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SIMULTANEOUS DETERMINATION OF LORATADINE AND DESCARBOETHOXYLORATADINE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) – ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-MASS SPECTROMETRY (APCI-LC-MS) METHOD

ODREĐIVANJE LORATADINA I DEZKARBOETOKSILORATADINA U SERUMU PRIMENOM METODE TEČNE HROMATOGRAFIJE SPREGNUTE SA JONIZACIJOM POD ATMOSFERSKIM PRITISKOM I MASENO-SPEKTROMETRIJSKOM DETEKCIJOM (APCI-LC-MS)

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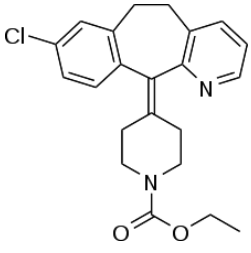
Ključne reči
loratadin, dezkarboetoksiloratadin, LC-MS metoda, studija bioekvivalence

Apstrakt

A sensitive and selective liquid chromatography coupled with atmospheric pressure chemical ionization-mass spectrometry (APCI-LC-MS) method for quantification of loratadine (L) and its active metabolite descarboethoxyloratadine (DCL) in human serum was validated. The proteins were precipitated using acetonitrile. At pH 10, L and DCL were extracted by 5 ml of mixture diethyl ether:n-hexane (1:1).
Analysis was performed on Lichrospher RP select B 60, 250 x 4, 5 µm column at the room temperature. The used mobile phase was the mixture of acetonitrile and demineralized water with addition 1% of acetic acid, with flow of 1.5 mL/min.
The detection of L and DCL was in SIM mode, with m/z 383 for L, and 311 for DCL. Retention times of L and DCL were 10 minutes and 12 minutes, respectively. Calibration curves were generated over the range of 0.5-20 ng/mL for both, L and DCL, with values for coefficient of determination greater than 0.99. The limits of quantification were established at 0.1 ng/mL of L and 0.2 ng/mL of DCL, respectively, with an accuracy and precision less than 9%. Recoveries of both analytes were around 80%. The validated LC/MS method has been applied to a pharmacokinetic study of loratadine after single oral dose of 20 mg given to 48 healthy volunteers.

INTRODUCTION

Loratadine is a long-acting tricyclic antihistamine with selective peripheral histamine H(1)-receptor antagonist activity. It is indicated for the symptomatic relief of alergy such us allergic rhinitis, urticaria and other skin allergies.



Picture 1. *Chemical structure of loratadine*

Loratadine is well absorbed from the gastrointestinal tract and has rapid first-pass hepatic metabolism. It is extensively metabolized in the liver to descarboethoxyloratadine (DCL) which is largely responsible for the antihistaminergic effects. Biological half-life of loratadine is

8-14 h, and DCL 17-28 h. Oral dose of loratadine of 20 mg leads to maximum plasma concentrations of about 11 and 10 ng/mL for loratadine and DCL respectively.

Determination of loratadine and its metabolite in plasma is necessary for monitoring of drug pharmacokinetic. The methods for determination of loratadine and descarboethoxyloratadine in biological materials were described with fluorescence and UV detection [1-8].

Quantitative analysis of therapeutic compounds and their metabolites in biological matrix (such as plasma, serum or urine) nowadays requires sensitive and selective methods to allow the determination of concentrations in the ng/mL range. New methods are using liquid chromatography with mass spectrometric detection [9-15].

We described a sensitive, accurate and precise method of liquid chromatography combined with atmospheric pressure chemical ionization-mass spectrometry (APCI-LC-MS) for determination of loratadine and its metabolite descarboethoxyloratadine in serum samples after single oral dose (20 mg) given to 48 volunteers.

METHODS

Chromatography

The method uses liquid chromatograph Thermo Separation Products Spectra System (Autosampler AS3000, HPLC binary pump P2000, Degasser SCM1000), mass spectrometer with atmospheric pressure chemical ionization source (Finnigan MAT SSQ7000 LC-MS – APCI System), a column Lichrospher RP select B 60, 250 x 4,5 µm (Merck), with guard column Lichrochart 4 x 4 RP-select B, at the room temperature, with injector loop volume 100 µL.

The mobile phase was the mixture of 49.5 mL of acetonitrile and 49.5 mL of demineralized water with addition of 1 mL gacial acetic acid per 100 mL, filtrated and degassed by membrane degasser with flow of 1.5 mL/min.

Mass detector worked in SIM mode. Protonized ion masses were m/z 311 amu for descarboethoxyloratadine and m/z 383 amu for loratadine.

Retention times of descarboethoxyloratadine and loratadine were about 10 min. and 12 min. respectively.

Materials

Analytical standard of loratadine (USP Refefence standard, cat. No. 9039/01), descarboethoxyloratadine 99.77% (USP Refefence standard, cat. No. 008/A10/2001) is obtained from HEMOFARM – Vršac.

Acetonitrile, Ammonium hydroxide, Glacial acid acetic diethyl ether and n-hexane were of HPLC and p.a. purity, obtained from MERCK.

Sample preparation

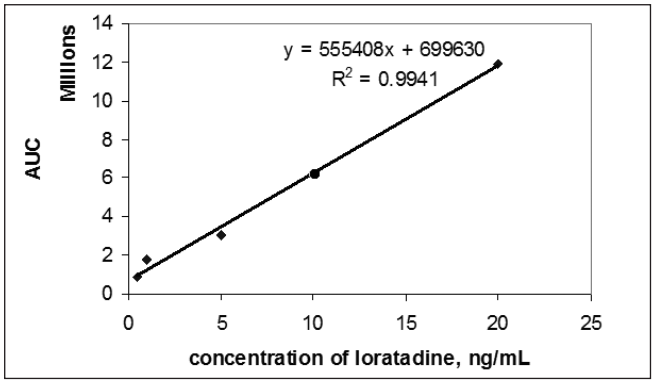
After melting to the room temperature in 1 mL of serum sample was added 1 mL acetonitrile then was alkalized to pH 10 with ammonium hydroxide. Loratadine and DCL were extracted with 5 ml mixture diethyl ether:n-hexane (1:1). This mixture was mixed 20 min. using a mechanical mixing extractor. After centrifugation 10 min at 3000 rpm, organic phase was separated and dried at room temperature under a stream of air. Dried content was dissolved in 1 mL of mobile phase and filtered by 0,22 µm filter.

Calibration and quality control samples were prepared by adding loratadine and DCL solution in blank ("drug-free") human serum to achieve calibration concetrations expected to meet thetherapeutic levels in serum of volunteers. The calibration curves for serum spiked by loratadine and DCL were obtained by plotting peak areas for the concentrations range from 0.5 to 20 ng/mL.

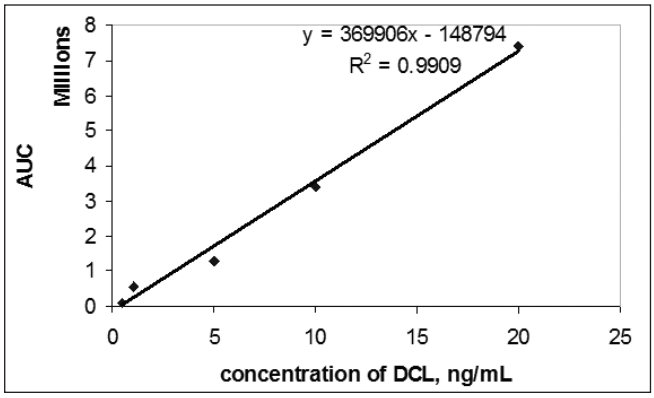
RESULTS

Concentration of loratadine and DCL were calculated by using linear regression function. Picture 2. and 3. show calibration curves for serum spiked by loratadine and DCL.

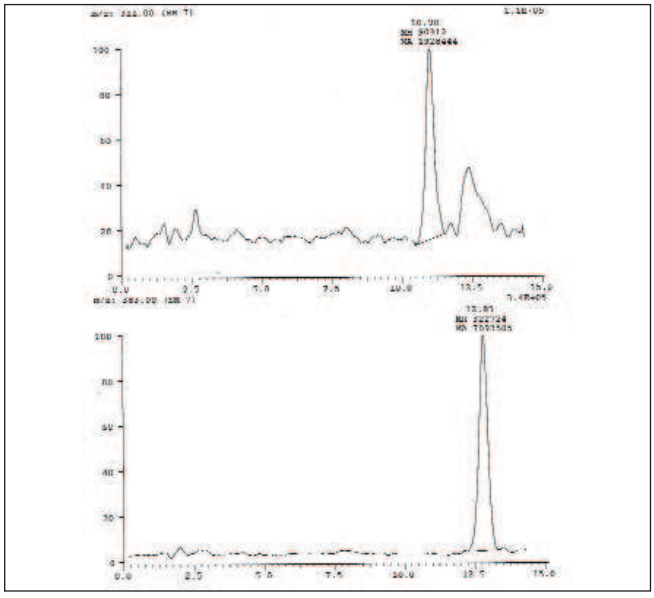
Unknown concentrations of loratadine and descarboethoxyloratadine in serum samples of volunteers were calculated using the corresponding factor from calibration curves.



Picture 2. *Calibration curve for serum spiked with loratadine*



Picture 3*. Calibration curve for serum spiked with DCL*



Picture 4*. LC-MS chromatogram of spiked serum*

Recovery was determined, for each concentration, as the mean of three samples by comparing the peak areas of the extracted and non-extracted samples. Analytical recovery for loratadine and DCL were 84.1 % (from 82.85% to 89.49%) and 86.7 % (from 83.25 to 94.84%) respectively.

Picture 4. shows chromatogram of serum spiked by loratadine and DCL solution concentration of 10 ng/mL.

Limits of detection and quantification were determined by injection of spiked serum in 100 µl loop, and represented the amounts of 1 ng/mL loratadine and DCL at signal/noise = 7.00. The results are in Table 1.

***Table 1**. Limits of detection (LOD), limits of quantitation (LOQ), standard deviations (SD) and coefficients of variation (CV), for loratadine and DCL*

	LOD (ng/mL)	LOQ (ng/mL)	SD	CV (%)
Loratadine	0.07	0.1	9737	6.03
DCL	0.09	0.2	3368	7.10

Selectivity of the method is cheked by using the mass spectrometer programmed in SIM mode. Loratadine and DCL were monitored with m/z 383 and m/z 311 amu respectively. Described analytical conditions obtained reliable, selective and accurate peaks for identification and quantification of mentioned compounds in serum of volunteers. Their identities were also tested by protonized full mass spectra.

DISCUSSION

The most using method for isolation of loratadine and DCL is liquid-liquid extraction. Different organic solvent could be used for extraction.

Kunicki described extraction of loratadine with 2-methylbutane-hexane (2:1) [5].

Due to the differences in pKa values and polarities of loratadine and DCL it is difficult to chromatograph both the analyte and metabolite in a single chromatographic run.

Simultaneous determination of loratadine and DCL is more beneficial for clinical pharmacokinetic studies, as they have similar pharmacological activity.

Different kinds of extraction for loratadine and DCL require high volume of sample. These methods have higher possibility of fault, lower recovery and require long time for sample preparation.

Precipitation of proteins from human plasma with acetonitrile is simple method [10]. Samples produced on that way of preparation are not clear. Moreover, adding of acetonitrile into plasma samples lead to dissolution of initial sample, and decreasing of analyites concentration.

Sutherland at al. described four step extraction which consist of two main steps: extraction of loratadine and DCL with toluene, and back extraction of DCL with formic acid. Extract with loratadine and back-extract with DCL was combined and analyzed by LC-MS technique.

The advantage of our method is in one step extraction with mixture of diethyl ether:n-hexane (1:1). We get extract which contains the both compound with good recovery (84.1 and 86.7% for loratadine and DCL respectively). The extracts were clean and there were not co-eluting compounds that could influence on ionization of the analytes.

In described HPLC methods various columns and mobile phase were used (C18 or cyano columns and mobile phases with acetonitrile, methanol and acetate buffer, with pH range from pH 6.8 to 11)[7-8,10-15].

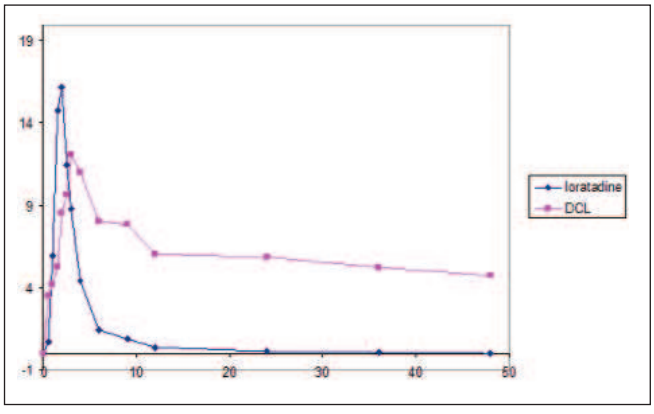
Because loratadine and DCL have different pKa values and polarities, it was difficult to set chromatographic conditions that produce sharp peak and adequate response. This included mobile phase selection, pH of solution, flow rate, column type and injection volume. We get the best results with RP select B colum and acid mobile phase with acetonitrile.

Total run time of our method was about 13 min. which enables analysis of high number of blood samples for the purpose of pharmacokinetic study.

Limits of quantitation for loratadine and DCL were 0.1 and 0.2 ng/mL. Other authors described lower limit of quantitation, but they used more sensitive tandem mass spectrometric method (LC-MS-MS) then our LC-MS method.

Applying to bioequivalence study

We applied LC-MS method for determination of loratadine i DCL to bioequivalence study. The bioequivalence study of two loratadine tablet formulations was assessed in 24 healthy volunteers who received a single 20 mg dose od each loratadine formulation. The study was conducted using an open, randomized, two sequences, two period crossover design with a 2-week washout period. Serum samples were obtained over a 48 h period and analyzed by LC-MS method. A multiplicative/additive statistic model was used for comparison of pharmacokinetic parameters describing the rate and extent of bioavailability. For loratadine, the respective 90% CI of the individual ratio geometric means were 91.2 to 103.9 for AUC (0-inf.) and 86.9 to 107.8 for Cmax, while corresponding 90% confidence limits for descarboethoxyloratadine were 102.9 to 113.6 [AUC(0-inf)] and 101.1 to 135.0 [Cmax], respectively. Since both 90% CI for the long-transformed AUC an Cmax geometric mean ratios were included either in the 80%-125% interval, or in the wider 70%-143% range, test drug (Pressing tablets, Hemofarm) was considered bioequivalent to the reference (Claritine tablets, Shering-Plough).



***Picture 5.** Pharmacokinetic profile of loratadine and DCL in healthy volunteers*

CONCLUSION

Analytical LC-MS method developed and validated in our work for loratadine and descarboethoxyloratadine determination in human serum found to perform satisfactory requirements in sensitivity to detect very low concentrations, high extraction recovery, valuable quantification and high mass spectral specificity. Applied method is more complex than classical HPLC methods, but it is accurate and reproducible for clinical studies and routine analysis of loratadine and descarboethoxyloratadine in serum.

Sažetak

Validovana je osetljiva i selektivna metoda tečne hromatografije spregnute sa jonizacijom pod atmosferskim pritiskom i maseno-spektrometrijskom detekcijom (APCI-LC-MS) za određivanje koncentracije loratadina (L) i njegovog aktivnog metabolita dezkarboetoksiloratadina (DCL) u serumu. Proteini su staloženi upotrebom acetonitrila. Loratadin i DCL su ekstrahovani smešom dietiletar-n-heksan (1:1). Analiza je izvršena na Lichrospher RP select B 60, 250 x 4,5 µm koloni na sobnoj temperaturi. Korišćena je mobilna faza koja se sastojala od acetonitrila i vode uz dodatak 1% sirćetne kiseline sa protokom od 1,5 mL/min.

Detekcija L i DCL vršena je u SIM modu sa jonskim masama od m/z 383 za L i 311 za DCL. Retenciono vreme loratadina bilo je oko 10 min, a DCL 12 min. Kalibraciona kriva je pravljena u opsegu koncentracija od 0,5 do 20 ng/mL za oba ispitivana jedinjenja, sa koeficijentom r>0.99. Limit kvantifikacije za L iznosio je 0,1 ng/mL, a za DCL 0,2 ng/mL uz tačnost i preciznost <9%. Prinos ekstrakcije za oba analita bio je veći od 80%. Validovana LC-MS metoda je primenjena u farmakokinetičkom ispitivanju loratadina nakon primenjene pojedinačne oralne doze od 20 mg koja je data zdravim dobrovoljcima.

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