Research Article

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Bioanalysis

Development of an UPLC–MS/MS micromethod for quantitation of cinitapride in plasma and its application in a pharmacokinetic interaction trial

Aim: Cinitapride (CIN) is a benzamide-derived molecule used for the treatment of gastroesophageal reflux and dyspepsia. Its pharmacokinetics are controversial due to the use of supratherapeutic doses and the lack of sensitive methodology. Therefore, a sensitive and accurate micromethod was developed for its quantitation in human plasma. **Results:** CIN was extracted from 300 µl of heparinized plasma by liquid–liquid extraction using cisapride as internal standard, and analyzed with an ultra performance liquid chromatograph employing positive multiple-reaction monitoring–MS. **Conclusion:** The method proved to be rapid, accurate and stable within a range between 50 and 2000 pg/ml and was successfully validated and applied in a pharmacokinetic interaction trial, where it was demonstrated that oral co-administration of simethicone does not modify the bioavailability of CIN.

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Keywords: cinitapride • gastroesophageal reflux • HPLC–MS • simethicone

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Cinitapride (CIN) is a benzamide-derived molecule (4-amino-N-[1-(3-cyclohexen-1-yl--methyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide) with esophageal and gastric prokinetic properties. The structure presents moderately basic (pk_a = 9.74) and highly hydrophobic (logP = 3.7) behavior. Although CIN is slightly soluble in water (0.0141 mg/ml), its hydrogen tartrate salt (CAS no. 66564-14-5) is used for oral formulations, which renders a more highly soluble and bioavailable drug [1,2].

CIN is a selective 5-HT_4 serotoninergic receptor agonist that also possesses antagonist effects on 5-HT_2 and dopaminergic D_2 receptors. Its therapeutic properties lie in its capacity to increase the tone of the lower esophageal sphincter concomitantly with a potent gastrokinetic effect, which produces significant emptying of the bowel. In addition to this, CIN also reduces anterograde esophageal movements, with the consequent decrease of gastroesophageal reflux episodes [3].

The pharmacokinetics (PKs) of CIN were formerly described with supratherapeutic doses because of the lack, at that time, of sensitive methodology; however, such data have been refreshed with a single oral dose of 1 mg/tablet in German population. This study, conducted under fasting conditions, reported a maximal plasma concentration of 0.33 ± 0.17 ng/ml at 1.4 ± 0.6 h, with an elimination half-life of 8.4 ± 15 h (mean ± standard deviation). Oral absorption of CIN is rapid and it is distributed widely in the body, including in the myenteric plexus, CNS and conductive cardiac tissue [4]. More than 70% of an oral dose is metabolized via CYP450, and hepatic biotransformation is extensive; at least 15 metabolites have been reported, which correspond to mono- and dihydroxylated species; additionally, N-oxide has been reported. Metabolites are eliminated by feces [5].

CIN has been approved since 1990 in Spain, Latin America and recently, in Asia

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for the treatment of gastroesophageal reflux, irritable bowel syndrome and functional dyspepsia [6,7]; however, only two methods for plasma drug quantitation have been reported [4,8]. CIN metabolites were studied with an UPLC method coupled with an ion trap, with only qualitative purposes [5]. Current PK data were obtained with back-flushed HPLC with MS, reaching an LOQ of 0.1 ng/ml with 0.5 ml of plasma (however, no additional validation data are included) [4]. Finally, a recent HPLC method with MS/MS has been reported for the determination of free levels of CIN in plasma, with a long extractive procedure by solid phase, achieving up to 20 pg/ml of sensitivity [8], but with succinct information on its chromatography.

With respect to simethicone (SIM), this is an inert silicone dioxide polymer (not absorbed into systemic circulation, not metabolized), with tensoactive and foam-dissolving properties. Its surfactant action contributes to facilitating gas elimination from the bowel, alleviating symptoms of abdominal fullness, bloating and a sense of slow digestion occurring during dyspeptic syndrome [9]. SIM has been used empirically alone or in combination to antispasmodic agents, anti-acids or other surfactants, with succinct scientific information [10].

For these reasons, it appeared reasonable to generate data concerning CIN PKs when it is coformulated and administered with SIM. Thus, the aim of present work was to develop and validate a robust and sensitive high-throughput method based on UPLC–MS/MS for the measurement of CIN in human plasma and its application in a PK interaction trial.

Materials & methods

Chemicals & reagents

Cinitapride bitartrate (Symed Labs, Ltd., Hyderabad, India; 99.7% pure) was acquired through Helm de México (Mexico City, Mexico). Cisapride (CIS; United States Pharmacopeia standard, 95.3% pure) was purchased via PROQUIFA (Mexico City, Mexico). HPLC-grade methanol and acetonitrile, in addition to isopropanol, diethyl ether and ethyl acetate analytical grade, were purchased from JT Baker (Xalostoc, Mexico), while ammonium formate and formic acid was obtained from Sigma-Aldrich-Fluka (Toluca, Mexico). Ultra-pure water was obtained from a Milli-Q™ system (Millipore, MA, USA).

Chromatographic conditions

Ultra-performance Liquid Chromatography (Acquity™ Class- I/; Waters Co., MA, USA) coupled with a tandem mass spectrometer (Xevo™ TQ-S Waters Micromass, Manchester, UK) was employed, interfaced by MassLynx™ Ver. 4.1s software. CIN separation from possible endogenous interferences was carried out by means of a BEH C18 column (2.1 × 50 mm, 1.7-µm particle size, Acquity UPLC; Waters, Ireland). Flow rate was fixed at 0.35 ml/min with isocratic conditions, and mobile phase was optimized as aqueous ammonium formate 2 mM (adjusted with formic acid to pH = 3)/acetonitrile (74:26 v/v). Column was maintained at 40°C and autosampler temperature was set at 10°C. Volume of sample injection was 5 µl and, under these conditions, a complete run took 3 min.

Mass spectrometer settings

Tuning for MS/MS was performed under positive ESI with 100-ng/ml solutions from each molecule in water: methanol (40:60 v/v) with an infusion of 10 µl/min and a mobile-phase flow rate of 200 µl/min. Analytes were measured through multiple-reaction monitoring employing the following ionic transitions: m/z^{1+} 403.28 > 209.09 for CIN, and m/z^{1+} 466.23 > 184.09 for CIS. Table 1 presents the general settings of the tandem mass spectrometer.

Table 1. Mass spectrometer setting parameters for positive electrospray and multiple-reaction monitoring of cinitapride and cisapride detection.

ESI (+) parameters	Values	Analyzer parameters	Values
Capillary energy	1.0 kV	L & H MR 1	2.9/14.9
Cone energy	40 V	Ion energy 1	0.5
Source temperature	150°C	Collision energy	27 (CIN), 30 (CIS)
Desolvation temperature	450°C	L & H MR 2	2.8/14.9
Cone gas flow	150 l/h	Ion energy 2	0.5
Desolvation gas flow	1000 l/h	Dwell	66 ms
Collision gas flow	0.14 ml/min		

Collision energy is expressed as electron-volts.
CIN: Cinitapride; CIS: Cisapride; L & H MR: Low and high mass resolution.

Preparation of stock solutions, calibration standards & quality control samples

CIN and CIS (internal standard of technique) stock solutions (1 mg/ml) were prepared individually in 100% HPLC-grade methanol, protected from photodegradation. Working solutions of CIN (1, 2, 5, 10, 20, 30 and 40 ng/ml) were prepared in water: methanol (60:40 v/v); in addition, the internal standard solution of CIS (7.5 ng/ml) was prepared in the same diluent and all of these were also maintained at 4°C.

Separated calibration curves (50, 100, 250, 500, 1000, 1500 and 2000 pg/ml for CIN) were prepared daily by spiking 950 µl of drug-free citrated pooled human plasma (blank plasma), with 50 µl of corresponding working solution. Quality control (QC) points at low, medium and high levels (150, 750 and 1600 pg/ml for CIN, respectively) were prepared in the same manner to validate the technique.

Sample preparation & plasma extraction

All unknown plasma samples from volunteers, calibration standards, QC points and plasma blanks were processed identically as follows: 300 µl of human plasma was pipetted into 2-ml microcentrifuge tubes. Samples were added to 30 µl of CIS working solution (internal standard). Tubes were briefly vortex-mixed, and then plasma was added with 1200 µl of cold extractive phase (diethyl ether: ethyl acetate 70:30 v/v; 4°C). Samples were vortex-mixed for 1 min at 2000 rpm for liquid–liquid extraction (Talboys-Troemmers, NJ, USA), and centrifuged at 17,000 × *g* at 4°C for 5 min. Then, samples were frozen at -70°C and supernatants were decanted into glass tubes and evaporated to dryness in a TurboVap LV™ concentration workstation (Biotage, Stockholm, Sweden) at 40°C with nitrogen steam for 10 min. Samples were reconstituted with 200 µl of Water: acetonitrile (50:50 v/v) and transferred onto 96-well 'V-shaped' polypropylene plates, covered with preslit silicone mats and placed into the autosampler for their subsequent injection.

UPLC–MS/MS method validation

The analytical method was validated according internal standard operation procedures based on Mexican Regulatory Normativity and US FDA Guidelines [11,12].

Selectivity

Selectivity was evaluated by analyzing six lots of drug-free human plasma kindly donated by the Blood Bank of the Obstetrics Clinic (IMSS-Tlalnepantla, Mexico). The absence of interfering components was considered as being no signal above 20% of low limit of quantitation for CIN and 5% for CIS. Selectivity was also tested by preparing LLOQ in lipemic or 2% hemolyzed plasma. LLOQ was also spiked with aspirin, chlorphenamine, acetaminophen, butylhyoscine, ketorolac, loratadine, (internal approved drugs for the medical staff used during the management of slight-to-mild adverse events during clinical trials), and also cotinine and caffeine; independent responses of CIN and CIS were compared with fresh LLOQ, expecting a variability of <20%.

Matrix effects

Matrix influences were assayed by measuring the ionization response in low and high QC samples prepared in the six lots of plasma chosen during selectivity. Matrix factor (Mf) was determined by comparing peak area ratios of analyte/IS of spiked samples after extraction to peak area ratios of neat solutions at the same concentration, as follow: Mf = spiked ratios/neat ratios; %CV of Mf must be ≤15%.

Carryover

Carryover was assayed by bracketing each running batch by injecting a blank of plasma immediately after an ULOQ, employing the same acceptance criteria used during selectivity.

Precision & accuracy

QC points were utilized to determine within- and between-day precision and accuracy, by analyzing six separate times during three consecutive days. Acceptance criteria were ≤15% CV, and 85–115% of nominal concentrations, for precision and accuracy, respectively.

Stability

Plasma stability (long-term at -70°C, benchtop at room temperature, three 24-h freeze and thaw cycles and processed samples inside the autosampler) were evaluated. No impact on precision and accuracy must be observed.

Linearity

Quantification was achieved by employing the seven-point calibration curve based on CIN/CIS peak area ratios versus CIN concentration in a 1/x-weighted linear model obtained by means of least-squares regression; residual analyses were performed in order to choose the best weighting factor. Linearity was assayed by using three independent single calibration curves (50–2000 pg/ml; no replicates at each level) during three consecutive days.

PK interaction trial

The developed method was employed to investigate the effect of SIM on the oral bioavailability of CIN 1-mg immediate-release tablets. Twenty-six volunteers

of indistinct gender were enrolled after having complied with the following inclusion criteria: age between 18 and 55 years; nonsmokers or having quit smoking 72 h prior to dose administration; BMI between 18 and 27 kg/m^2; normal clinical history (including electrocardiogram); normal values in laboratory tests (hematology, blood chemistry, hepatic function assays and urine analysis); and negative results for AIDS, type-C and type-B hepatitis, pregnancy and antidoping tests. Withdrawal criteria were defined as any allergic reaction, dietetic transgression or vomiting up to 3 h post-dose [11].

All subjects received, in a randomized crossover design, a single oral dose of two 1-mg CIN immediate-release tablets (Pemix®, batch no. 501J, expiration date May 2017; Industrias Farmacéuticas Almirall, Spain, for Ind. Farmacéutica Andrómaco, S.A. de C.V., México), or two 1-mg CIN plus 200-mg SIM immediate-release tablets (Rogastril Plus®, batch no. L00057, expiration date July 2017; Roemmers SAICF, Argentina, for Siegfried Rhein, S.A. de C.V., México) with 250 ml of tap water after a 10-h overnight fasting period, with a 1-week washout period between treatments. Blood samples of 5 ml were taken through a catheter placed in the participant's forearm vein in vacuum tubes with sodium heparin at 0.0 (predose sample) and at 0.16, 0.33, 0.50, 0.66, 0.83, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 12.00 and 24.00 h post-dose. Plasma was separated by centrifugation at 3500 rpm at room temperature for 10 min, transferred into labeled cryovials and stored at -70°C until CIN quantitation.

Statistical analysis

All statistical calculations for PKs were performed using Phoenix™ WinNonlin ver. 6.4 software (Pharsight Co., CA, USA). PK parameters were calculated according to the Mexican Norm NOM-177 Statistical Appendix by programming plasma data, a single extravascular dose and a noncompartmental model.

Maximal plasma drug concentration (C_{max}), time to reach maximal plasma concentration following drug administration (T_{max}), elimination half-life (t½), area under the plasma concentration-time curve from time zero to last measurable concentration ($AUC_{0-24\ h}$), AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$), relative volume of distribution after non-intravenous administration (Vd_{app}), apparent total clearance of the drug from plasma after oral administration (CL_{app}) and mean residence time comprised the software outputs.

No PK interactions were assayed based on a bioequivalence approach, building 90% CIs of log-transformed relationships for C_{max} and AUC_{0-t} between both formulations.

Results & discussion

Mass spectrometry

Due to its basic nature, CIN was ionized in positive electrospray, delivering an abundant and stable signal. The transition obtained (m/z^{1+} 403.28 > 209.09) had been previously reported [4,8] and resulted very useful for quantitative purposes due to its intensity and stability. In addition, CIS also exhibited a quite clean and stable transition (m/z^{1+} 466.23 > 184.09), which contributes to the robustness of the technique. During multiple-reaction monitoring methods development, the selection of different fragments was critical to avoid cross-talking between analyte and internal standard channels. Choosing CIS as internal standard, a drug with a similar structure to that of the main analyte, notes the advantages of possessing a nonexpensive, commercially available, nondeuterated molecule that shares very similar physicochemical properties with CIN (ionization mode, logP = 3.4; pk_a = 8.24 and chromatographic behavior). Proposed fragmentation patterns are illustrated in Figure 1.

Roy *et al.* [8] used Risperidone (logP = 2.7; pk_a = 8.76) as internal standard, a molecule that does not share as many physicochemical properties with CIN; the authors did not demonstrate the suitability of the molecule (no chromatograms, k′, %CV, among others), having as internal standard a preponderant effect on quantitation by MS. Marques *et al.* [5] did not use internal standard because their method was only for qualitative metabolic screening purposes from incubation of CIN with human liver microsomes.

Chromatography

Regarding chromatography, both CIN and CIS exhibited well-defined, symmetrical sharp peaks at 1.03 min (k′ = 2.21) and 2.23 min (k′ = 5.96), respectively, with a full running-time of 3 min under isocratic conditions. During optimization of mobile phase, there is always a compromise between best ionization and best retention; regarding the pH and considering the very low CIN plasmatic levels expected with a single oral dose of 2 mg, mobile phase was adjusted to pH = 3.0 (ammonium acetate, formic acid and acetic acid were also tested) because of the strong and stable ionization induced in CIN, due to that the majority of the analyte is protonated during positive ESI. Concerning the organic part of mobile phase, when methanol was assayed, suppression was observed in the CIN and CIS signal, and a loss of chromatographic retention; thus, acetonitrile was selected, and the aqueous-organic proportion was optimized to give adequate retention to both analyte and internal standard. Figure 2A depicts a stable and clean baseline without interferences of endogenous peaks, while Figure 2B exhibits an LLOQ

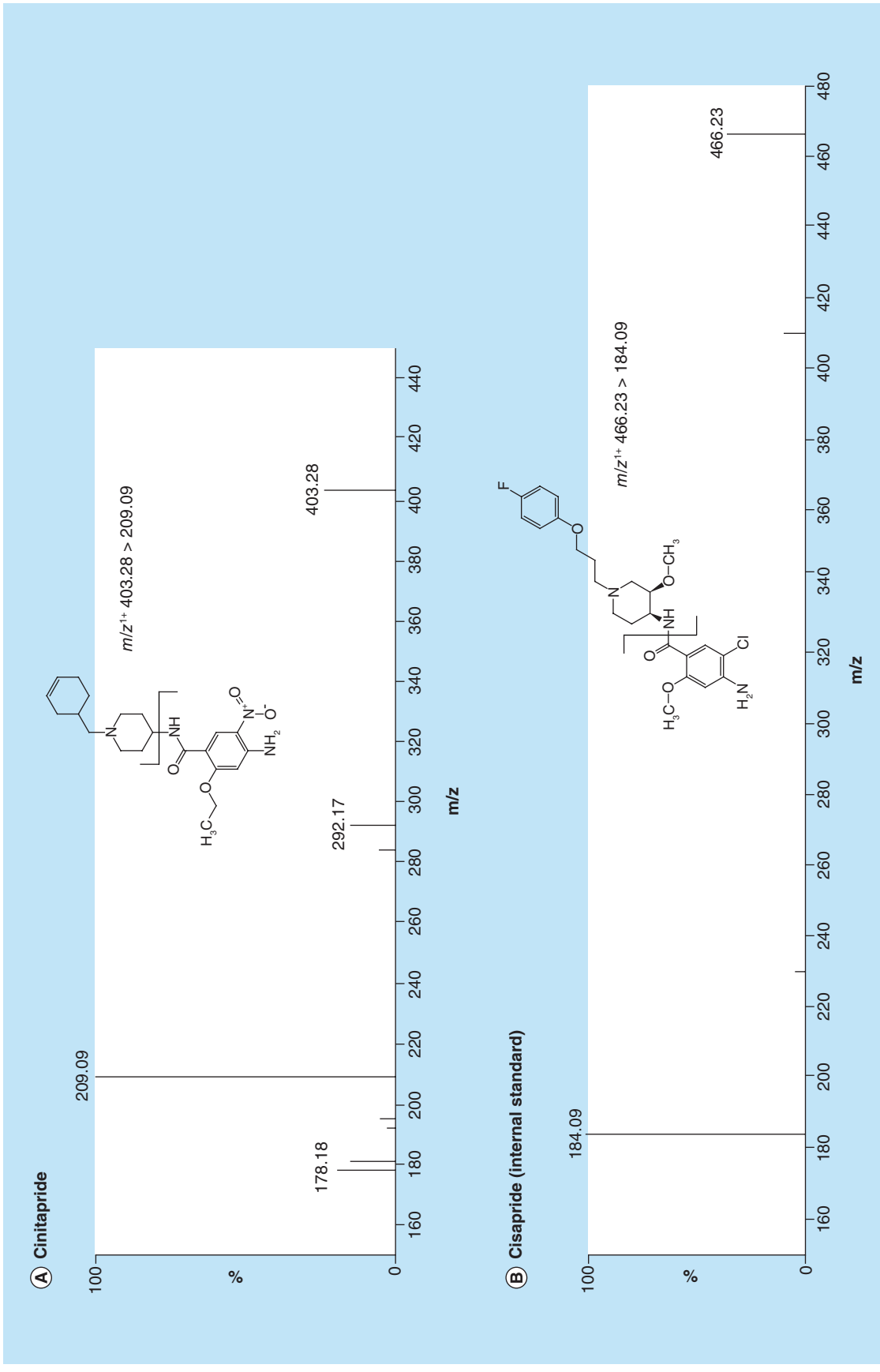

Figure 1. Mass spectra and possible fragmentation patterns of (A) cinitapride, and (B) cisapride (internal standard). The most intense and stable fragments generated under positive multiple-reaction monitoring electrospray ionization (ESI^+) were used for the quantitative analytical technique.

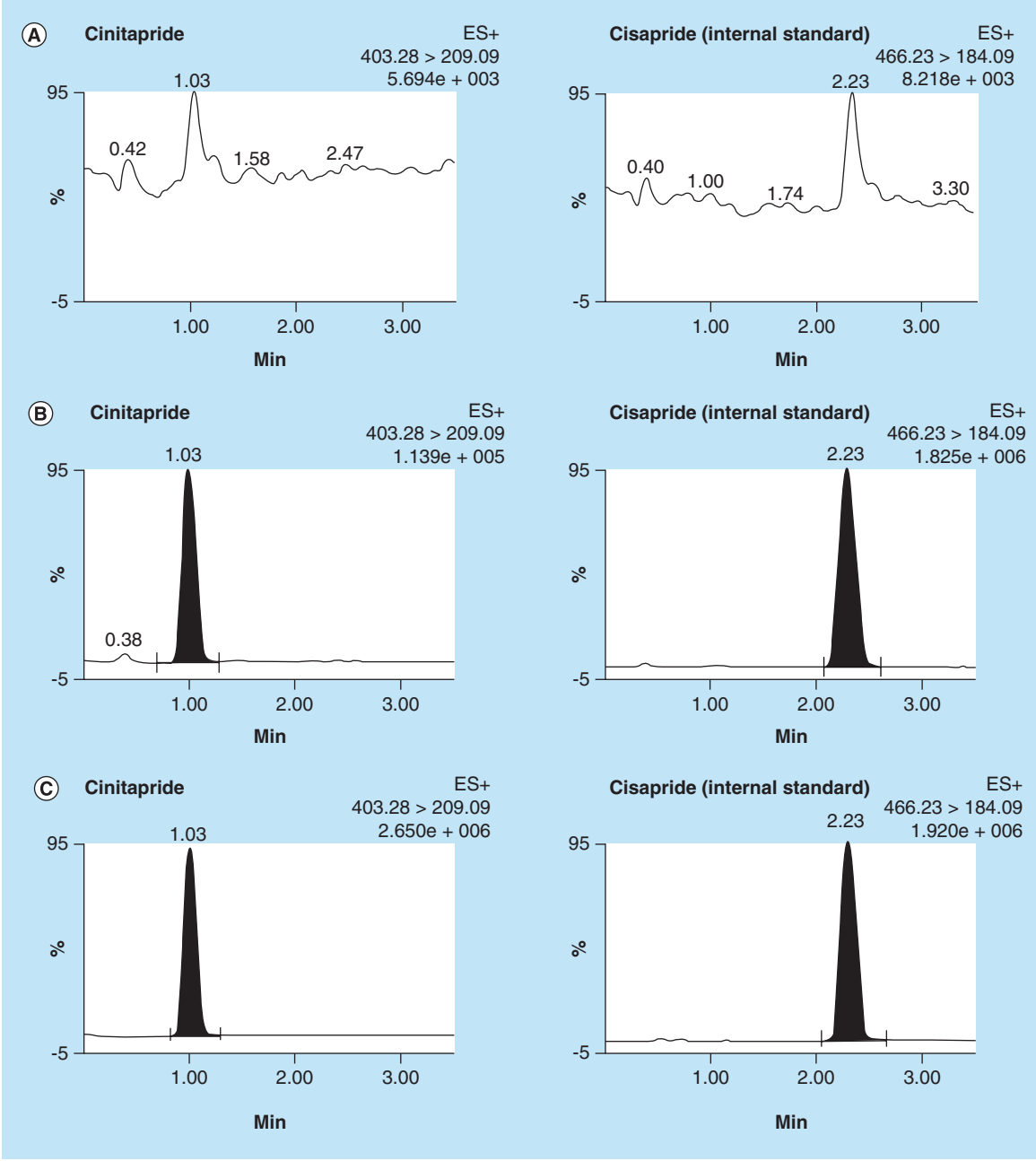

Figure 2. Chromatograms from individual channels of cinitapride and cisapride. (A) Processed blank plasma, **(B)** lower limit of quantification (LLOQ = 50 pg/ml) and **(C)** sample of a volunteer exhibiting maximal cinitapride plasma concentration.

(50 pg/ml) with a signal-to-noise ratio of around 100:1 (calculated with respect to blank CIN channel). Data sampling frequency provided around 15 points-per-peak; all of these parameters contributed to ensure high chromatographic quality [13].

The former method reported by Robert *et al.* [4] employed a mobile phase consisting of acetonitrile: water: 0.25% aqueous ammonia, an unstable phase in terms of pH and volatility, but no more data concerning method validation were included.

Sample processing

One of the most important issues in bioanalysis is to avoid unspecific reconversion of metabolites into 'parent drug' during extractive and/or instrumental procedures and also limited changes in the original drug due to extreme conditions (such as acidic protein precipitation). Thus, liquid–liquid extraction results in a conservative, specific and highly efficient technique to recovering and concentrating free CIN for human plasma.

Several organic solvents were assayed (hexanes and methyl *tert*-butyl ether); however, diethyl ether plus ethyl acetate yielded adequate polarity for extracting CIN in a cleaner manner, and this was reproducible from only 300 µl of biological matrix in a micro-format. Reconstitution with 200 µl of acetonitrile: water gave a concentrating factor of 1.5, and such a volume contributes to minimize sample evaporation inside autosampler. Acetonitrile: water (50:50 v/v) provided cleaner and stable samples, more reproducible retention times and without a harmful effect on chromatographic separation.

Robert *et al.* [4] reported the use of an on-line cleaning up and concentrating process; CIN analysis was performed by introducing centrifuged plasma with a back-flushing technique, coupled to a semi-prep and an analytical column with a multiport valve and having a running time of 10 min. However, the authors did not provide information on possible carryover derived from the HPLC-introducing sample technique, or matrix effects on ionization due to cleaning efficiency. On the other hand, Roy *et al.* [8] employed an expensive and time-consuming SPE, also processing 500 µl of human plasma and reporting recoveries of 55 and 70% for CIN and its internal standard, respectively.

Method validation

The method proved to be linear, precise and accurate during the whole quantification interval. Validation results are included in Table 2.

Selectivity for possible concomitant medication (aspirin, chlorphenamine, acetaminophen, butylhyoscine, ketorolac and loratadine, and also caffeine and cotinine), as well as lipemia and hemolyzed plasma, were tested, exhibiting no interferences.

Table 2. Summary of validations of the assayed method.

Validation parameters	Cinitapride		
	LQC (150 pg/ml)	MQC (750 pg/ml)	HQC (1600 pg/ml)
Between-days (3 day) accuracy and precision			
Mean (n = 18)	153.01	733.04	1530.17
Accuracy (%)	101.90	97.74	95.64
RSD (%)	9.29	4.99	5.36
Within-day accuracy and precision			
Mean (n = 6)	134.12	707.30	1524.32
Accuracy (%)	89.32	94.28	95.27
RSD (%)	10.46	1.57	3.22
Bench-top stability (at least 4 h)			
Mean (n = 6)	138.25	710.72	1506.01
Accuracy (%)	92.07	94.74	94.13
RSD (%)	2.17	4.82	1.13
Autosampler stability (at least 21 h)			
Mean (n = 6)	154.96	766.80	1582.56
Accuracy (%)	103.20	102.21	98.91
RSD (%)	0.24	1.31	5.89
Long-term stability (127 days)			
Mean (n = 6)	146.89	707.24	1553.50
Accuracy (%)	97.92	94.28	97.09
RSD (%)	6.97	6.02	10.43
Three freeze-and-thaw cycles			
Mean (n = 6)	165.88	697.29	1412.08
Accuracy (%)	110.59	92.98	88.26
RSD (%)	4.84	4.25	2.51

HQC: High-quality control; LQC: Low-quality control; MQC: Medium-quality control; RSD: Relative standard deviation.

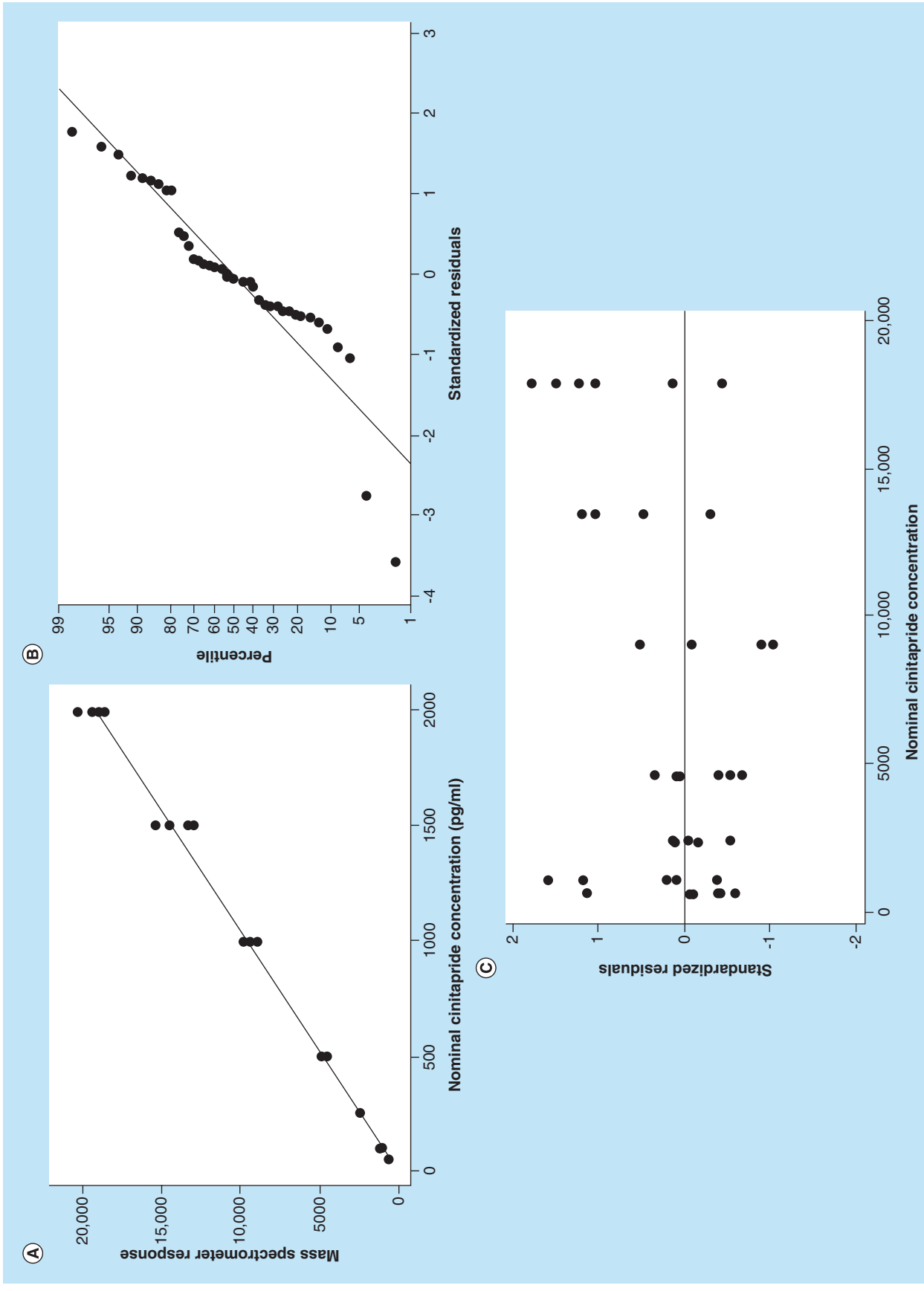

Figure 3. Analysis of linear regression of the calibration curve. **(A)** Scatterplot of Response (cinitapride [CIN] area/cisapride area) versus nominal CIN concentration, **(B)** Probability graphic adjustment to normal distribution of residuals from the linear regression data and **(C)** chart of standardized residuals distribution versus nominal CIN concentration, depicting a homogeneous (nontrendy) distribution that supports the 1/x-weighting regression.

Calibration curves and volunteers' samples should be utilized in the same anticoagulant; however, drug-free plasma supplied by a blood bank is normally citrated. Thus, Mf assays did not show differences between heparinized samples from volunteers and citrated plasma.

During validation, CIN and CIS were processed while exposed to white light, and instability was not observed. Also, a dilution of 1:4 of four-times-concentrated high QC points were assayed and demonstrated that the method is capable of quantifying CIN with precision and accuracy. In addition to all of these, imperceptible carryover was detected (0.94% of LLOQ for CIN, and 1.13% of CIS area).

One of the most important issues during quantitative MS is not only the selection of the regression model, but also the analysis of the residuals, in order to establish the need for weighting the regression [14]. During analysis of the present data, linear regression was simplest, best-fit ($y = 9.417x + 157.88$; $r^2 = 0.9948$) and homogeneous residual distribution that justified 1/x-weighting (see Figure 3). An inadequate regression model (i.e., polynomial function) or weighting factor may exert a profound influence on the extremes of the calibration curve (LLOQ and ULOQ), resulting in underestimation of high concentrations (i.e., C_{max}, which is a punctual criterion of bioequivalence) or overestimation of very low plasma concentration, the latter translated as the calculation of extended residual elimination half-lives with no clinical relevance, which could be merely statistical artifacts.

Clinical trial

All volunteers finished the clinical trial, forming a homogeneous population in terms of age (28 ± 7 years), weight (63.6 ± 6.5 kg) and BMI (23.01 ± 1.6 kg/m^2). Both formulations were well-tolerated, and a sole adverse effect (oropharyngeal and proximal esophageal pain) was reported, which did not require medical treatment.

Pharmacokinetics

The method developed was successfully applied in the PK interaction study of CIN and SIM. Concentration-time profiles of the 26 volunteers for CIN are presented in Figure 4, indicating that 12 h was sufficient sampling time for describing >90% of $AUC_{0-\infty}$. All calculated PK parameters are summarized in Table 3. As can be noted, no significant differences were observed in both C_{max} and AUC_{0-t}, (90% CI

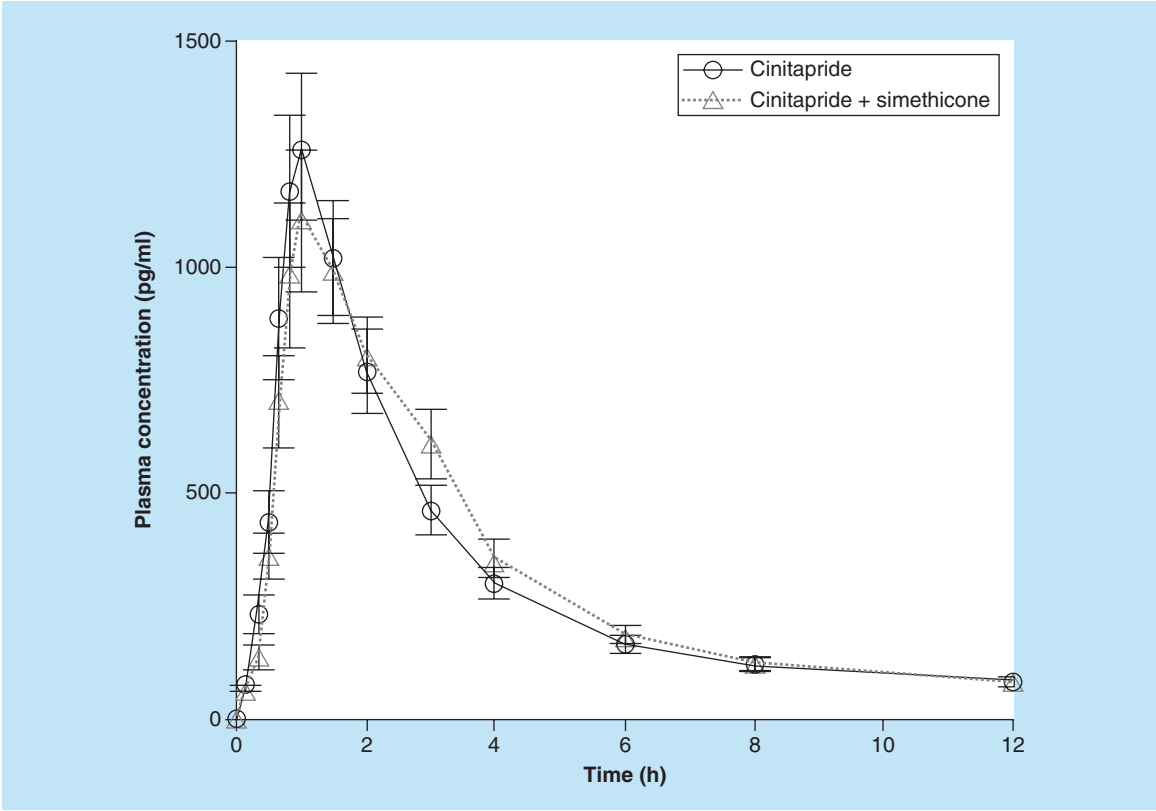

Figure 4. Plasma concentration–time profiles of cinitapride after a single oral dose of two 1-mg tablets (Pemix™) or two 1-mg cinitapride plus 200-mg simethicone tablets (Rogastril Plus™), in a healthy Mexican population under fasting conditions. Data are expressed as means ± standard error.

Table 3. Pharmacokinetic parameters of cinitapride after a single oral dose of two 1-mg tablets (Pemix™) or two 1-mg cinitapride plus 200-mg simethicone tablets (Rogastril Plus™) in a healthy Mexican population under fasting conditions.

Pharmacokinetics	Pemix™	Rogastril Plus™
C_{max} (pg/ml)	1398.66 (±824.45)	1308.46 (±753.99)
T_{max} (h)	1.17 (±0.49)	1.26 (±0.60)
$AUC_{0–12\,h}$ (pg*h/ml)	3464.46 (±2126.89)	3704.02 (±2199.39)
$AUC_{0–\infty}$ (pg*h/ml)	3779.93 (±2220.46)	3998.40 (±2300.62)
$t_{1/2}$ (h)	2.68 (±1.03)	2.76 (±1.31)
Vd_{app} (l)	2446.83 (±1187.12)	2327.32 (±1072.68)
Cl_{app} (l/h)	681.88 (±313.09)	649.62 (±320.23)
MRT (h)	2.75 (±0.72)	3.10 (±0.76)

Data are expressed as mean (±standard deviation).
AUC: Area under the curve; Cl_{app}: Apparent clearance; C_{max}: Maximal drug plasma Concentration; MRT: Mean residence time; T_{max}: Time to reach C_{max}; t½: Plasma elimination half-life; Vd_{app}: Apparent volume of distribution.

were 81.26–106.45 and 96.27–118.27, respectively). Thus, during coformulation, SIM appears not to exert any influence on the bioavailability of CIN. SIM is a nonsoluble dimethyl-siloxane polymer that may adsorb and capture some drugs, with the delivery and oral bioavailability of the coformulated agents becoming erratic. SIM had been previously combined with another serotoninergic prokinetic drug (CIS), with adequate results in the treatment of functional dyspepsia [15]; however, the clinical use of CIS has been discouraged due to the risk of ventricular arrhythmia induction [16].

Furthermore, CIN plasma concentrations exhibit a high dependence on metabolic polymorphism. Robert *et al.* [4] reported a C_{max} of 330 pg/ml with a single dose of 1-mg CIN oral solution immediately before breakfast in German population, while Roy *et al.* [8] reported a C_{max} of 561 pg/ml with the same dose but under different conditions (a 1-mg tablet under fasting conditions) in an Indian population. Current data in Mexican population show a C_{max} of 1398 pg/ml with a 2-mg dose under fasting conditions.

Conclusion

The present UPLC–MS/MS method was, to our knowledge, the first fully validated method according FDA guidelines and recent international recommendations and that was successfully applied in a PK interaction study. The method proved to be rapid, robust, accurate and precise, with low consumption of solvents and biological matrix.

Coformulation in immediate-release tablets and oral administration of SIM plus CIN does not exert any influence on the bioavailability of the latter. Apparently, CIN would entertain a slight bias from linear PKs.

Future perspective

Gastroesophageal reflux and functional dyspepsia are common syndromes in pediatric and in adult patients, where the use of prokinetics are recommended. The knowledge of CIN PKs in our populations becomes an optimized therapeutics, and the lack of influence of SIM on the oral bioavailability of CIN will allow the formulation and commercialization of safe-and-effective oral pediatric suspension and chewable tablets of CIN plus SIM in Latin America.

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Financial & competing interest disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that clinical protocol was reviewed and approved by an independent Ethics Committee. In addition, they have obtained COFEPRIS approval for the conduction of present study. Volunteers signed informed consent, which was formulated according to the latest version of Declaration of Helsinki (64th General Meeting, Fortaleza, Brazil; October 2013).

Executive summary

Clinical use of cinitapride

- Cinitapride (CIN) is a well-tolerated prokinetic drug prescribed for the treatment of gastroesophageal reflux with a safe cardiac-risk profile.

UPLC–MS/MS

- Liquid–liquid micro-extraction is a highly efficient and conservative extractive procedure for CIN bioanalysis, avoiding extraction and/or reconversion of metabolites.
- Cisapride proved to have good performance as internal standard, becoming an accurate and precise quantitative method.
- Positive electrospray provides an intense and stable signal for both molecules, giving a very sensitive technique with a LLOQ up to 50 pg/ml, useful for clinical approaches.

Pharmacokinetic interaction results

- Mexican population exhibits a slightly higher C_{max} under fasting conditions than other populations.
- Oral pharmacokinetics of CIN was not modified by the coformulation of oral immediate-release tablets with simethicone.

References

Papers of special note have been highlighted as:
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