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Determination of dimenhydrinate in human plasma by liquid chromatography–electrospray tandem mass spectrometry: Application to a relative bioavailability study

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Abstract

Here we present a sensitive and specific liquid chromatography–tandem mass spectrometric method for the quantification of dimenhydrinate (I) in human plasma. Sample preparation is conducted using citalopram (II) addition as an internal standard (IS), liquid–liquid extraction with basified plasma using a mixture hexane/acetate (1:1, v/v) as the extracting solvent, and the final extract reconstituted in the mobile phase. I and II (IS) were injected in a C8 column with the mobile phase composed of methanol:isopropanol:water:formic acid (78.00:19.92:2.00:0.08, v/v/v/v) and monitored using a positive electrospray source with tandem mass spectrometry analyses. The selected reaction monitoring (SRM) was set using precursor ion and product ion combinations of m/z 256.0 > 167.0 and m/z 325.0 > 109.0 for I and II, respectively. The limit of quantification (LOQ) was 0.4 ng/mL, the dynamic range being 0.4–200 ng/mL. Validation results on linearity, specificity, accuracy, precision and stability, as well as on application to the analysis of plasma samples taken up to 24 h after oral administration of 100 mg of dimenhydrinate in healthy volunteers demonstrated its applicability to bioavailability studies.

Keywords: Mass spectrometry; Bioavailability; Dimenidrynate

1. Introduction

Dimenhydrinate (I) (CAS - 523-87-5, 2-benzhydryloxy-*N*,*N*-dimethyl-ethanamine; 8-chloro-1,3-dimethyl-7H-purine-2,6-dione) is a salt of two drugs diphenhydramine and 8-chloro-theophyllinate used to prevent motion sickness. It is well absorbed, with a peak plasma concentration (C_{max}) of 14.5 ng/mL following a single dose of 25 mg; the time to achieve maximum plasma concentration (t_{max}) following a single dose of 25 mg is 2.6 h [1].

Mass spectrometric detection coupled to liquid chromatography has been considered as the state-of-art technique to perform bioanalytical analysis with maximum selectivity and sensitivity [2]. The use of HPLC–MS/MS technique has become the first choice in bioavailability studies due to the fast, sensitive, and reliable results generated by its use [3].

The coupling of these powerfull techniques allowed to study a broad range of pharmaceutical compounds in very low LOQ specially polar compounds [4]. Other techniques have been previously used to determine dimenhydrinate in plasma, including high performance liquid chromatography (HPLC) [5,6]. This paper describes a sensitive and specific liquid chromatography tandem mass spectrometry method for dimenhydrinate quantification in human plasma and its application for bioavailability studies.

2. Experimental

2.1. Materials and reagents

Dimenhydrinate was obtained from U.S. Pharmacopeia (Rockville, MD) and citalopram (internal standard) was

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obtained from Farma Center (Montevideo, Uruguay). Formic acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany) and HPLC grade acetonitrile was purchased from J.T. Baker (Deventer, Netherlands). Hexane, ethyl acetate, and isopropanol were GR grade and purchased from E. Merck. Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Analyses conditions and instrumentation

Elution was performed using methanol:isopropanol:water: formic acid (78.00:19.92:0.20:0.08 v/v/v/v) as the mobile phase, at a flow-rate of 0.12 mL/min with time between injections being 3.0 min. The X-terra C-8 (50 mm × 20 mm, 5 μm) column (Waters, USA) was used at room temperature (25 °C) and aliquots (5 μL) of the plasma extracts were injected into a Shimadzu LC system (Shimadzu, JAPAN). Mass spectrometric analysis was performed using a Micromass Quattro Micro (Waters, USA) tandem mass spectrometer, equipped with an electrospray (ESI) source. The temperatures of the dessolvation gas and source block were 450 °C and 120 °C, respectively. The electrospray source was operated in the positive ionization mode (ESI+) at 3650 V, and selected reaction monitoring mode (SRM), *m/z* 256.0 > 167.0 and *m/z* 325.0 > 109.0 were used for quantification of dimenhydrinate and citalopram (used as Internal Standard, IS), respectively. Cone voltage, collision energy and collision gas pressure (argon) were 16.0 V, 7.0 V, and 2.99×10^{-3} Torr, respectively for dimenhydrinate, and 35.0 V, 20.0 V, and 2.99×10^{-3} Torr for citalopram, respectively.

2.3. Standards and quality control samples preparation

Stock solutions of dimenhydrinate (800 ng/mL, 120 ng/mL, and 40 ng/mL) were prepared in deionized water while citalopram stocks (1 μg/mL) were prepared in acetonitrile. Aliquots of the 40 ng/mL and 800 ng/mL solutions were used to spike blank human plasma in order to obtain calibration standards of 0.4, 4.0, 8.0, 40.0, 120.0, 200.0 ng/mL. Aliquots of the 120 ng/mL, and 800 ng/mL solutions were used to spike blank human plasma in order to obtain three levels of quality controls (QCs) fixed at 1.2, 80.0, and 160.0 ng/mL (low, medium and high) were prepared using the same blank plasma. All spiked plasma was stored at −20 °C.

2.4. Sample extraction

Plasma samples collected from the volunteers were stored as 200 μL aliquots at −20 °C; aliquots were thawed at room temperature before processing. A twenty-five-microliter aliquot of internal standard solution (1 μg/mL) was added and briefly mixed for 1 min. Fifty microliters of 1.0 M NaOH and 1.0 mL of hexane-ethyl acetate (1:1, v/v) were added and vortexed for 5 min. The tube was centrifuged for 10 min at 10800 × *g* and the upper organic phase (800 μL) was transferred to another tube and evaporated to dryness under an air stream at room temperature. The residue was dissolved in 200 μL of mobile phase, the sample was then transferred to the glass autosampler vial and 5 μL was injected into the chromatographic system.

2.5. Recovery

The efficiency of the extraction of dimenhydrinate from human plasma was measured analyzing three levels of quality control samples. The recovery of each of the three concentrations was determined by comparing peak areas obtained from the spiking plasma samples (QCs) and the standard solution spiked with the blank plasma residue. The recovery of IS was also evaluated using the same procedure.

2.6. Limit of quantification

The limit of quantification (LOQ) was defined as the lowest concentration at which precision and accuracy, expressed by relative standard deviation (RSD), were lower than 20%.

2.7. Analytical curves

The analytical curves were constructed using concentration values ranging from 0.4 to 200.0 ng/mL of dimenhydrinate in human plasma. Linear analytical curves were obtained by weight linear regression (weighing factor: 1/*x*), the ratio of dimenhydrinate peak area to citalopram peak area was plotted versus the ratio of dimenhydrinate concentration to that of the internal standard, in ng/mL. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

2.8. Accuracy and precision

Quality controls of dimenhydrinate (1.2, 80.0 and 160.0 ng/mL) were analyzed using the corresponding standard curves and were used to calculate accuracy and precision. The accuracy of the method was shown as relative error (RE) and calculation based on the difference between the mean calculated and nominal concentrations, whereas precision was evaluated by calculating the within- and between-run relative standard deviations (RSDs).

2.9. Freezing and thawing stability

Freezing and thawing stability for dimenhydrinate in plasma samples was studied after three cycles and the analytical process with control concentrations in four plasma batches. Samples were frozen at −20 °C in three cycles of 24, 36 and 48 h. In addition, the long-term stability of dimenhydrinate in QC samples was also evaluated by analysis after 3 months storage at −20 °C. Autosampler stability was studied over a 24 h storage period in the autosampler tray with control concentrations.

2.10. Pharmacokinetics and statistical analysis

Pharmacokinetics parameters were calculated from plasma levels applying a non-compartmental statistic using WinNon-

Lin 5.0 software (Pharsight, USA). Blood samples were drawn up to a period of 3–5 times the terminal elimination half-live ($t_{1/2}$) resulting a pharmacokinetic curve (concentration versus time) following Food and Drug Administration (F.D.A.) guidelines [7]. The C_{max} and T_{max} values were determined considering the plasma dimenhydrinate concentration–time profiles. The area under the concentration–time curve (AUC_{0-t}) was obtained by the trapezoidal method. The total area under the curve ($AUC_{0-\infty}$) was calculated up to the last measureable concentration and extrapolations were obtained using the last measureable concentration and the terminal elimination rate constant (K_e). The terminal elimination rate constant, K_e, was estimated from the slope of the terminal exponential phase of the plasma of dimenhydrinate concentration–time curve (by means of the linear regression method). The terminal elimination half-life, $t_{1/2}$, was then calculated as $0.693/K_e$. Results are indicated as mean ± standard deviation. Regarding AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, relative bioavailability was assessed by means of an analysis of variance (ANOVA) and by calculating the standard 90% confidence intervals (90% CIs) of the ratios test/reference (logarithmically transformed data). The relative bioavailability was considered when the ratio of averages of log-transformed data was within 80–125% for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. In this study we considered the tablet as the reference formulation (R) and the capsule as the test formulation (T).

3. Results and discussion

3.1. Method development

The MS tuning was carried out by direct infusion of solutions of both dimenhydrinate and citalopram (IS) into the ESI source of the mass spectrometer. The critical parameters in the ESI–MS/MS equipment includes the ionization in the solution (mobile phase), flow rate, needle (ESI) voltage and polarity (charge separation), drying gases (ion evaporation model and charged residue model) [8], and ion transmission. In our case, the formation of protonated ionic dimenhydrinate and citalopram (IS) molecules were observed (Fig. 1).

A collisionally-activated dissociation (CAD) product ion spectrum for dimenhydrinate yielded high-abundance fragment ions of *m/z* 167.0 (Fig. 2).

Scheme 1 shows the proposed dissociation mechanism for the protonated dimenhydrinate of *m/z* 256.0, a protonated ether by *N,N*-dimethyl-amine-2-ethanol loss forming the product ion of *m/z* 167.0 that is a tropilium ion stabilized by resonance.

After the SRM channels were tuned, the mobile-phase was changed from an organic phase to a more aqueous phase with acid dopant to obtain a fast and selective LC method. The better signal was obtained using methanol:isopropanol:water:formic acid (78.00:19.92:0.20:0.08 v/v/v/v) that was tested using three triplicate curves with 5 QCL, 5 QCM, 5 QCH.

3.2. Specificity

The analysis of dimenhydrinate and citalopram using the SRM function was highly selective, with no interfering compounds or significative ion suppression from endogenous substances observed at the retention times for dimenhydrinate and citalopram, as shown in Fig. 3. The chromatographic run was executed using a short (50 mm) HPLC XTERRA column, which is convenient for running a high throughput of samples. There was no chromatographic separation, due to the high degree of similarity shared by the two structures, with the adjusted retention time being as short as 1.4 min, in order to increase the

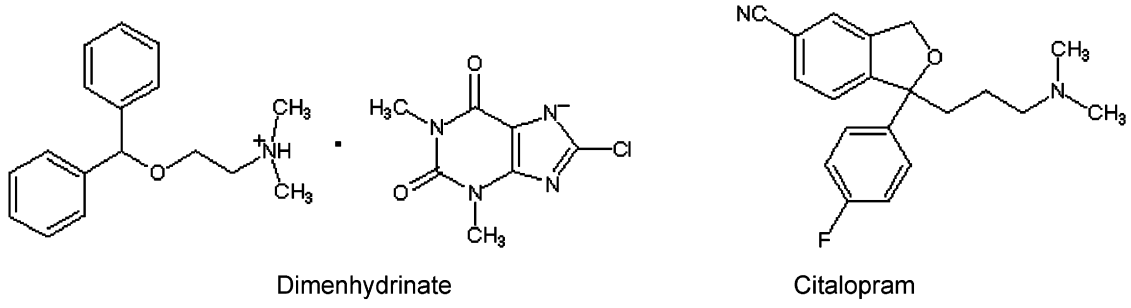


Fig. 1. Chemical structures of dimenhydrinate and citalopram (IS).

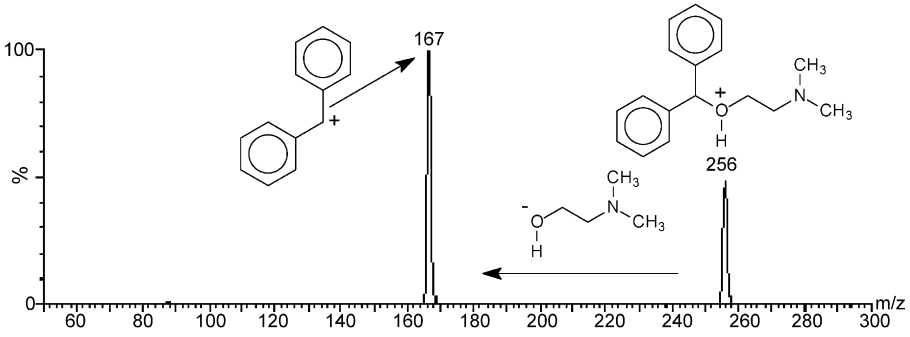
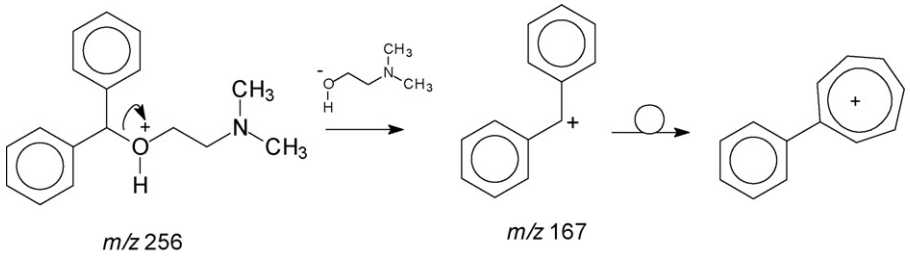


Fig. 2. CAD mass spectra of the dimenhydrinate protonated molecule.



Scheme 1.

analytical capability (Fig. 3a and b). Chromatograms obtained from plasma spiked with dimenhydrinate (1.2 ng/mL) and citalopram (125.0 ng/mL) are shown in Fig. 3c and d.

The matrix effect was evaluated directly extracting blank plasma and again following spiking it with the analyte at the LOQ concentration. There was no difference observed on the signal for the solution and the spiked extract at the LOQ concentration.

3.3. Linearity, precision and accuracy

Calibration curves were plotted as the peak area ratio (drug/I.S.) versus drug concentration. The assay was linear in the concentration range of 0.4–200 ng/mL. The RSD were less than 4%. The relative error of the mean of the measured concentrations ranged from 3.61 to −6.65% (Table 1). The determination coefficients (r^2) were greater than 0.998 for all curves. Precision and accuracy for this method were controlled by calculating the intra-batch and inter-batch variation at three concentrations (1.20, 80.00 and 160.00 ng/mL) of QC samples in five replicates. As shown in Table 2, the intra-batch RSDs and REs were less than 7%. These results indicate that the method is reliable and reproducible within its analytical range.

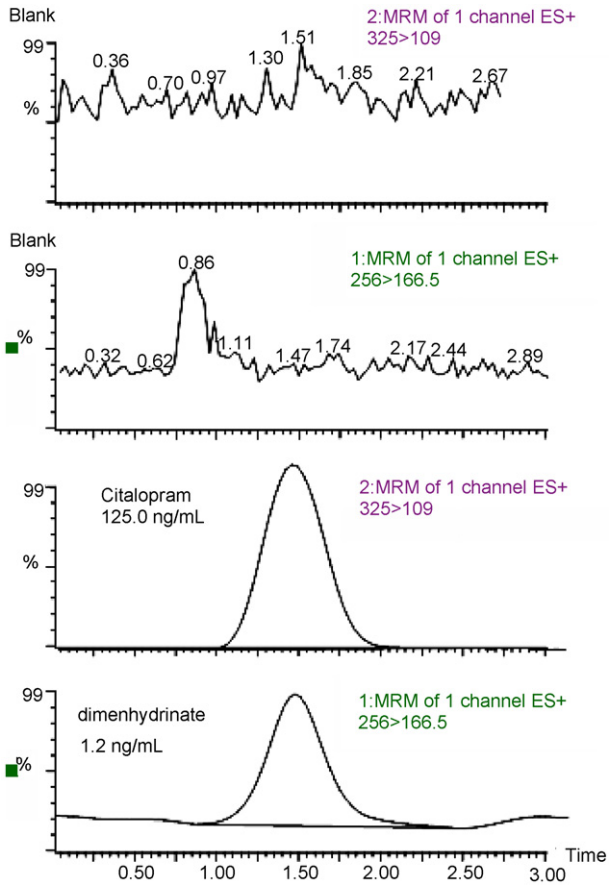


Fig. 3. Representative SRM chromatograms of dimenhydrinate in human plasma: (a) dimenhydrinate and (b) citalopram blank human plasma; (c) dimenhydrinate and (d) citalopram spiked human plasma containing 1.20 ng/mL dimenhydrinate and internal standard.

3.4. Freezing and thawing stability

The results of the freeze–thaw stability studies are shown in Table 3. Quantification of the analyte in plasma subjected to a number of freeze–thaw (−20 °C to room temperature) cycles showed that the analyte is stable after three cycles. No degradation of the analyte had taken place over a 24 h storage period in the autosampler tray with the final concentrations of dimenhydrinate ranging from 108.66 to 101.38% of the theoretical values. In addition, the long-term stability of dimenhydrinate in QC samples after 97 days of storage at −20 °C was also evaluated. The concentrations ranged from 93.7 to 108.66% of the theoretical values. Dimenhydrinate was therefore stable in human plasma for at least 97 days at −20 °C.

3.5. Recovery

The recovery for the liquid–liquid extraction with hexane/ethyl acetate (1:1, v/v) was calculated by comparing the peak area ratios of dimenhydrinate in plasma samples and area of dimenhydrinate standard solution spiked with the blank plasma

Table 1
Calibration curves from one batch of the validation section

Spiking plasma concentration (ng/mL)	Concentration measured (mean) (ng/mL)	RSD[a] (%) ($n = 3$)	Relative error[b]
0.4	0.42	3.37	5.00
4	4.08	0.68	2.00
8	8.01	1.63	0.13
40	37.51	3.32	−6.22
120	117.91	0.43	−1.74
200	204.49	0.85	2.25

[a] Standard deviation/mean concentration measured.
[b] [(Mean concentration measured − spiked plasma concentration)/spiked plasma concentration] × 100.

Table 2
Precision and accuracy (analysis with spiking plasma samples at three different concentrations)

Spiking plasma concentration (ng/mL)	Within-run			Between-run		
	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)	Relative error[b] (%)	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)	Relative error[b] (%)
1.20	1.26	4.16	4.92	1.21	4.32	1.17
80.00	84.86	1.95	6.07	83.33	4.13	4.16
160.00	169.45	2.23	5.91	165.61	4.74	3.51

[a] Standard deviation/mean concentration measured.
[b] [(Mean concentration measured − spiked plasma concentration)/spiked plasma concentration] × 100.

Table 3
Freeze and thaw stability of the samples

Sample concentration (ng/mL)	Initial (0 h)		Cycle 1 (24 h)		Cycle 2 (36 h)		Cycle 3 (48 h)	
	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)	Concentration measured (mean ± SD) (ng/mL)	RSD[a] (%) ($n = 5$)
1.20	1.25 ± 0.02	1.60	1.28 ± 0.03	2.34	1.28 ± 0.04	3.13	1.25 ± 0.07	5.6
80.00	86.93 ± 1.00	1.15	82.67 ± 1.56	1.89	85.05 ± 1.66	1.95	83.11 ± 3.10	3.73
160.00	172.85 ± 1.20	0.69	162.21 ± 3.42	2.11	170.11 ± 3.78	2.22	165.71 ± 4.99	3.01

[a] Standard deviation/mean concentration measured.

residue. The recovery of dimenhydrinate, determined at three different concentrations (1.20, 80.00 and 160.00 ng/mL), were 58.8, 64.2 and 63.2%, respectively; the overall average recovery was 62.1%.

3.6. Application to biological samples

The proposed analytical method was applied to a pilot study to compare the bioavailability of two dimenhydrinate formulations: 100 mg tablet (R) and 50 mg capsule (T). The study was conducted with 6 subjects under single oral dose (one 100 mg tablet versus two 50 mg capsules) and randomized crossover design. Typical plasma concentration versus time profiles is shown in Fig. 4. Plasma concentrations of dimenhydrinate were in the standard curve range and remained above the 0.4 ng/mL quantitation limit for the entire sampling period. The observed maximum plasma concentration (C_{max}) was 80.07 ± 24.56 ng/mL for the reference and 80.17 ± 28.31 ng/mL for the test. The corresponding time of maximum concentration (T_{max}) was 2.46 ± 0.46 h for the reference and 2.17 ± 0.49 h for the test. The value of the area under the curve from time 0 to the last sampling time (AUC_{0-t}) was 700.57 ± 296.62 ng h/mL for the reference and 631.35 ± 257.03 ng h/mL for the test, and area under the curve from 0 to ∞ ($AUC_{0-\infty}$) was 788.43 ± 341.22 ng h/mL for the reference and 726.48 ± 312.29 ng h/mL for the test.

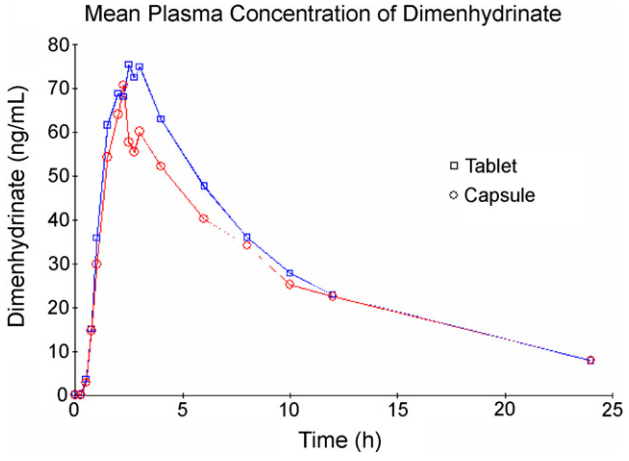


Fig. 4. Mean plasma concentrations of test and reference formulations after 100 mg single oral dose (six healthy volunteers).

The elimination half-life ($t_{1/2}$) was 7.44 ± 1.5 h for the reference and 8.05 ± 0.91 h for the test. The pharmacokinetic data obtained were similar to those reported by Blyden et al. [9]. In addition, the mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ was higher than 80%, complying with the Food and Drug Administration Bioequivalence Guideline [10].

The ratio test/reference (T/R) and ninety percent confidence intervals (90 CIs) for overall analysis were comprised within the previously stipulated range (80–125%).

The ratio T/R and 90 CIs (in parenthesis) were 99.41% (80.5–122.77%) for C_{max}, 91.62% (80.0–105.03%) for AUC_{0-t} and 93.58% (80.56–108.71%) for $AUC_{0-\infty}$. Therefore, the results demonstrated the relative bioavailability of the two formulations of dimenhydrinate.

4. Conclusion

In conclusion, the use of LC–MS/MS allows an accurate, precise and reliable measurement of dimenhydrinate concentrations in human plasma for up to 24 h after oral administration of 100 mg to healthy volunteers. The described method has proven to be fast and robust, with each sample requiring less than 3 min

analysis time. The assay method is also highly specific due to the inherent selectivity of tandem mass spectrometry and has significant advantages over other techniques previously described for measuring dimenhydrinate in biological fluids. The sensitivity of the assay is sufficient to follow accurately the pharmacokinetics of this drug.

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