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Short communication

Validation of a levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic studies

S. Siewert *

Institute for Clinical Pharmacology, Department of Pharmacology and Toxicology, Faculty of Medicine, University of Rostock, Schillingallee 70, 18057 Rostock, Germany

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Abstract

An HPLC method with fluorescence detection suitable for routine determination of levofloxacin in plasma and dialysate has been validated. Sample preparation was assured by one-step protein precipitation for plasma or direct injection of the dialysate solution, respectively. Separation occurred on an YMC Pro C18 RP column (150 mm × 2 mm) with an acidic binary gradient mobile phase and detection at excitation and emission wavelengths of 296 and 504 nm. The assay was linear between 0.1 and 6 μg/ml for plasma and 0.1 and 5 μg/ml for dialysate with intra- and inter-day precision and accuracy lower than 10%. No degradation of levofloxacin was observed under the applied conditions for both matrices. The method was successfully applied to an in vitro pharmacokinetic study and patient samples as well.

Keywords: Levofloxacin; Plasma; Dialysate; Pharmacokinetic studies

1. Introduction

Levofloxacin (levo), the levorotatory isomer of ofloxacin, exhibits activity against a broad spectrum of Gram-positive and Gram-negative bacteria [1] and is therefore administered to treat various infectious diseases, e.g. community acquired and nosocomial pneumonia, skin and skin structure infection, urinary tract infections or sepsis [2].

In patients, especially critically ill patients, pharmacokinetic parameters as volume of distribution, half-life and clearance differ substantially from those seen in volunteers. Also the variability of these parameters increases significantly. These facts make the prediction of drug exposure in the individual patient more complicated [3]. Therefore, a monitoring of levo could be reasonable and allow a rapid estimation of drug exposure to optimize efficacy of treatment.

Numerous analytical studies have been reported for the determination of levo or the racemic ofloxacin in various biological fluids [4–6], including a broad spectrum of analytical techniques [4–13]. A survey of these papers revealed that some of them are quite complex and lengthy, mostly because of the sample preparation, like solid phase extraction [5,7], liquid–liquid extraction [10], protein precipitation combined with time consuming centrifugation steps [4,6,11] or expensive automated sample analysis requiring column switching [12]. These methods are not appropriate for routine use in clinical pharmacology, because the necessary equipment is not common, sample preparation used to be tedious or selectivity and stability were not sufficient.

In this report, a previously published HPLC assay for the determination of moxifloxacin (moxi) in serum and pancreas tissue [14], which used ofloxacin as internal standard, was adapted to allow the simple and rapid determination of levo in plasma and dialysate, using moxi as internal standard. Since the assay was also aimed to serve for pharmacokinetic studies of levo during continuous haemodialysis measurement of the drug in dialysate was necessary. Because dialysate is an uncommon medium and contains various electrolytes in high concentrations, stability of both drugs in this medium was evaluated extensively. In vivo the method was used to monitor plasma concentration in patients hospitalized in the clinic of internal medicine of the University of Rostock. Validation was performed according to the FDA Guidance for Industry: Bioanalytical Method Validation for Human Studies [15].

* Tel.: +49 381 4945784; fax: +49 381 4945782.
E-mail address: Saskia-Siewert@web.de.

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2. Experimental

2.1. Instrumentation

In agreement with the moxi assay [14], the HPLC–instrumentation consisted of a Shimadzu HPLC 10 A system, comprising a thermostated column compartment, a quaternary pump, a degasser, a system controller, an auto injector with sample cooler and spectrofluorometric detector. Data integration was performed using the CLASS LC 10® software (Shimadzu Europe GmbH, Duisburg, Germany). Chromatographic separation occurred at 20 °C on an YMC Pro C18 150 mm × 2 mm column (120 A, 5 μm particle size) with a 10 mm × 2 mm guard column, filled with the same material.

2.2. Chemicals

Pure levo and moxi were kindly provided from Aventis Pharmaceuticals (Frankfurt, Germany) and Bayer Vital GmbH (Leverkusen, Germany). Methanol and water in HPLC gradient grade were obtained from Merck (Darmstadt, Germany). Concentrated trifluoroacetic acid, acetic acid and ammonium acetate were purchased from Mallinckrodt Baker B.V. (Deventer, Netherlands) and Fluka Chemie GmbH (Buchs, Switzerland), respectively. SH 04 solution, used for dialysis and the calibration standards, was obtained from B. Braun Medizintechnologie GmbH (Melsungen, Germany) and composed as follows: natrium 138 mmol/l, kalium 2 mmol/l, calcium 2 mmol/l, magnesium 0.75 mmol/l, chloride 112 mmol/l and lactate 34 mmol/l.

2.3. Chromatographic conditions

The composition of the mobile phase and the determinants of the gradient were incurred as published [14]. Flow rate was set at 0.25 ml/min. Detection was performed using a fluorescence detector set up to an excitation wavelength of 296 nm and an emission wavelength of 504 nm with attenuation 5.

2.4. Preparation of standards and quality controls

Stock solutions of 1 mg/ml were prepared for levo and moxi as well. For levo, separate stock solutions for calibration samples and quality controls were used. By spiking drug-free human plasma and SH 04 dialysate with working solution, calibration standards in the concentration range of 0.1–6 μg/ml for plasma and 0.1–5 μg/ml for dialysate were obtained. Quality controls were prepared in the same way, using the second stock solution of levo, to yield concentrations of 0.1, 0.2, 3 and 5 μg/ml. Concentration of the internal standard moxi was 6 μg/ml in plasma and 3 μg/ml in dialysate.

2.5. Sample preparation

For the analysis of the plasma samples, 50 μl aliquots of the samples were vortex-mixed for 30 s with 60 μl plasma, containing the internal standard moxi, and 10 μl water. Twenty-five microlitres trifluoroacetic acid 50% (freshly prepared) were added and after another mixing step the solution was centrifuged at 6000 × *g* for 4 min. A 100 μl volume of the supernatant was added to 26 μl ammonium acetate buffer (5 M) to achieve a pH of 3.6. For dialysate 50 μl of the sample were vortex-mixed with 50 μl of a solution of the internal standard in SH 04 and then directly injected into the HPLC system. For both matrices calibration standards were treated in the same way as described, using 50 μl aliquots of the standards.

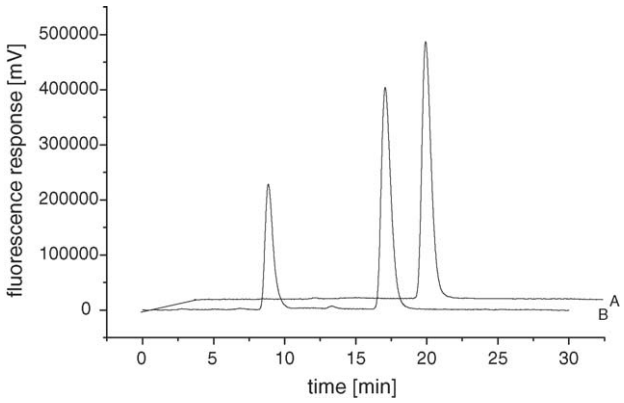

Fig. 1. Chromatogram of plasma: (A) plasma of a patient before administration of levo and (B) 24 h after administration of levo (1.1 μg/ml), co-medication of the patient: digitoxin, spironolactone, furosemide, celecoxib, metoprolol.

3. Results and discussion

The chromatographic separation of levo and moxi is demonstrated in Fig. 1, showing a representative chromatogram of a patient, receiving co-medication, before and after administration of levo. Retention time (t_R) in plasma as well as in dialysate was 8.3 min for levo and 16.7 min for moxi, respectively.

Several HPLC assays have been published to determine levo in plasma [5,6,10–13]. Not all of these assays are appropriate for routine monitoring of levo concentration to adjust drug dosage or pharmacokinetic studies. The used method has the advantage to be simple and rapid. Sample preparation is shortened to less than 5 min, requiring only a small sample volume (50 μl). Used HPLC equipment and mobile phases additives are cheap and widely common, thus the assay can easily applied in other laboratories.

A new aspect is the determination of levo in dialysate. In contrast to plasma, methods for the measurement of levo in this medium are sparse and refer mostly to the microdialysate technique [13], without reporting validation data.

3.1. Validation

The method was validated according to the FDA Guidance and satisfies all requirements for bioanalytical method validation [15]. Selectivity was evaluated in plasma samples received from patients within the scope of therapeutic drug monitoring (TDM) and in drug-free dialysate medium. For both matrices no interfering peaks were observed. Recovery determined from the quality controls ranged from 97.2 to 104.7% for both matrices, respectively. Linearity was demonstrated over a range from 0.1 to

Table 1
Intra- and inter-day precision (CV, %) and accuracy (R.S.D., %) for levo in plasma and dialysate

C_{nom} (μg/ml)	Intra-day ($n=7$)		Inter-day ($n=15$)	
	CV (%)	R.S.D. (%)	CV (%)	R.S.D. (%)
Plasma				
0.1	2.83	6.02	7.24	9.96
0.2	1.50	8.71	4.97	5.62
3	0.89	5.69	3.67	1.77
5	0.45	−3.60	4.12	3.64
Dialysate				
0.1	3.71	19.45	12.27	9.99
0.2	1.95	4.82	4.67	0.44
2.5	0.52	3.24	5.88	0.44
5	0.36	0.6	5.29	−0.47

6 μg/ml for plasma and 0.1–5 μg/ml for dialysate; fitting the concentrations found in patients after oral or i.v. therapeutic doses of levo. Correlation coefficients received from regression analysis of the calibration curves ranged from 0.9994 to 0.9999. Lower limit of the calibration range represented the LLOQ (0.1 μg/ml) and allowed measurement of concentration down to the minimal inhibitory concentration (MIC_{90}) of relevant pathogens. Intra- and inter-day accuracy and precision for the LLOQ and the other quality control samples are demonstrated in Table 1. Except the LLOQ, overall mean precision and accuracy are ≤10%. Thus, the assay is reliable, reproducible and accurate. Stability was evaluated for the stock solutions over 14 days at 4 °C (plasma: 94.18–108.43%; dialysate: 96.9–108.43%), at room temperature for 12 h (varying between 99.22 and 104.65% for plasma and 93.75 and 101.14% for dialysate) and for 15 h in the autosampler after sample preparation (99.67–102.65% of the nominal plasma concentration and 99.5–100.3% of the concentration set up in dialysate). Values determined for the freeze thaw stability ranged from 91.8 to 105.79% for plasma and from 99.4 to 102.69% for dialysate. Long-term stability at −20° was established for 6 months in plasma and for 3 months in dialysate.

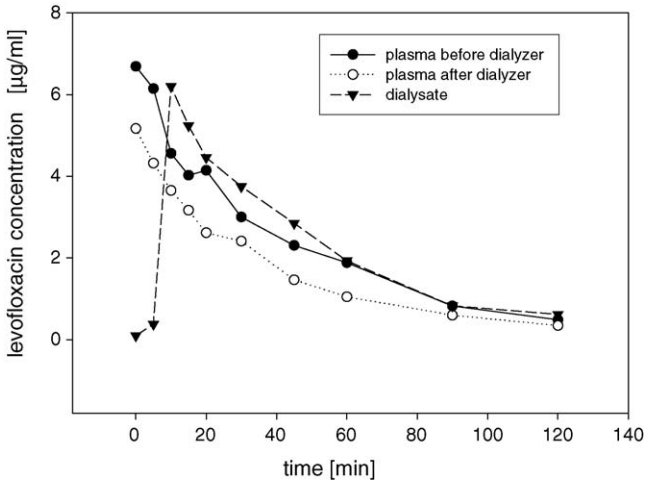

Fig. 2. Mean levofloxacin concentration during continuous veno-venous hemodialysis: the closed circles represent the plasma concentration in front of the dialyzer (●), the empty circles the plasma concentration after passing the dialyzer (○) and the triangles represent levofloxacin concentration in dialysate (▲).

3.2. Application

The validated method was first applied to an in vitro study of the elimination of levo by continuous veno-venous hemodialysis (CVVHD). A representative concentration–time curve of levo during CVVHD in the diverse matrices is shown in Fig. 2. In vivo the assay was used for the purpose of a therapeutic drug monitoring of levo as demonstrated in Fig. 1.

4. Conclusion

The used method involves a rapid, specific, reliable assay for the determination of levo in plasma and dialysate. Sample preparation is quick and cheap, therefore the method permits the analysis of a large number of samples. Frequently co administered drugs did not interfere the assay. Application of the method to pharmacokinetic studies and patient samples has been successfully. Thus, the assay is suitable for routine use in clinical pharmacology and therapeutic drug monitoring.

References

[1] G.M. Eliopoulos, C.B. Wennersten, R.C. Moellering Jr., Diagn. Microbiol. Infect. Dis. 25 (1996) 35–41.
[2] D.S. North, D.N. Fish, J.J. Redington, Pharmacotherapy 18 (1998) 915–935.
[3] G.L. Drusano, S.L. Preston, C. Fowler, M. Corrado, B. Weisinger, J. Kahn, J. Infect. Dis. 189 (2004) 1590–1597.
[4] S. Bottcher, H. von Baum, T. Hoppe-Tichy, C. Benz, H.G. Sonntag, J. Pharm. Biomed. Anal. 25 (2001) 197–203.
[5] S. Djabarouti, E. Boselli, B. Allaouchiche, B. Ba, A.T. Nguyen, J.B. Gordien, J.M. Bernadou, M.C. Saux, D. Breilh, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 799 (2004) 165–172.
[6] C. Immanuel, A.K. Hemanth Kumar, J. Chromatogr. B Biomed. Sci. Appl. 760 (2001) 91–95.
[7] M. Hernandez, F. Borrull, M. Calull, Electrophoresis 23 (2002) 506–511.
[8] J.A. Ocana, M. Callejon, F.J. Barragan, Analyst 125 (2000) 1851–1854.
[9] O. Ballesteros, J.L. Vilchez, A. Navalon, J. Pharm. Biomed. Anal. 30 (2002) 1103–1110.
[10] F.A. Wong, S.J. Juzwin, S.C. Flor, J. Pharm. Biomed. Anal. 15 (1997) 765–771.
[11] H. Liang, M.B. Kays, K.M. Sowinski, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 772 (2002) 53–63.
[12] H.A. Nguyen, J. Grellet, B.B. Ba, C. Quentin, M.C. Saux, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 810 (2004) 77–83.
[13] F.C. Cheng, T.R. Tsai, Y.F. Chen, L.C. Hung, T.H. Tsai, J. Chromatogr. A 961 (2002) 131–136.
[14] R. Wacke, S. Park, R.G. Mundkowski, N. Block, A. Kuhn-Thiel, B. Drewelow, Chemotherapy 49 (2003) 167–171.
[15] FDA, http://www.fda.gov/cder/giudance/4252fnl.pdf, 2001.