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Short communication

Determination of loratadine in human plasma by high-performance liquid chromatographic method with ultraviolet detection

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Abstract

A HPLC–UV determination of loratadine in human plasma is presented. After simple liquid–liquid extraction with 2-methylbutane–hexane (2:1) and evaporation of organic phase the compounds were re-dissolved in 0.01 *M* HCl, evaporated again and finally separated on a Supelcosil LC-18-DB column. The analyses were done at ambient temperature under isocratic conditions using the mobile phase: CH_3CN–water–0.5 *M* KH_2PO_4–H_3PO_4 (440:480:80:1, v/v). UV detection was performed at 200 nm with a limit of quantification of 0.5 ng/ml. The precision was found to be satisfactory over the whole range tested (0.5–50 ng/ml) with relative standard deviations of 2.3–6.3 and 5.2–14.1% for intra- and inter-assays, respectively.

Keywords: Loratadine

1. Introduction

Loratadine (LORA) is an antihistamine drug used as first-line agent for the treatment of urticaria and allergic rhinitis [1]. The drug is rapidly absorbed and extensively metabolised in the liver and descarboethoxyloratadine (DCL) is the major metabolite also possessing pharmacological activity similar to the parent drug [1,2]. The formation of DCL is proceeds through two isoforms of cytochrome P450 namely CYP2D6 and CYP3A4 resulting in genetic polymorphism [1,3,4]. Thus, great individual variability in LORA concentration occurs among patients. LORA is generally well tolerated having considerable advantages over older antiallergic drugs [1,4,5]. It is widely used and generic formulations are also introduced. A sensitive and simple LORA determination method for bioavailability studies and for therapeutic drug monitoring is then expected. Several techniques have been reported for LORA quantification in plasma samples; radioimmunoassay [2], gas chromatography (GC) [6,7] and high-performance liquid chromatography (HPLC) [8]. The limit of quantification (LOQ) has been reached at a level of 0.1–0.5 ng/ml. To our knowledge no HPLC method with commonly used UV detection is available for LORA determinations at therapeutic concentrations in plasma. A method for analysis of LORA and DCL in human plasma has been reported by Zhong and Blume [8] using fluorescence detection with an LOQ of 0.5 ng/ml, but unfortunately the internal standards were produced especially for that purpose, which complicates the analytical procedure. Also there

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were no data describing the yield of extraction and only limited data on precision and accuracy.

This paper presents a simple and sensitive procedure for LORA determination in human plasma suitable for therapeutic drug monitoring, pharmacokinetics, successfully applied to bioavailability studies.

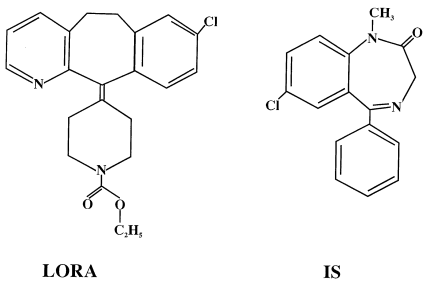
2. Experimental

2.1. Chemicals

The pure substance of LORA was from Glaxo-Wellcome (Poznań, Poland). Diazepam (internal standard, I.S.), was obtained from Polfa (Warsaw, Tarchomin, Poland). Chemical structures are presented in Fig. 1. A stock solution of LORA (1 mg/ml) was prepared in methanol and a stock solution of I.S. (1 mg/ml) prepared in acetonitrile; the solutions were stable for 3 months when stored at 4°C. HPLC-grade acetonitrile and methanol were from Promochem (Wesel, Germany), 2-methylbutane was from Sigma–Aldrich (Gillingham, UK), hexane, water, Na_2CO_3, KH_2PO_4 and H_3PO_4 were obtained from BDH (Poole, UK). All other chemicals were of analytical grade.

2.2. Chromatography

The HPLC isocratic system (Thermo Separation Products, San Jose, CA, USA) consisted of a pump (P100), an injector with a 100-μl loop (Model 7125; Rheodyne, Cotati, CA, USA), a UV detector (UV150) and an integrator (ChromJet 4400). The separation of compounds was made on a Supelcosil LC-18-DB (250×4.6 mm, 5 μm) column protected with a Supelguard LC-18-DB (20×4.6 mm, 5 μm) pre-column (Supelco, Bellefonte, PA, USA) at ambient temperature. The mobile phase was a mixture of CH_3CN–water–0.5 *M* KH_2PO_4–H_3PO_4 (440:480:80:1, v/v, pH 3.0) pumped at a flow-rate of 1.8 ml/min. Detection was set at a wavelength of 200 nm.



LORA IS

Fig. 1. Chemical structures of loratadine (LORA) and diazepam (I.S.).

2.3. Sample preparation

A 1-ml volume of plasma was transferred to a 10-ml Pyrex glass test tube, then mixed with 25 ng (20 μl) of diazepam methanolic solution (I.S.) and with 500 μl of 10% Na_2CO_3 solution. Next, a 2-ml aliquot of extraction medium: 2-methylbutane–hexane (2:1) was added and the sample was vigorously shaken for 4 min. After centrifugation (3000 *g*) for 5 min and freezing at −20°C the organic layer was quantitatively transferred to a 10-ml Pyrex conical glass centrifuge tube and evaporated to dryness in a water bath at 56°C under a stream of argon. Then 200 μl of 0.01 *M* HCl was added and the tube was carefully vortex-mixed within 30 s, next 180 μl of obtained solution was quickly pipetted into a second 10-ml Pyrex conical glass centrifuge tube and evaporated to dryness in a water bath at 56°C under the stream of argon. Then the dried extract was reconstituted in 125 μl of a mobile phase and a 100-μl aliquot was injected onto the column.

2.4. Calibration

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution in methanol. Working solutions were added to drug-free plasma to obtain the LORA concentration levels of 0.5, 2, 5, 10, 20, 50 ng/ml. The following procedure was as described above for sample preparation. The method was fully validated.

3. Results

3.1. Separation

LORA and I.S. were well separated from the biological background under the described chromato-

graphic conditions at retention times of 4.1 and 4.7 min, respectively (Fig. 2). The peaks were of good shape, completely resolved one from another in therapeutic concentrations of LORA (resolution factor R_s=1.10). No interference with constituents from plasma matrix was observed. The mobile phase used guaranteed good repeatability of retention times. Plasma samples injected directly after first evaporation step were contaminated with endogenous substances – which required subsequent clean up. Classic re-extraction did not give satisfactory results mainly because of still interfering plasma constituents and also of some technical problems with complete separation of two phases. Surprisingly, good results were obtained when dried extract after evaporation was simply dissolved in diluted acid. Such a procedure allows one to leave the majority of impurities on the probe walls.

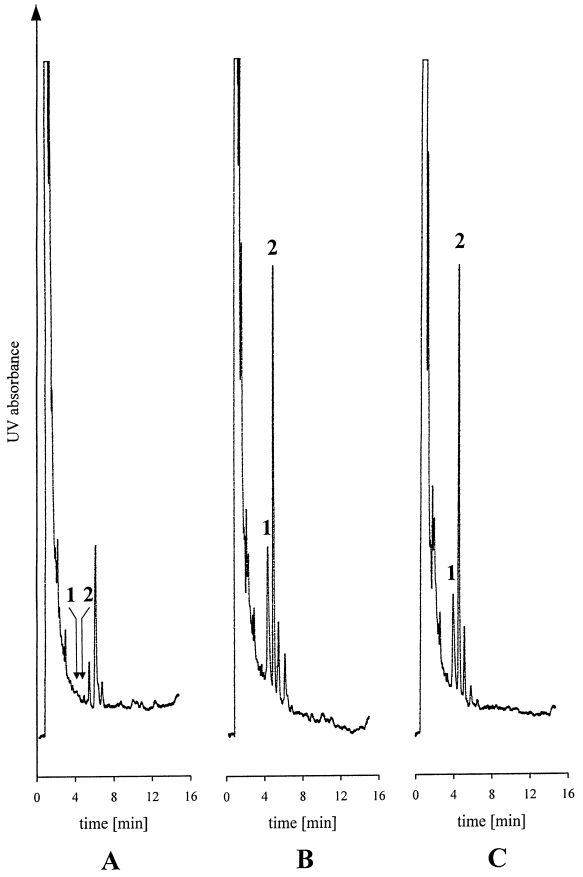


Fig. 2. Chromatograms of plasma samples analyzed as described in Experimental (attenuation 8): (A) drug-free plasma, (B) drug-free plasma spiked with LORA to obtain the concentration of 10 ng/ml, (C) plasma sample taken from the patient treated with loratadine containing 7.7 ng/ml of LORA. LORA, 4.1 min (1), I.S., 4.7 min (2).

LORA concentration in patients' samples is about a few ng/ml, thus, the analytical wavelength was selected according to maximal LORA absorbance with respect to stable baseline.

3.2. Calibration

The calibration curve was obtained by analyzing six samples for each of six tested concentrations (two samples for 0.5 ng/ml, one for 2 ng/ml and one for 10 ng/ml were not included for computation because of contamination). The curve was linear in whole range tested (0.5–50 ng/ml) and described by following equation: $y=0.052710x-0.019376$ ($r=0.999861$, $r^2=0.999721$).

3.3. Extraction

LORA is not easily extracted from biological matrices using common extraction solvents. The best yield of extraction (70–80%) was obtained with 2-methylbutane, nevertheless it is extremely volatile so difficult to use alone. The addition of one third of hexane decreased the extraction efficiency insignificantly (to about 70%). Neither increase of extraction mixture volume and/or extraction time nor repeating the extraction increase the yield of the process greater than 5%. The absolute extraction recovery was analyzed by comparing the peak areas for extracted calibration standards with those obtained from direct injection of equivalent quantities of standards (after evaporation and reconstitution in mobile phase) and I.S. working solution. The yield of extraction was 70.89±2.88% (66.38–74.82) for LORA being stable for the concentrations covering the calibration ranges (data in parentheses) and 57.93±3.72% (n=32) for the I.S.

3.4. Validation

The precision of the method was examined using the data from calibration for intra-assay and analyzing standard samples on different days for inter-assay precision. The relative standard deviation (RSD) for

Table 1
Precision of the method[a]

Concentration added LORA (ng/ml)	Intra-assay			Inter-assay		
	Factor determined[b]	RSD (%)	*n*	Factor determined	RSD (%)	*n*
0.5	0.0269±0.0009	3.45	4	0.0260±0.0037	14.11	6
2	0.097±0.0061	6.28	5	0.092±0.0112	12.21	10
5	0.240±0.0125	5.22	6	0.228±0.0150	6.57	11
10	0.488±0.0223	4.59	5	0.474±0.0276	5.83	10
20	1.017±0.0410	4.04	6	0.997±0.0517	5.18	11
50	2.627±0.0598	2.28	6	2.455±0.1779	7.25	11

[a] The data for precision are expressed as a mean±S.D.
[b] Factor determined – the ratio of peak area of LORA to peak area of internal standard.

LORA determinations was below 6.3% for intra-assay. The accuracy computed from intra-assay data as well as detailed information on precision is included in Tables 1 and 2.

The limit of detection (signal-to-noise ratio 3:1) was experimentally set at 0.1 ng of LORA standard injected onto the column, which corresponds to about 0.175 ng/ml of serum sample. The limit of quantification was also taken experimentally as the lowest concentration on calibration curve and set at 0.5 ng/ml. The range of the method is then 0.5–50 ng/ml.

3.5. Quality control and clinical application

Quality control samples were prepared fresh daily and analyzed as described. Average results and accuracy during 2 months of clinical application (Fig. 3) of this method lay between 98.09 and 98.85% of target values: 1.96±0.187 ng/ml (accuracy: −1.91%) for 2 ng/ml, $n=29$; 4.94±0.405 ng/ml (accuracy: −1.15%) for 5 ng/ml, $n=29$ and 9.88±0.824 ng/ml (accuracy: −1.23%) for 10 ng/ml, $n=29$.

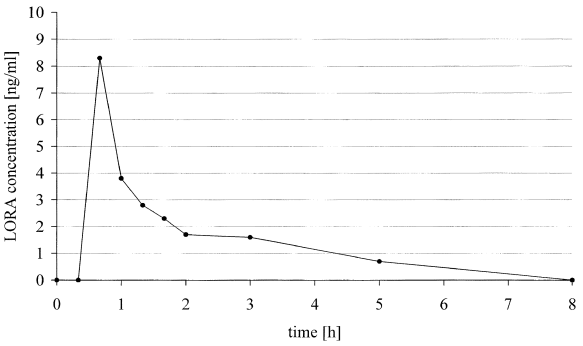


Fig. 3. Example of a concentration–time curve for LORA taken from a volunteer (23 years, 82 kg, male) to whom 20 mg (two tablets) of LORA was given orally.

4. Discussion

The described method was established as an analytical tool in bioequivalence study which requiring high precision and LOQ at least 0.5 ng/ml. The parameters of the assay obtained in course of validation processes presented above in Results section were considered as satisfactory for its clinical appli-

Table 2
Accuracy of the method[a]

Concentration added LORA (ng/ml)	Concentration determined LORA (ng/ml)	Accuracy (%)
0.5	0.52	+4.99
2	1.88	−5.85
5	4.68	−6.38
10	9.36	−6.40
20	19.28	−3.60
50	46.93	−6.14

[a] Concentration determined was calculated using calibration curve data.

cation. Uncomplicated two-steps analytical procedure based on simple liquid–liquid extraction followed by re-dissolving and evaporation gives clean probe for HPLC with satisfactory recovery. The purity of sample injected is confirmed by surprisingly long life of pre-column which allows to measure more than 500 samples (injected volume=100 μl!). The only disadvantage caused by very short analytical wave (200 nm) and extraction procedure is that sometimes the system needs to be rinsed after more or less 20 injections from very late eluting impurities.

Instead of LORA chemical analog used as internal standard by Zhong and Blume [8], commonly available diazepam was introduced to presented method. Its recovery although not very impressive (58%) was stable enough among the samples. Fluorescence detection is generally more sensitive than UV however, by minimizing sample dilution during preparation procedure and decreasing the analytical wavelength we are able to reach a comparable level for the LOQ.

In conclusion, this isocratic HPLC–UV method for loratadine in human plasma may be an economic analytical tool recommended for therapeutic drug monitoring, pharmacokinetic analysis and bioequivalence studies.

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References

[1] M. Haria, A. Fitton, D.H. Peters, Drugs 48 (1994) 617.
[2] J. Hilbert, E. Radwanski, R. Weglein, V. Luc, G. Perntesis, S. Symchowicz, N. Zampaglione, J. Clin. Pharmacol. 27 (1987) 694.
[3] N. Yumibe, K. Huie, K.-J. Chen, M. Snow, R.P. Clement, M.N. Cayen, Biochem. Pharmacol. 51 (1996) 165.
[4] M.A. Gonzalez, K.S. Esles, Int. J. Clin. Pharmacol. Ther. 36 (1998) 292.
[5] P.B. Van Cauwenberge, Drug Invest. 4 (1992) 283.
[6] J. Martens, J. Chromatogr. B 673 (1995) 183.
[7] R. Johnson, J. Christensen, C.-C. Lin, J. Chromatogr. B 657 (1994) 125.
[8] D. Zhong, H. Blume, Pharmazie 49 (1994) 736.