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Sensitive liquid chromatography–tandem mass spectrometry method for the determination of loratadine and its major active metabolite descarboethoxyloratadine in human plasma

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Abstract

A sensitive method for the simultaneous determination of loratadine and its major active metabolite descarboethoxyloratadine (DCL) in plasma was developed, using high-performance liquid chromatographic separation with tandem mass spectrometric detection. The samples were extracted from plasma with toluene followed by back-extraction into formic acid (2%) for DCL after which the toluene containing the loratadine was evaporated, the analyte reconstituted and combined with the DCL back-extract. Chromatography was performed on a Phenomenex Luna C_{18} (2) 5-μm, 150×2.1-mm column with a mobile phase consisting of acetonitrile–0.1% formic acid using gradient elution (10 to 90% acetonitrile in 2 min) at a flow-rate of 0.3 ml/min. Detection was achieved by a Perkin-Elmer API 2000 mass spectrometer (LC–MS–MS) set at unit resolution in the multiple reaction monitoring mode. TurboIonSpray ionisation was used for ion production. The mean recovery for loratadine and descarboethoxyloratadine was 61 and 100%, respectively, with a lower limit of quantification at 0.10 ng/ml for both the analyte and its metabolite. This is the first assay method described for the simultaneous determination of loratadine and descarboethoxyloratadine in plasma using one chromatographic run. The method is sensitive and reproducible enough to be used in pharmacokinetic studies.

Keywords: Validation; Loratadine; Descarboethoxyloratadine

1. Introduction

Loratadine, ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo [5,6]-cycloheptal[1,2-*b*]-pyridin-11-ylidine)-1-piperidinecarboxylate is a selective peripheral histamine H_1-receptor antagonist devoid of any substantial effects on the central and autonomic nervous system [1–3]. Loratadine is extensively metabolised in the liver to an active metabolite, descarboethoxyloratadine (DCL), which is then further oxidized to several products [1]. The elimination half-life of loratadine is 8–14 h, and that of DCL 17–24 h [4]. An oral dose of loratadine (20 mg) leads to maximum plasma concentrations of ~11 and 10 ng/ml for loratadine and DCL, respectively, which stresses the need for a sensitive assay method for pharmacokinetic studies [4].

Different methods (radioimmunoassay, high-performance liquid chromatography, gas chromatog-

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raphy, gas chromatography–tandem mass spectrometry) exist for the determination of loratadine in plasma with lower limits of quantification (LLOQs) ranging from 0.1 to 0.6 ng/ml [4–7]. Due to the differences in their pK_a values and polarities it is difficult and time consuming to chromatograph both the analyte and the metabolite in a single chromatographic run. The GC method of Johnson et al. [6] describes the determination of DCL and loratadine during separate chromatographic runs. Zhong and Blume [8] also describe a simultaneous assay for loratadine and DCL but determined the two analytes on two separate HPLC systems. Recently a liquid chromatography (LC)–tandem mass spectrometry (MS–MS) method was described for the separation of loratadine, DCL and some more polar metabolites of loratadine in one chromatographic run [9]. The method is however more suitable for qualitative work as the long run times (50 min) are not favourable for high-throughput quantitative analysis. This report describes a high-throughput quantitative method which involves the use of a mass-selective detector with MS–MS capabilities in tandem with gradient LC for the simultaneous determination of loratadine and DCL in human plasma.

2. Experimental

2.1. Materials and chemicals

A Phenomenex Luna C_{18} (2) 5-μm, 2.1×150-mm column (Phenomenex, Torrance, CA, USA) was used for separation at a flow-rate of 0.3 ml/min after injecting 30 μl of the extract onto the column. The mobile phase was delivered by a Hewlett-Packard Series 1100 quaternary pump (Hewlett-Packard, Palo Alto, CA, USA) and the samples injected by a Perkin-Elmer Series 200 autoampler. Detection was performed by a Perkin-Elmer Sciex API-2000 detector (Perkin-Elmer Sciex, Ont., Canada) using TurboIonSpray ionisation (ESI) for ion production.

Formic acid (high purity) was obtained from BDH (Poole, UK), toluene and acetonitrile (Burdick and Jackson, high purity) were obtained from Baxter, USA, and sodium hydroxide (analytical-reagent grade) from Fluka (Buchs, Switzerland). All chemicals were used as received. Water was purified by RO 20SA reverse osmosis and Milli-Q polishing system (Millipore, Bedford, MA, USA).

Loratadine, $C_{22}H_{23}ClN_2O_2$, and DCL, $C_{19}H_{19}ClN_2$, were supplied by Quimica Sintetica. Fluoxetine and fluspirilene, external standards for DCL and loratadine, respectively, were taken from the Farmovs Research Centre internal pure substance reference material library.

2.2. Extraction procedure

Loratadine and DCL standard solutions were made up in methanol and used immediately to spike plasma and discarded thereafter. Calibration standards and quality control standards were prepared in normal human plasma by spiking a pool of normal plasma which was then serially diluted with normal blank plasma to attain the desired concentrations (0.10–20.2 ng/ml for loratadine and 0.10–20.3 ng/ml for DCL). The calibration standards and quality control standards were aliquoted into tubes and stored under the same conditions as the trial samples at −20°C. The extraction procedure consisted out of four main steps: simultaneous extraction of loratadine and DCL out of plasma into toluene (step 1); back-extraction of DCL into 2% formic acid (step 2); evaporation of the organic phase (containing only loratadine) followed by reconstitution of the analyte (step 3); and finally the combination of the two extracts (step 4).

2.2.1. Step 1

To 1 ml plasma in a 10-ml amber glass ampoule was added 100 μl sodium hydroxide (1 *M*) and 3 ml of toluene. The sample was vortex mixed for 2 min and centrifuged at 1300 *g* for 3 min at 8°C. The aqueous phase was frozen at −30°C on a Fryka Polar cooling plate (Kältetechnik, Esslingen) and the organic phase decanted into a clean 5-ml amber glass ampoule.

2.2.2. Step 2

To the organic phase was added 200 μl of a 2% formic acid solution containing the external standard for DCL (fluoxetine, 45.2 ng/ml). After vortex mixing for 2 min and centrifuging (1300 *g* for 3 min at 8°C) the aqueous phase (2% formic acid) con-

taining DCL and fluoxetine was frozen (−30°C on a Fryka Polar cooling plate) and the organic phase containing only loratadine was transferred to a clean 5-ml amber glass ampoule. The back-extract was thawed and any residual organic phase present was evaporated under a stream of nitrogen at 45°C for 1.5 min.

2.2.3. Step 3

The remaining organic phase from step 2, containing only loratadine, was evaporated under vacuum on a Savant SpeedVac (Savant Instruments, NY, USA) rotary evaporator at ambient temperature. The extract was reconstituted with 200 μl acetonitrile–0.1% formic acid (50:50, v/v) containing the external standard for loratadine (fluspirilene, 43.0 ng/ml) by vortex mixing for 1 min.

2.2.4. Step 4

Finally 100 μl of the back-extract obtained in step 2 was combined with 100 μl of the reconstituted extract obtained in step 3 in an autosampler vial insert and after brief vortexing, 30 μl was injected onto the HPLC column.

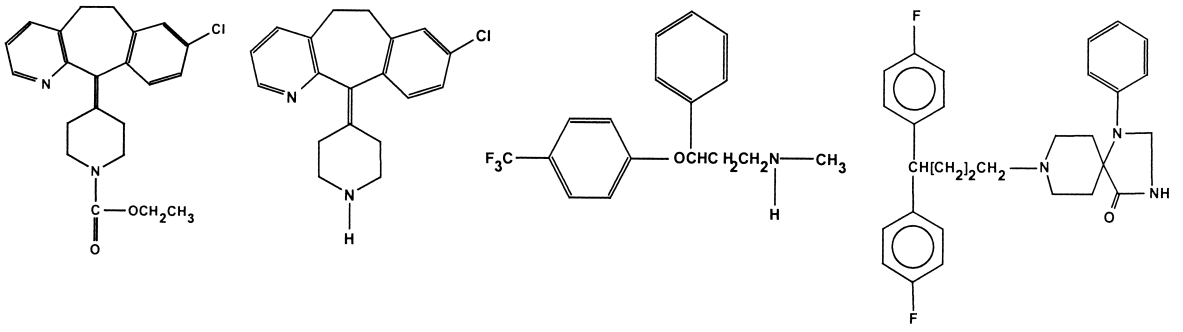
2.3. Liquid chromatography

All chromatographic solvents were sparged with helium before use. Chromatography was carried out at ambient temperature with a mobile phase consisting of acetonitrile–0.1% formic acid using gradient elution at a flow-rate of 0.3 ml/min. The acetonitrile content was changed following injection from 10 to 90% in 2 min and the final mixture maintained for 1 min. The mobile phase content was then immediately changed back to the pre-injection composition (acetonitrile–0.1% formic acid, 10:90, v/v) and maintained for 3.4 min giving a total run time of 6.4 min.

2.4. Mass spectrometry

Electrospray ionisation was performed in the positive ion mode with nitrogen as the nebulizing, turbo spray and curtain gas with the optimum values set at 80:50:55 (respective arbitrary values). The TurboIonSpray temperature was set at 400°C. The instrument response was optimised for loratadine, DCL, fluoxetine and fluspiriline by infusing a constant flow of a solution of the drugs dissolved in mobile phase into the stream of mobile phase eluting from the column. The pause time was set at 5 ms and the dwell time at 200 ms.

The Perkin-Elmer Sciex API 2000 LC–MS–MS detector was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions m/z 382.7, 311.1, 309.9 and 476.0 to the principal product ions m/z 336.8, 259.0, 43.6 and 98.1 (for loratadine, DCL, fluoxetine and fluspirilene, respectively). Figs. 1–4 show the single parent to product ions MS–MS for loratadine, DCL, fluoxetine and fluspirilene. The collision energies for loratadine, DCL, fluoxetine and fluspirilene were 27, 29, 33 and 27 V, respectively. ESI was used for ion production and the collision gas (N_2) set at 3 (arbitrary value). The instrument was interfaced to an Apple Macin-



Loratadine DCL Fluoxetine Fluspirilene

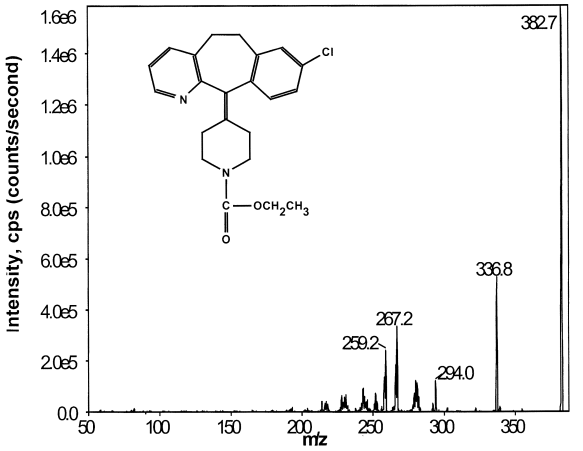


Fig. 1. Full mass spectrum of the protonated loratadine molecular ion (m/z 382.7, molecular structure given) and the principal product ion formed at m/z 336.8 after collision (MS–MS).

tosh computer running Perkin-Elmer MassChrom version 1.1 with MacQuan version 1.6 software.

2.5. Validation

The method was validated by analysing plasma quality control samples five times at eight different concentrations, i.e. 16.1, 8.95, 4.47, 1.12, 0.50, 0.38, 0.25 and 0.13 ng/ml for loratadine and DCL to determine the accuracy and precision of the method.

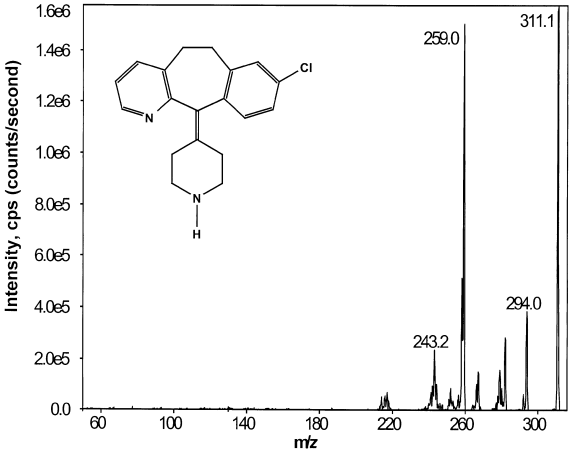


Fig. 2. Full mass spectrum of the protonated descarboethoxyloratadine (DCL) molecular ion (m/z 311.1, molecular structure given) and the principal product ion formed at m/z 259.0 after collision (MS–MS).

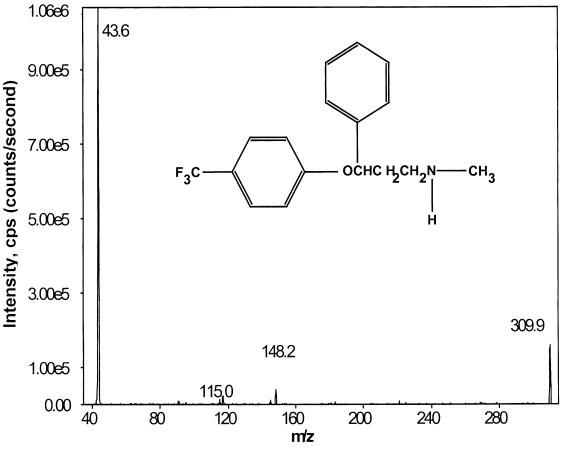


Fig. 3. Full mass spectrum of the protonated fluoxetine molecular ion (m/z 309.9, molecular structure given) and the principal product ion formed at m/z 43.6 after collision (MS–MS).

The quality control values were calculated from a standard regression curve containing nine different concentrations spanning the concentration range (20.2–0.10 ng/ml for loratadine and 20.3–0.10 ng/ml for DCL). Calibration graphs were constructed using a weighted linear regression (1/concentration^2) of the drug/external standard peak-area ratios of the product ions for loratadine, DCL and their respective external standards, versus nominal drug concentrations.

The matrix effect (coeluting, undetected endogenous matrix compounds that may influence the analyte

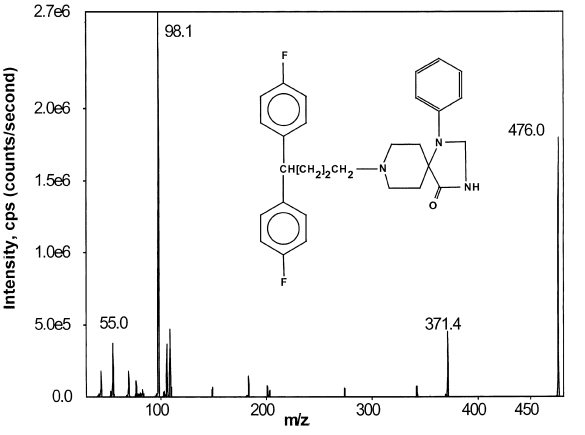


Fig. 4. Full mass spectrum of the protonated fluspirilene molecular ion (m/z 476.0, molecular structure given) and the principal product ion formed at m/z 98.1 after collision (MS–MS).

ionisation) was investigated by extracting 'blank' biological fluids from six different sources, reconstituting the final extract in mobile phase containing a known amount of the analyte, analysing the reconstituted extracts and then comparing the peak areas of the analytes.

Absolute recoveries of the analyte and metabolite were determined in triplicate in normal plasma by extracting drug free plasma samples spiked with loratadine and DCL. Recoveries were calculated by comparison of the analyte peak areas of the extracted samples with those of the unextracted system performance verification standards representing 100% recovery. The system performance verification standards were prepared by spiking blank extracts with known amounts of the analyte and metabolite.

3. Results and discussion

The mean absolute recovery of loratadine and DCL determined in triplicate at high, medium and low concentrations was 61 and 100%, respectively. No matrix effect for loratadine (RSD = 3.0%) and a marginal matrix effect for DCL (RSD = 8.9%) was observed for the six different plasma pools indicating that the extracts were 'clean' with very little to no undetected co-eluting compounds that could influence the ionisation of the analytes.

The LLOQ is defined as that concentration of loratadine and DCL which can still be determined with acceptable precision (RSD < 20%) and accuracy (bias < 20%) and was found to be 0.10 ng/ml for loratadine and its metabolite. Results from the intra-day validation assays indicate a valid calibration range of 0.10–20.2 ng/ml for loratadine and 0.10–20.3 ng/ml for the DCL metabolite. The intra and inter-day assay method performance statistics are presented in Tables 1–4.

On-instrument stability was inferred from special stability samples which were prepared and included in the validation batch. No significant degradation could be detected in the cooled samples (4°C) left on the autosampler for at least 8 h.

Due to the high specificity of MS–MS detection, no interfering or late eluting peaks were found when chromatographing blank plasma extracts from six different sources.

Table 1
Intra-day quality control results of loratadine

Nominal concentration (ng/ml)	Loratadine ($n = 5$), mean concentration found (ng/ml)	RSD (%)	% Nominal
16.1	15.1	4.6	94
8.95	8.12	7.0	91
4.47	4.51	3.6	101
1.12	1.13	8.2	101
0.50	0.54	9.1	108
0.38	0.44	10.5	115
0.25	0.28	10.3	113
0.13	0.14	6.4	108

Table 2
Intra-day quality control results of DCL

Nominal concentration (ng/ml)	DCL ($n = 5$), mean concentration found (ng/ml)	RSD (%)	% Nominal
16.1	15.2	5.4	94
8.95	8.08	6.3	90
4.47	4.20	8.3	94
1.12	1.09	5.7	97
0.50	0.50	6.4	100
0.38	0.35	6.5	93
0.25	0.23	12.0	93
0.13	0.14	12.1	109

Table 3
Inter-day quality control results of loratadine

	Nominal (ng/ml)				
	0.13	0.38	1.12	4.47	8.95
Mean	0.14	0.41	1.19	4.83	9.23
RSD	12.6	8.8	7.3	8.9	11.0
% Nominal	111	108	106	108	103
n	17	17	14	17	17

Table 4
Inter-day quality control results of DCL

	Nominal (ng/ml)				
	0.13	0.38	1.12	4.47	8.95
Mean	0.12	0.38	1.09	4.47	9.00
RSD	9.5	9.0	8.9	14.3	11.3
% Nominal	98	100	97	100	101
n	16	16	18	17	18

Several extraction procedures were tested to find a suitable method using a single extraction procedure for both loratadine and DCL, including solid-phase and liquid–liquid extraction methods using different organic solvents. Although both analytes could be extracted simultaneously from plasma into toluene, DCL (unlike loratadine) had a poor recovery and gave irreproducible results when the organic phase was evaporated and the extract reconstituted. It was therefore necessary to back-extract the DCL out of the toluene with 2% formic acid, before evaporation of the organic phase, after which the DCL back-extract was combined with the reconstituted loratadine extract to ensure optimum results. The different pK_a value and polarity of loratadine (to that of DCL) ensured that loratadine was not also back-extracted into the formic acid. Due to the un-availability of deuterated analogues (for loratadine and DCL) and suitable internal standards, external standards had to be used that eluted as close as possible to loratadine and DCL in order to minimise any potential matrix effects. Different concentrations of acetic acid, formic acid and ammonium acetate were tested for optimum ionisation of the analytes and 0.1% formic acid was found to give the best result.

Retention times were 5.18 and 4.47 min for loratadine and DCL, respectively, and 4.63 and 4.59 min for the external standards fluspirilene and fluoxetine, respectively. A total chromatography run time of 6.4 min was allowed, which made it possible to analyse batches of up to 150 samples per day on one instrument.

Fig. 5A and B shows representative chromatograms obtained of the calibration standard at the LLOQ for loratadine (A) and DCL (B) and of study samples close to the limit of quantification at the late elimination phase of the pharmacokinetic profiles for each analyte.

The method was employed to analyse plasma samples containing loratadine and DCL obtained after a single oral dose of 20 mg loratadine per treatment phase in 24 healthy volunteers. Concentration versus time profiles were constructed for up to 20 h for loratadine and 80 h for DCL. The maximum loratadine plasma concentrations obtained varied between 2.20 and 19.47 ng/ml and between 2.89 and 14.46 ng/ml for the DCL metabolite. Fig. 6 shows the average loratadine and DCL pharmacokinetic profiles in a study with 24 trial subjects (two phases) after each subject received a 20-mg oral dose of loratadine.

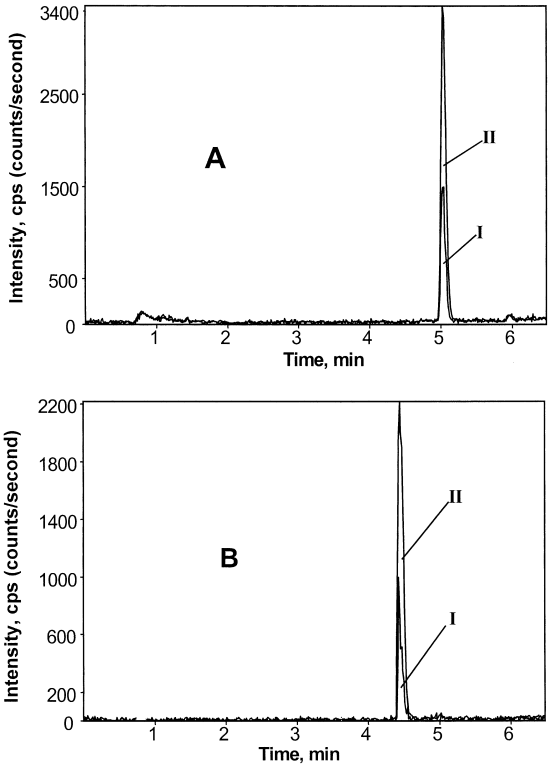


Fig. 5. High-performance liquid chromatograms of the calibration standard at the limit of quantification (I) containing 0.1 ng/ml loratadine (A) and 0.1 ng/ml descarboethoxyloratadine (DCL) (B) and of study samples near the limit of quantification (II) at the late elimination phase of the pharmacokinetic profiles for each analyte containing 0.27 ng/ml loratadine (A) and 0.28 ng/ml DCL (B).

In this method we made use of the increased sensitivity and selectivity of MS–MS detection which allowed us to use a harsh gradient to force both analytes to elute in one chromatographic run without the risk of interference. A possible problem with such an approach could be increased matrix effects which we found to be marginal in this method. The rapid gradient also allowed us to regenerate the column during the chromatographic run, the gradient was applied for 2 min, the solvent ratios maintained for 1 min and then immediately returned to the pre-injection status for 3.4 min. This leads to a much faster turnover time (7.4 min) per

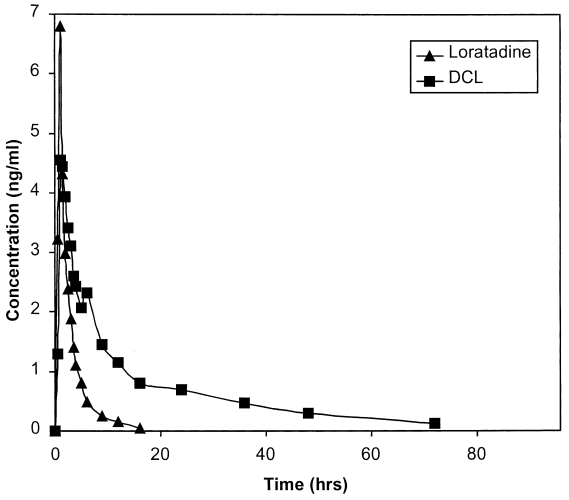


Fig. 6. Representative loratadine and descarboethoxyloratadine (DCL) plasma concentrations versus time profiles as obtained after a single 20-mg oral dose of loratadine (24 subjects, two phases).

sample which increases productivity. The gradient, especially the organic content, was also crucial for peak shape as even slight changes to the gradient ratios led to bad chromatography.

4. Conclusion

A highly sensitive and selective method for the simultaneous determination of loratadine and its major active metabolite DCL in plasma was developed, using high-performance liquid chromatographic separation with tandem mass spectrometric detection. Plasma concentrations of loratadine and DCL could be quantified from 0.10 to 20.2 and 20.3 ng/ml, respectively, making it possible to do pharmacokinetic studies after a single oral dose of 20 mg loratadine. The comparatively fast chromatographic time allows for high-throughput quantitative analysis, making the method more productive and thus more cost effective.

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