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Extractionless and sensitive method for high-throughput quantitation of cetirizine in human plasma samples by liquid chromatography–tandem mass spectrometry ☆

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Abstract

Following a single 10-mg oral dose of cetirizine dihydrochloride to 24 healthy volunteers, the analyte was quantified in human plasma. Protein precipitation using acetonitrile (ACN) was followed by reversed-phase liquid chromatography and tandem mass spectrometry. The MS/MS method was optimised using a PE Sciex API 2000 triple quadrupole mass spectrometer in selected reaction monitoring (SRM) mode, using electrospray with positive ionisation. Oxybutynin was used as the internal standard. The assay method represents a robust, high-throughput, highly specific and sensitive quantitative assay procedure, with 0.5 ng/ml being the lowest plasma concentration that could be reliably quantified. The procedure involves minimal sample preparation, and is well suited to clinical studies of the drug involving large numbers of generated samples. Pre-dose as well as post-dose samples up to and including 48 h were quantified, and the data generated were used to determine the pharmacokinetic profile of the drug.

Keywords: Cetirizine

1. Introduction

Cetirizine (2-[2-[4[(4-chlorophenol) phenylmethyl]-1-piperazinyl]ethyloxy] acetic acid) (Fig. 1), a member of the cyclizine class of compounds, is an H_1-receptor antagonist [1] and is an active metabolite of hydroxyzine, a first generation H_1-receptor antagonist. Cetirizine's marked affinity for peripheral histamine H_1 receptors results in antiallergic properties, but has the advantage that it lacks the CNS depressant effects often encountered in antihistamines [2]. This is owing to the fact that it is highly selective and has less affinity for calcium channel receptors, adrenergic α_1-, dopamine D_2-, serotonin5-HT_2 receptors and muscarinic receptors than do

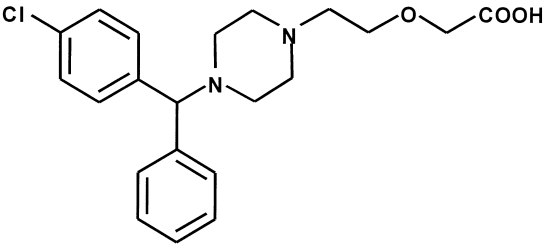

Fig. 1. Cetirizine.

☆Sample assays performed by FARMOVS-PAREXEL, clinical study executed in Ireland.

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other common antiallergic drugs. In recent times, cetirizine has been quantified by TLC [3] in a bioavailability study involving two oral formulations of cetirizine. In similar studies, reversed-phase HPLC with UV detection was employed [4–6]. Moreover, a GC method has been described [7] involving esterification of cetirizine. The present paper deals with a bioavailability study involving two oral formulations of cetirizine dihydrochloride, but to the authors' knowledge, is the first description of an LC–MS/MS assay method for the quantitation of cetirizine in human plasma. The chief benefit of the present method is the minimal sample preparation involved, as the procedure is a simple protein precipitation procedure involving acetonitrile, with oxybutynin as internal standard. Although liquid–liquid extraction procedures have most commonly been described [4,6], Moncrieff [5] also used a protein precipitation procedure, but using perchloric acid (this option was not investigated owing to the type of detector being used). Further benefits are improved sensitivity and specificity, which are chiefly due to instrumental advances, and in particular LC–MS/MS with electrospray ionisation.

The present LC–MS/MS method is well suited to studies generating large numbers of samples. In addition, much improved sensitivity was achieved, with a lower limit of quantification (LLOQ) of 0.5 ng/ml.

2. Experimental

2.1. Reagents and materials

Cetirizine was obtained from Cipla (Cipla Ltd., Mumbai). The internal standard, oxybutynin (Fig. 2) HCl, was supplied by SELOC (Limay, France). HPLC-grade acetonitrile and methanol (B&J brand™) were purchased from Baxter (Muskegon, MI, USA). Formic acid (BDH) was used without further purification. All water used was purified by RO 20SA reverse osmosis and Milli-Q® polishing system (Millipore, Bedford, MA, USA).

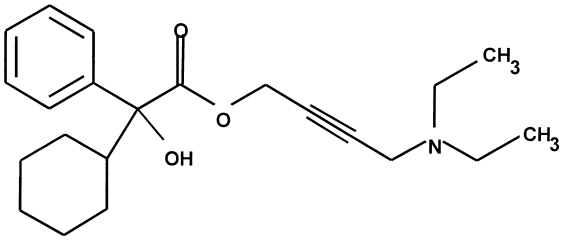

Fig. 2. Oxybutynin.

2.2. Preparation of standards

Calibration standards were prepared by dissolving cetirizine in methanol, and spiking 100 μl of this stock solution into a pool of blank plasma. This resulted in the highest calibration standard (808 ng/ml). This calibration standard was then serially diluted with blank plasma (1:1) 10 times, which resulted in a calibration standard range between 808 and 0.5 ng/ml. This entire calibration range was validated. Similarly, quality control standards were prepared (using the same methodology) spanning a range between 719 and 0.68 ng/ml. Sufficient calibration standards and quality controls were prepared to validate the method and assay all sample batches. These were stored together with the study samples at −20 °C until used for sample processing.

2.3. Sample preparation

To 100 μl plasma in a 1.5-ml Eppendorf® microfuge tube (Eppendorf, Hamburg, Germany) was added 200 μl acetonitrile containing oxybutynin (300 ng oxybutynin/ml acetonitrile). The samples were immediately vortexed for 20 s, and centrifuged at 5750 *g* for 10 min, using an Eppendorf® 5416 high-speed centrifuge. One hundred μl of the supernatant layer was transferred to an autosampler vial containing a glass insert and 8 μl injected onto the HPLC column. Automatic injection was done by a Perkin-Elmer series 200 autosampler, fitted with a Peltier cooling plate that cooled samples to 4 °C while awaiting injection. The chromatographic eluent was diverted to waste for 1.4 min after each sample injection in order to keep the ion source as clean as possible.

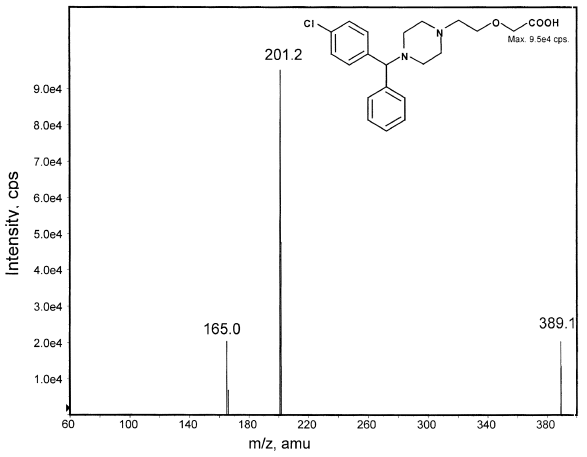

Fig. 3. Cetirizine spectra depicting the protonated molecular ion (m/z 389.1) and the predominant daughter ion (m/z 201.2).

3. Results and discussion

3.1. Optimization of instrumental and chromatographic conditions

Detection of cetirizine and oxybutynin (I.S.) was achieved using a PE Sciex API 2000 LC–MS/MS mass spectrometer (Perkin-Elmer, Foster City, CA, USA) using an electrospray (TurboIonSpray) ion source. Instrumental conditions for the SRM transition of the analyte (Fig. 3) and internal standard were automatically optimized using Analyst™ version 1.1 software. Instrumental conditions were optimised using a syringe pump infusion (5 μl/min). Infusions of cetirizine and internal standard were done in alkaline and acidic medium, and it was found that the analyte was able to act both as a proton donor and acceptor, which resulted in similar sensitivity. It was, however, decided to use positive ionisation owing to the fact that the internal standard responded best to positive ionisation.

High-flow gas flow parameters (curtain, nebuliser and heater gas) were optimised by making successive flow injections, while introducing mobile phase into the ionisation source at 200 μl/min. Instrumental parameters are summarised in Table 1.

Various combinations of acetonitrile, methanol, acetic acid and formic acid were investigated with a view to arriving at an optimal mobile phase. A number of combinations were found to be suitable and it was decided to use a mobile phase that was both simple to prepare, and resulted in minimal matrix effects.

The final mobile phase consisted of acetonitrile and a 0.2% (v/v) formic acid solution (44+56). The formic acid solution was prepared by adding 2 ml concentrated formic acid to 1998 ml water. The mobile phase was delivered at 0.25 ml/min by a Perkin-Elmer series 200 analytical pump onto a Phenomenex Luna® $C_8(2)$ 5μ, 150×2.1 mm analytical column used for compound retention (Phenomenex, Torrance, CA, USA). A $C_{18}(2)$ column by the same manufacturer was also investigated, which resulted in similar chromatography, but the $C_8(2)$ stationary phase was ultimately used owing to the slightly superior peak shape under the given conditions.

3.2. Analyte stability in matrix

Baltes et al. [7] report that cetirizine is stable in human plasma for at least one calendar month when stored at −20 °C. Response of the calibration standards included in sample batches relative to a freshly prepared system suitability standard confirmed these findings.

3.3. On-instrument stability

In order to ascertain on-instrument stability, a single prepared sample that had been extracted was

Table 1
LC–MS/MS instrumental parameters

Parameter	Analyte	I.S.
Curtain gas (arbitrary unit)	50	50
Nebuliser gas (arbitrary unit)	70	70
Heater gas (arbitrary unit)	70	70
Protonated molecule (m/z)	389.0	358.0
Dwell time (ms)	150	150
Product ion (m/z)	201.1	142.0
Declustering potential (V)	21	41
Focusing potential (V)	360	360
Entrance potential (V)	−7	−11
Collision cell entrance potential (V)	18	22
Collision energy (V)	25	33
Collision cell exit potential (V)	7.3	6.0

split and injected from eight different positions on the autosampler during the intra-day validation batch. From this, it was established that the cetirizine response increased by 2.74% and the internal standard by 4.39% over the 5.5 h during which these eight samples were injected. This slight increase in response is not uncommon in API sources but was compensated for by the concomitant increase in sensitivity for the internal standard, even though an isotopically labelled internal standard was not used.

3.4. Chromatography

The retention times of the analyte and internal standard were 1.87 and 1.90 min, respectively (Fig. 4).

3.5. Extraction efficiency

Determinations at high, medium and low plasma concentrations of cetirizine (360, 89.8 and 1.63 ng/ml, respectively) were done ($n=5$) in order to ascertain recovery of analyte in the supernatant layer, following sample preparation. This was done by comparing the response of extracted samples to the response of a standard solution (prepared in the supernatant layer resulting when blank plasma was precipitated with acetonitrile). It was found that recovery did not vary significantly over the range tested, and that the mean recovery of the cetirizine was 92%.

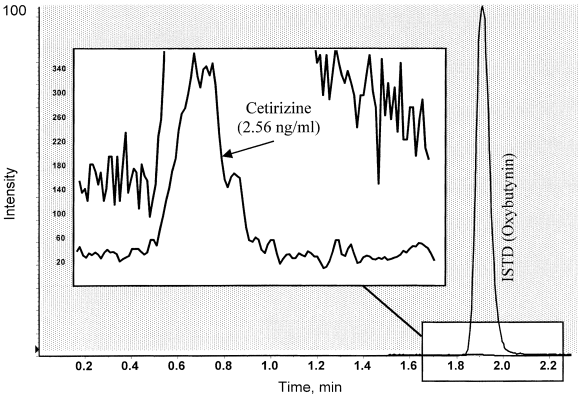

Fig. 4. Full-scale chromatogram of a calibration standard, showing analyte and internal standard. The insert shows an expanded view of the baseline, with the analyte peak at 2.56 ng/ml.

Similarly, the recovery of the internal standard was determined (10-fold) at a single concentration (184 ng/ml). It was found that the internal standard was fully recovered in the supernatant layer.

3.6. Sensitivity

Even though samples were not enriched, owing to the elementary sample preparation procedure, this assay method improved on the sensitivity of existing methods described in the literature. This is primarily due to the improved sensitivity afforded by the mass spectrometer. A plasma calibration standard of 0.5 ng/ml was reliably quantified with a C.V. of 10% ($n=12$) and a mean signal–noise ratio of 30 when injecting 8 μl onto the HPLC column.

3.7. Matrix effects

Matuszewsky et al. [8] report that undetected matrix components, which co-elute with analytes, may adversely affect the reproducibility of analyte ionisation in a mass spectrometer's electrospray source. For this reason, blank plasma was obtained from 10 different sources, and precipitated with acetonitrile containing cetirizine and the internal standard (these determinations were done at an effective concentration of 800 ng/ml). The C.V.% of the analyte and internal standard peak areas for these 10 determinations was calculated. From the calculated C.V. of 1.85% for the analyte and 2.60% for the internal standard, it was concluded that if the analyte and internal standard did co-elute with endogenous matrix components, this did not adversely affect the reproducibility of ionisation.

3.8. Method validation

Three method validations were performed. Each of these three batches included an 11-point calibration line and quality controls (included in sixfold) at five different levels of the calibration line. The calculated concentrations of the quality controls included in the three validation batches are summarised in Table 2.

Table 2
Summary of validation quality controls

	QC I (360 ng/ml)	QC H (180 ng/ml)	QC G (89.8 ng/ml)	QC B (1.63 ng/ml)	QC A (0.680 ng/ml)
Mean ($n=18$)	364	182	91.4	1.61	0.656
C.V.%	3.8	2.7	3.1	4.1	8.0

Table 3
Summary of sample batch quality controls

	QC I (360 ng/ml)	QC H (180 ng/ml)	QC G (89.8 ng/ml)	QC B (1.63 ng/ml)	QC A (0.680 ng/ml)
Mean ($n=12$)	362	181	93.9	1.58	0.693
C.V.%	4.6	3.5	2.5	5.7	5.0

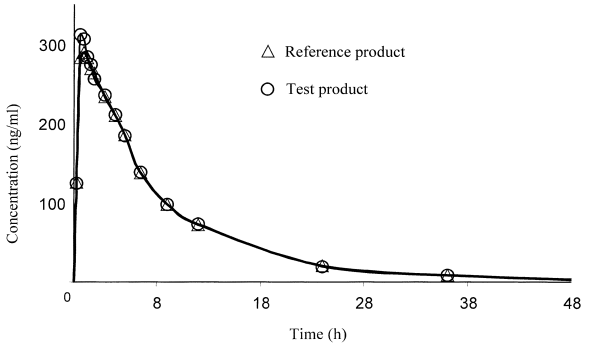

Fig. 5. Mean plasma concentration versus time profiles ($n=24$) following oral ingestion of 10 mg cetirizine dihydrochloride.

3.9. Batch processing

Following validation, 768 study samples were processed in six batches, each including a 10-point calibration line and 10 quality controls. A typical batch of ~160 samples (including calibrators) required 90 min of sample preparation time. Following sample preparation, samples were injected on the instrument within 8 h. Results of the quality controls included in the sample batches are summarised in Table 3.

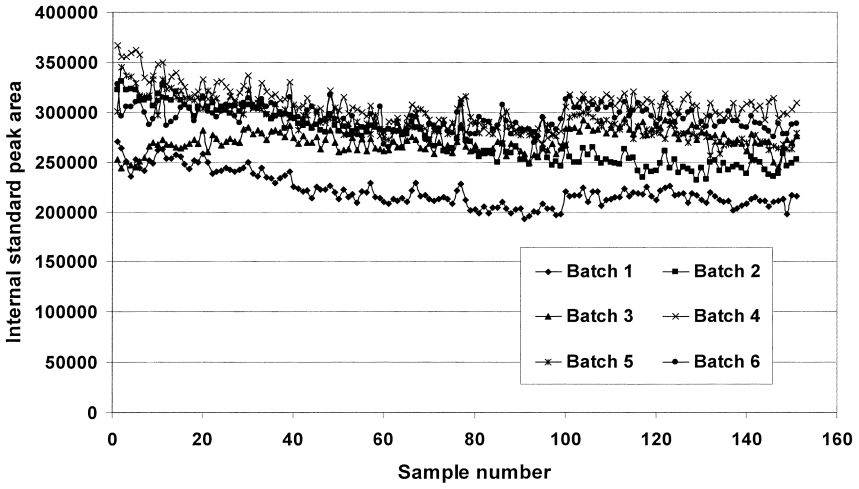

Fig. 6. Peak area of internal standard (oxybutynin) plotted against sample number in the 6 batches in which samples were processed, involving a 'dilute and shoot' sample preparation scheme. The first 1.4 min of chromatographic eluent was diverted to waste in order to keep the MS orifice as clean as possible.

Table 4
Summary of pharmacokinetic parameters

Parameter	*n*	Dose (mg)	C_{max} (ng/ml)	T_{max} (h)	AUC (ng ml^{-1} h)
Pandya et al.	8	10	390	2.2	$4462_{(0-24\ h)}$
Muscará and De Nucci	14	10	305	0.5	$2380_{(0-24\ h)}$
Baltes et. al	16	10	350	–	–
Present study	24	10	302	0.5	$2714_{(0-48\ h)}$

Parameters represent the average of two bioequivalent oral formulations of cetirizine.

3.10. Application

This newly developed assay method was used in a cross-over study to ascertain bioequivalence between two oral formulations of cetirizine after producing a plasma concentration versus time profile (Fig. 5). The samples generated during the said study were rapidly processed owing to the rapid sample preparation scheme and short chromatography time. Robust LC–MS/MS instrument performance was observed, with only slight variations in instrument response observed within batches (Fig. 6). The calculated pharmacokinetic parameters are in agreement with existing literature (Table 4), and the present method is an excellent analytical option for quantifying cetirizine in human plasma.

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