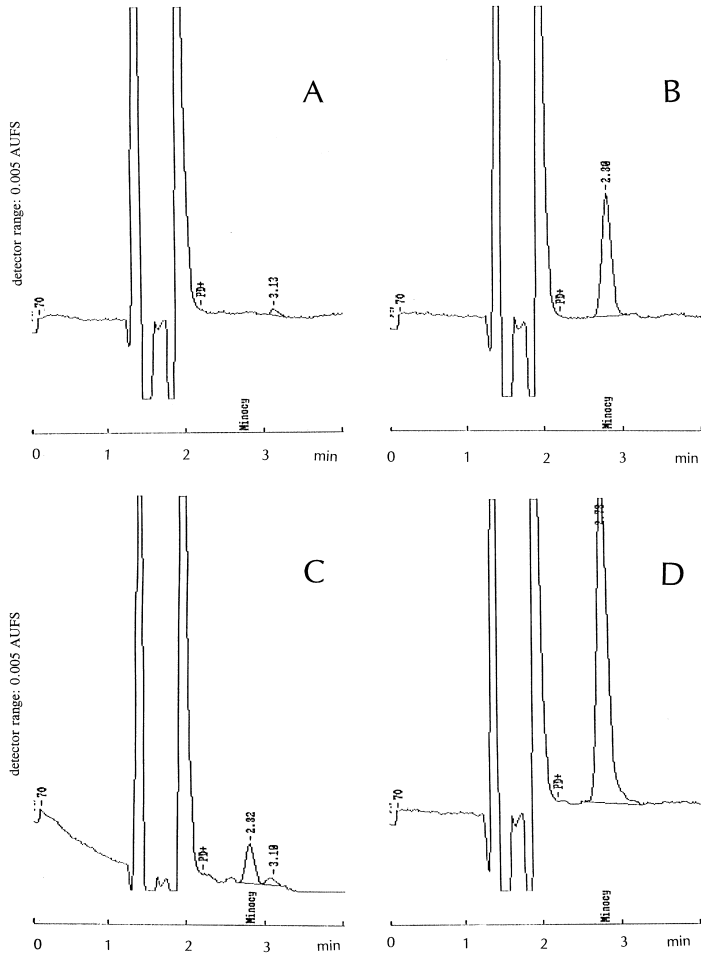

Fig. 1. (A) Drug-free plasma, (B) plasma spiked at a concentration of 148 ng/ml, (C) plasma sample taken from a volunteer 48 h after oral intake of 50 mg minocycline (concentration 37 ng/ml), (D) plasma sample taken from a volunteer 0.5 h after oral intake of 50 mg minocycline (concentration 447 ng/ml). Retention time of minocycline: approx. 2.8 min, detector range: 0.005 AUFS.

Table 1
Inter-day precision (reproducibility) of minocycline in plasma over four days

Concentration spiked ng/ml	S.D.	S.E.	R.S.D. (%)	Accuracy (%)
148.1	9.65	3.05	±6.57	−0.80
888.5	41.85	12.62	±4.46	+5.66
2742.9	113.27	34.15	±3.84	+7.43

S.D.: standard deviation; S.E.: standard error; ±R.S.D.: relative standard deviation.

3.2. Stability

For the determination of the counter stability, calibration samples at five concentrations were used. After spiking in plasma, aliquots were stored at room temperature for 4 h and 20 h, and one aliquot was frozen at −20°C, thawed and frozen again (results see Table 2).

Stability was also tested after sample preparation at three concentrations (148, 889 and 2743 ng/ml) at room temperature in 20 m*M* hydrochloric acid. No instability of minocycline was observed after 7 h and 14 h.

Table 2
Counter stability of minocycline in human plasma. (A) 4 h at room temp. in plasma; (B) 20 h at room temp. in plasma; (C) 2×thaw–freeze cycles

Concentration (ng/ml)	Deviation from the initial value (rel. %)		
	Treatment		
	A	B	C
182	2.5	−11.2	5.1
363	−8.6	−9.2	−2.0
726	6.4	−12.3	9.3
1452	14.9	−6.7	21.1
2900	−2.1	7.5	20.1

3.3. Recovery

Recovery was tested at a concentration of 1 μg/ml comparing plasma extracts with a solution without extraction and reextraction. The absolute recovery for minocycline was calculated as 76.4±0.37%.

4. Discussion

Our experience with doxycycline also showed us that protein precipitation with acids, e.g. perchloric acid, resulted in rather low recovery rates because of the protein binding. On the other hand, protein precipitation with acetonitrile would give high recovery rates, but it was not possible to inject higher volumes of the protein-free phase because of the difficult chromatographic behaviour of the tetracyclins. Injecting rather small volumes resulted in higher determination limits. Therefore, it was necessary when working with minocycline, which appears in lower concentration levels in plasma, to develop a more sensitive method. Some years ago, in response to this need, the liquid–liquid extraction procedure was developed.

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