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Human plasma quantification of droperidol and ondansetron used in preventing postoperative nausea and vomiting with a LC/ESI/MS/MS method

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ABSTRACT

An analytical method based upon liquid chromatography coupled to ion trap mass spectrometry (MS) detection with electrospray ionization interface has been developed for the simultaneous identification and quantification of droperidol and ondansetron in human plasma. The two drugs were isolated from 0.5 mL of plasma using a basic liquid–liquid extraction with diethyl ether/heptane (90/10, *v*/*v*) and tropisetron and haloperidol as internal standards, with satisfactory extraction recoveries. They were separated on a 5-μm C_{18} Highpurity column (150 mm × 2.1 mm I.D.) maintained at 30 °C. The elution was achieved isocratically with a mobile phase of 2 mM $HCOONH_4$ pH 3.8 buffer/acetonitrile (60/40, *v*/*v*) at a flow rate of 200 μL/min. Data were collected either in full-scan MS mode at *m*/*z* 100–450 or in full-scan MS–MS mode, selecting the $[M+H]^+$ ion at *m*/*z* = 294.0 for ondansetron, *m*/*z* = 285.2 for tropisetron, *m*/*z* = 380.0 for droperidol and *m*/*z* = 376.0 for haloperidol. The most intense daughter ion of ondansetron (*m*/*z* = 212.0) and droperidol (*m*/*z* = 194.0) were used for quantification. Retention times for tropisetron, ondansetron, droperidol and haloperidol were 2.50, 2.61, 3.10 and 4.68 min, respectively. Calibration curves were linear for both compounds in the 0.50–500 ng/mL range. The limits of detection and quantification were 0.10 ng/mL and 0.50 ng/mL, respectively. The intra- and inter-assay precisions were lower than 6.4% and intra- and inter-assay recoveries were in the 97.6–101.9% range for the three 3, 30 and 300 ng/mL concentrations. This method allows simultaneous and rapid measurement of droperidol and ondansetron, which are frequently co-administrated for the prevention of postoperative nausea and vomiting.

1. Introduction

Many patients suffer from postoperative nausea and vomiting (PONV) after general anaesthesia with volatile anaesthetics or propofol [1]. Receptors that can cause nausea or vomiting or both include dopamine type 2, serotonin type 3, histamine type 1, and muscarinic cholinergic type 1 receptors [2]. Two effective antiemetic agents are droperidol, a dopamine D2 receptor antagonist [3], and ondansetron, a serotonin type 3 receptor antagonist [4]. However, a significant portion of patients continue to suffer from PONV despite the use of an adequate dose of either drug [5,6]. A logical approach is to combine the two drugs because they act on two different receptors of the vomiting pathway. In high-risk patients, combining the two antiemetic drugs has been shown to be more effective than using only one agent alone [7]. Many studies have been published on this combination, focusing on drug pharmacodynamic or pharmacokinetic interactions [8–10]. Moreover, many analytical techniques have been published in the literature for measurement of droperidol [11–13] or ondansetron [14–19] alone, but to our knowledge, no method allowing the simultaneous measurement of both compounds has been published until now.

The present method is the first described for the simultaneous analysis of droperidol and ondansetron in human plasma by means of LC–MS–MS. It has been applied to the study of pharmacokinetic interactions between both compounds in a clinical trial evaluating the impact on QT interval prolongation of each compound used alone or in combination [9].

2. Materials and methods

2.1. Reagents

Ondansetron and droperidol free base were kindly supplied by GlaxoSmithKline (Marly-Le-Roi, France) and OTL Pharma (Paris,

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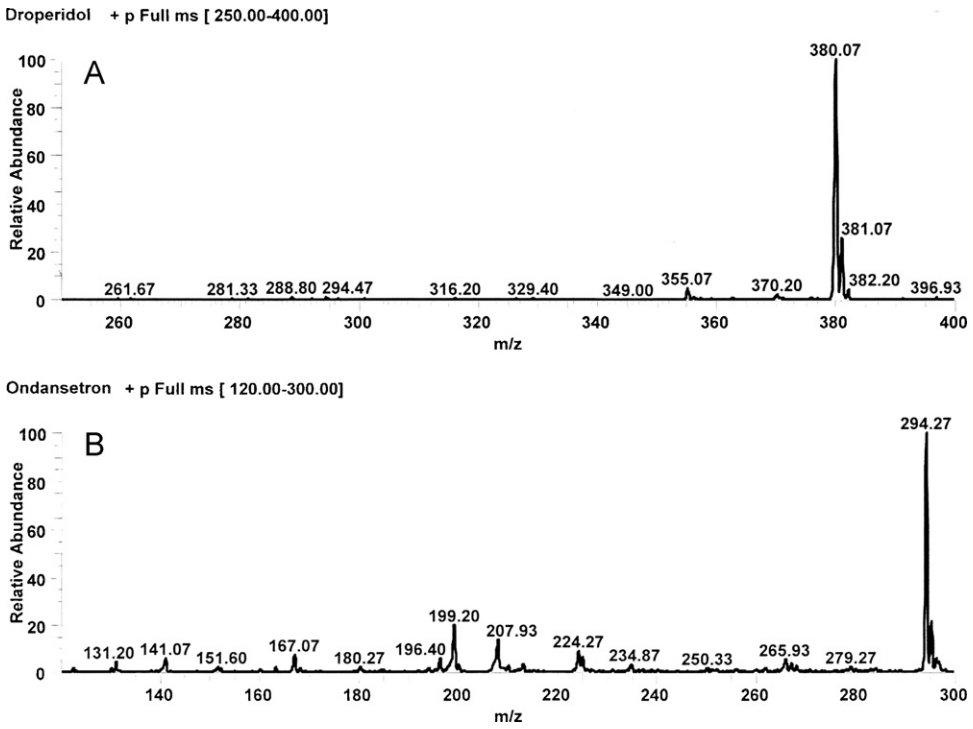

Fig. 1. (A) Full-scan (*m*/*z* 150–500) positive-ion mass spectrum of droperidol. (B) Full-scan (*m*/*z* 150–500) positive-ion mass spectrum of ondansetron.

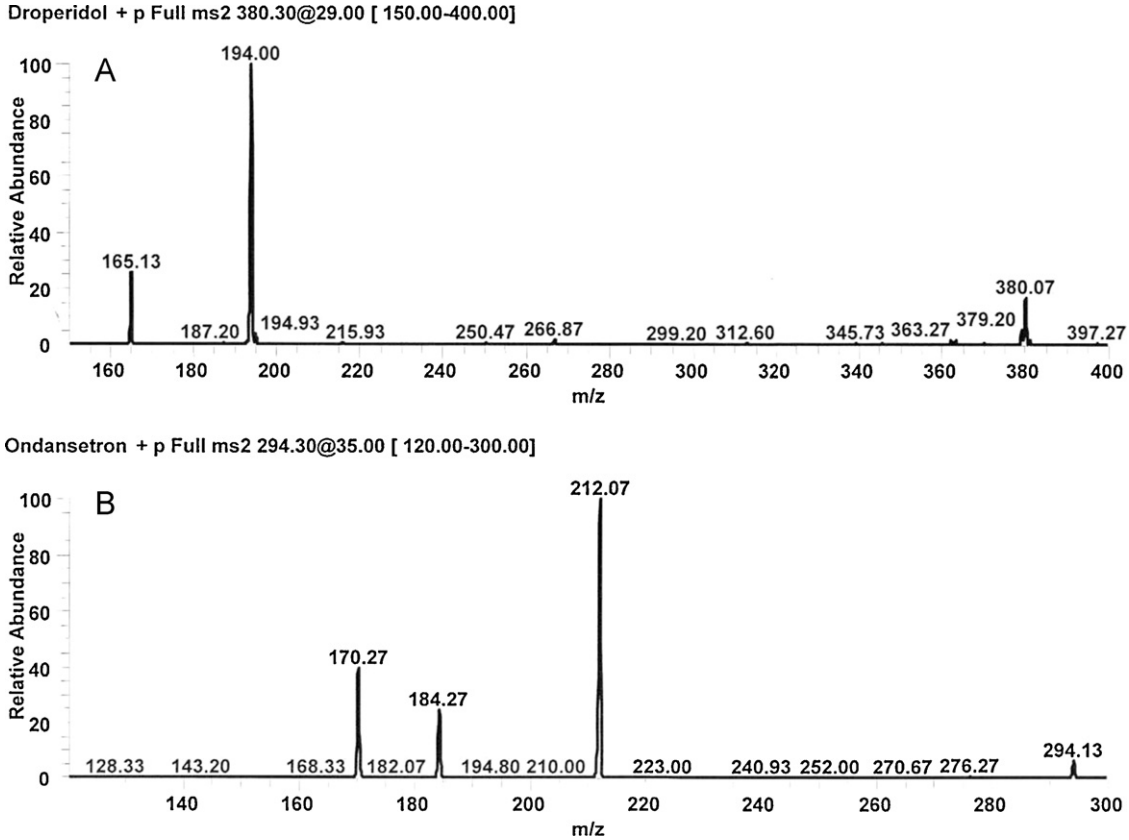

Fig. 2. (A) Full-scan MS–MS spectrum of droperidol with its most intense product ion *m*/*z* 194.0 used for quantification. (B) Full-scan MS–MS spectrum of ondansetron with its most intense product ion *m*/*z* 212.0 used for quantification.

France) Laboratories, respectively. Tropisetron HCl and haloperidol free-base were gift from Jansen-Cilag (Issy-Les-Moulineaux, France) and Novartis Pharma (Rueil Malmaison, France), respectively. Concentrated formic acid (HCOOH), ammonium formate ($HCOONH_4$) and heptane were purchased from Sigma–Aldrich (Paris, France). HPLC-grade acetonitrile, methanol and diethyl ether were obtained from Riedel De Haën (Paris, France), Prolabo (Paris, France) and E. Merck (Darmstadt, Germany), respectively. Purified water was obtained by ultrafiltration using a Direct-Q UV3 apparatus (Millipore Corp., Molsheim, France). Mobile phase buffer was prepared in purified water with a 2 mM $HCOONH_4$ solution (126.2 mg/L), adjusted at pH 3.8 with concentrated HCOOH. It was filtered through a 0.45 μm filter prior to use. Drug-free human plasma for preparing spiked samples was supplied by the local blood bank at the Etablissement Français du Sang (Le Chesnay, France). All other chemicals were of analytical grade.

2.2. Preparation of stock solutions

Primary stock solutions of droperidol and ondansetron were prepared at a concentration of 1.0 g/L in methanol. Working solutions (0.1 mg/L and 0.01 mg/L) were prepared by appropriate dilutions of stock solutions of both compounds in methanol. The stock and working solutions of haloperidol and tropisetron (IS) were prepared in methanol at 1.0 g/L and 0.1 mg/L, respectively. All stock and working solutions were stored at −20 °C in the dark for a maximum of three months and one month, respectively.

2.3. Preparation of calibration curves and quality control samples

Calibration curves were prepared by spiking drug-free plasma (0.5 mL) with appropriate volumes of the above mentioned working solutions to produce the calibration curve points equivalent to 0.5, 1, 5, 20, 100 and 500 ng/mL of droperidol and ondansetron. Six blank plasma samples from six different subjects were prepared and analyzed to check for peaks that might interfere with the detection of both compounds or the IS. Quality control (QC) samples were prepared at three levels, low level (3 ng/mL), medium level (30 ng/mL) and high level (300 ng/mL) by spiking three 10-mL plasma samples in bulk with appropriate volumes of droperidol and ondansetron working solution prepared from separate weighting. They were then aliquoted and frozen at −20 °C. Six aliquots of each level were thawed on each day of analysis.

2.4. Sample preparation

QC, calibration curve, blank plasma samples and patient samples were extracted using a liquid–liquid extraction technique. 50 μL of the internal standard working solution (haloperidol and tropisetron, 0.1 mg/L), 100 μL of 0.5 M NaOH and 4 mL of the extracting solvent diethyl-ether/heptane (90/10, *v*/*v*) were added to each tube containing 0.5 mL of plasma. The samples were then shaken for 10 min and centrifuged at 3500 × *g* for 5 min. The organic layer was introduced into another tube and was evaporated to complete dryness under nitrogen stream. Samples were reconstituted with 75 μL of mobile phase pH 3.8 buffer/acetonitrile (60/40, *v*/*v*), then vortexed for 15 s and transferred to a micro-vial (250 μL, 30 mm × 5 mm, ThermoFischer). 10 μL were injected into the LC system.

2.5. Equipment and chromatographic conditions

Chromatography was performed on Thermo Surveyor HPLC System (ThermoFischer, Les Ulis, France) with an autosampler injector, using a 5-μm C_{18} Highpurity (Thermohypersil, Les Ulis, France) column (150 mm × 2.1 mm I.D.) maintained at 30 °C. The device was completed with a precolumn (C_{18}, 5-μm, 10 mm × 2.0 mm I.D., ThermoFischer). The elution was achieved isocratically with a mobile phase of 2 mM $HCOONH_4$ pH 3.8 buffer/acetonitrile (60/40, *v*/*v*) at a flow rate of 200 μL/min.

2.6. Mass spectrometry

The compounds were detected by a LCQ Deca XP Plus ion-trap mass spectrometer (ThermoFischer, Les Ulis, France) equipped with an ESI source. The ThermoFischer Xcalibur software was used for system control, data acquisition and quantification. Nitrogen (Nitrox UHPLCMS 18, nitrogen generator, Domnick Hunter, Villefranche sur Saône, France) was employed as sheath and auxiliary gas at a pressure of 40 and 10 arbitrary units, respectively. The ESI source was working in the positive ionization mode, and an ion-spray voltage of +5.0 kV was applied. The capillary temperature was set to 250 °C under a voltage of +4 kV. The system was tuned using a continuous 5 μL/min infusion of an ondansetron (1 mg/L) solution in mobile phase pH 3.8 buffer/acetonitrile (60/40, *v*/*v*). The signal was optimized on the total ion current in MS mode. The protonated precursor molecular ions $[MH]^+$ of droperidol (*m*/*z* 380.0), ondansetron (*m*/*z* 294.2) and I.S (*m*/*z* 376.0 for haloperidol and *m*/*z* 285.2 for tropisetron) were trapped with a mass resolution of 1.0 amu, and fragmented by collision induced dissociation with an activation time of 30 ms and a collision energy of 28%, 28%, 35% and 30%, respectively. The daughter ions resulting from these fragmentations were monitored in full-scan. Droperidol was identified by the presence of two daughters' ions at *m*/*z* 194.0 (quantification ion) and *m*/*z* 165.0 (confirmation ion) and ondansetron at *m*/*z* 212.0 (quantification ion) and *m*/*z* 170.1 (confirmation ion). The ions used for quantification for haloperidol and tropisetron were *m*/*z* 165.0 and *m*/*z* 124.0, respectively.

2.7. Validation of the method

The absolute recoveries were evaluated at three levels (3, 30 and 300 ng/mL) by comparing the peak areas of the extracted samples ($n = 6$) with those of extracted blank plasma samples spiked afterwards with the same amount of compounds ($n = 6$). In order to evaluate matrix effect, the peak areas of ten different extracted blank plasma samples spiked afterwards with droperidol and ondansetron (3, 30 and 300 ng/mL) and IS (5 ng/mL for both IS in each case) were compared with those obtained by direct injection of the same amount of compounds ($n = 6$) in mobile phase pH 3.8 buffer/acetonitrile (60/40, *v*/*v*).

For linearity study, six calibration curves were obtained in four days. Linearity was tested by linear regression for the range of concentrations 0.5–500 ng/mL, employing standard calibration curves of at least six points. In addition, six blank and zero plasma samples were also analyzed to confirm absence of interferences. These samples were not used to construct the calibration curves. Quantification was performed by calculating the ratio between (1) the peak-area of droperidol and haloperidol and (2) between the peak-area of ondansetron and tropisetron.

The precision and accuracy of the method were carried out over 3 days. Each day, 1 calibration curve and 6 determinations of each QC level were analyzed. The values obtained were analyzed using analysis of variance (ANOVA), which separated the intra-day and inter-day standard deviations and consequently the corresponding coefficients of variation (CV). The intra-day CV took into account the variability of the 6 replicates each day for 3 days and the inter-day CV the variability of the days of analysis. The accuracy was determined by comparing the mean calculated concentration with the spiked target concentration of the QC samples.

The limit of detection (LOD) was defined as the lowest concentration of the compound that can be detected with a signal-to-noise

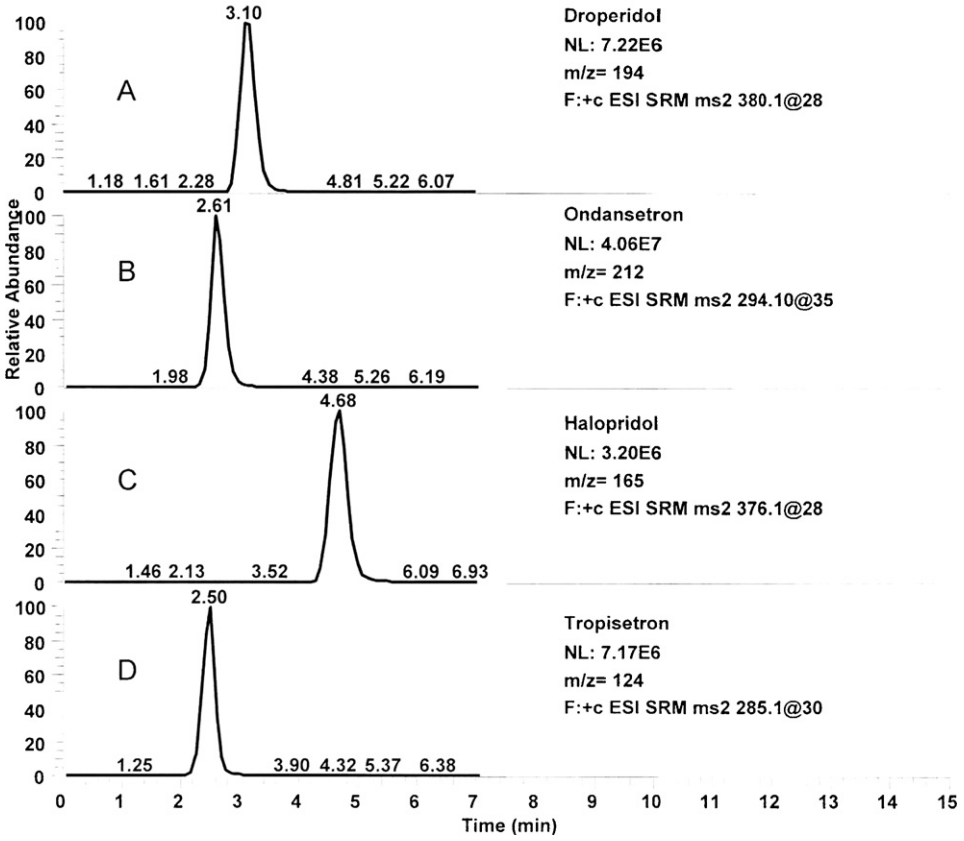

Fig. 3. LC–MS/MS chromatograms of droperidol (A: ion *m*/*z* 194.0), ondansetron (B: ion *m*/*z* 212.0), haloperidol (C: ion *m*/*z* 165.0) and tropisetron (D: ion *m*/*z* 124) obtained from extracted drug-free plasma spiked with 30 ng/mL droperidol and ondansetron and 10 ng/mL of both IS (haloperidol and tropisetron).

ratio greater than 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of droperidol and ondansetron that can be measured with both an accuracy of ±15% of the true value and a coefficient of variation (CV) ≤20%.

The ability to dilute samples originally above the upper limit of the standard curve was assessed by calculating accuracy and precision parameters for a plasma QC sample prepared at a nominal concentration of 1000 ng/mL and then diluted at 1:4.

3. Results

3.1. Separation and specificity

Fig. 1 presents the full-scan (*m*/*z* 120–400) positive-ion mass spectra of droperidol (A) and ondansetron (B). It was recorded from the continuous infusion of a 1.0 mg/L solution of each compound in mobile phase pH 3.8 buffer/acetonitrile (60/40, *v*/*v*). The spectra show major peaks at *m*/*z* 380.1 and *m*/*z* 294.3 due to protonated $[M+H]^+$ droperidol and ondansetron ions, respectively. The full-scan MS–MS spectra of droperidol and ondansetron obtained with collision energies of 29% and 35% are shown in Fig. 2A and B, respectively. For droperidol, the most intense product ion observed was *m*/*z* 194.0, which was used for quantification. The ion *m*/*z* 165.1 was used as a confirmation ion. For ondansetron, the most intense product ion observed was *m*/*z* 212.1 and was used for quantification. The ion *m*/*z* 170.3 was used as a confirmation ion. The full-scan positive-ion mass spectra of haloperidol and tropisetron show a major peak at *m*/*z* 376.0 and *m*/*z* 285.2 and the most intense product ions observed in the full-scan MS–MS mode with collision energies of 35% and 30% were *m*/*z* 165.0 and *m*/*z* 124.0, respectively (data not shown). Chromatograms of droperidol, ondansetron, haloperidol and tropisetron most intense daughter's ions, obtained from 0.5 mL of drug-free plasma spiked with 30 ng/mL of droperidol and ondansetron and with 10 ng/mL of IS, are shown in Fig. 3A–D, respectively. The retention times of the four compounds were 3.10, 2.61, 4.68 and 2.50 min, respectively, ensuring both a short analysis time and an adequate resolution. No endogenous peak was observed in all blank plasma samples tested.

3.2. Method validation

Results of the extraction recoveries determined for droperidol ranged from 75% at 3 ng/mL to 87% at 30 ng/mL and 300 ng/mL. For ondansetron, recoveries were 51% at 30 ng/mL, 65% at 30 ng/mL and 67% at 300 ng/mL. Analytical recoveries of internal standards were 88% for haloperidol and 93% for tropisetron. No matrix effect was observed in plasma samples versus mobile phase solution. Peak areas obtained in extracted blank plasma samples spiked afterwards with the four compounds were in the range 80–111% of those obtained after direct injection of the same amount of compounds in mobile phase.

The method exhibited a reliable linear response in the range 0.5–500 ng/mL for both compounds, and no weighting factor was useful. From six calibration curves over the range 0.5–500 ng/mL a high correlation ($r > 0.99$ in both cases) was observed between concentrations and peak-areas ratios of droperidol or ondansetron and the corresponding IS. The equations for the relationship between peak-area ratio (y) and plasma concentration (x) were $y = 0.1973x - 0.0315$ for droperidol and $y = 0.0385x + 0.0056$ for ondansetron. Goodness of fit was confirmed by comparison of back-calculated concentrations to nominal concentrations with linear regression and comparison of the slope to 1 and of the intercept to 0 by a Student's *t*-test. No significant difference was observed

Table 1
Intra-day and inter-day precisions and accuracies.

	Droperidol			Ondansetron		
Concentration (ng/mL)	3	30	300	3	30	300
Intra-assay (CV %)	4.0	2.3	2.7	3.2	4.8	4.8
Inter-assay (CV %)	4.4	3.6	2.2	4.5	2.0	6.4
Accuracies (%)	97.6	101.9	100.1	100.1	98.6	97.6

Six replicates of the three QC levels were analyzed three different days. The values obtained were analyzed using variance analysis (ANOVA).

between slope and 1 and between *y*-intercept and 0 for the two equations.

Accuracy and precision of the method were measured by analyzing 54 QC samples. Data are presented in Table 1. Intra-day precision was lower than 4.8% while inter-day precision was lower than 6.4% for both compounds. Accuracies were in the 97.6–101.9% range. LOD was set at 0.1 ng/mL and LOQ at 0.5 ng/mL for the two compounds. The ability to dilute samples originally above the upper limit of the standard curve was demonstrated since accuracy and precision parameters for a plasma QC sample prepared at a nominal concentration of 1000 ng/mL and then diluted at 1:4 were 8.2% and 100.4%, respectively ($n = 6$).

No significant difference in QC samples concentration was observed after a three-month storage at −20 °C.

3.3. Application

This method was applied to the study of pharmacokinetic interactions between both compounds in a clinical trial evaluating the impact on QT interval prolongation of each compound used alone or in combination. In this study, 16 volunteers were given a single dose of 1 mg droperidol or 4 mg ondansetron, either alone or in combination, or a placebo. Each study period was separated by a 48 h to 2 weeks drug-free interval. Fourteen blood samples were collected for each subject at each period. No significant difference was observed in pharmacokinetic parameters between droperidol and ondansetron when each drug was used alone or co-administered, either for C_{max}, area under the curve (AUC_{inf}) or terminal half-life [9].

4. Discussion

Postoperative nausea and vomiting constitutes a major unpleasant symptom in the postoperative period. The prevention of PONV is judged equally important as the prevention of postoperative pain. A number of studies have shown that the combination of droperidol and ondansetron provided better prophylaxis against early or overall PONV when compared with either drug alone [5,7] with side effects, notably QT interval prolongation, being not different when compared with that induced by droperidol alone [9]. However, to our knowledge, there was no method allowing the simultaneous measurement in plasma of both compounds published until now. This present method allows such a measurement, with acceptable validation criteria. Sensitivity may be a major criterion for a method devoted to the analysis of these compounds, since they were used in PONV at very low dosages. The LOD of our method is 0.1 ng/mL and the LOQ is low at 0.5 ng/mL, consequence of the MS–MS detection. The low LOQ of our method allowed the use of only 0.5 mL of sample, making it particularly convenient for droperidol and ondansetron quantification in human plasma in pharmacokinetic studies when many samples have to be collected as shown in our clinical pharmacokinetic study in which 14 blood samples were collected at each period during 4 periods.

A single-step liquid–liquid extraction with diethylether/heptane (90/10, *v*/*v*) was employed for reasons of speed and convenience. Results of the absolute recoveries were acceptable, ranging from 51% to 67% for ondansetron and 75% to 87% for droperidol. These absolute recoveries were evaluated by comparing the peak areas of the extracted samples with those of extracted blank plasma samples spiked afterwards with the same amount of compounds, in order to detach extraction efficiency from ionization suppression. When comparing the peak areas of the extracted samples spiked afterwards with those obtained by direct injection of the same amount of compounds in mobile phase, results were in the 80–111% range, showing no significant matrix effect for both compounds and their internal standards.

Linearity was validated up to 500 ng/mL, which appeared to be sufficient for therapeutic drug monitoring since in our pharmacokinetic study only one subject presented a C_{max} above this value (890 ng/mL).

Haloperidol and tropisetron were used as I.S. because of their chemical structure similar to that of droperidol and ondansetron.

5. Conclusion

To our knowledge, the present method is the first described for the simultaneous measurement of droperidol and ondansetron in human plasma by means of LC–MS–MS. Owing to the single-step liquid–liquid extraction, the use of 0.5 mL of sample and MS–MS detection, it is simple, rapid, and highly specific and sensitive. The acceptable validation criteria results of the method and the short chromatographic run time allows its use for the detection and quantification of droperidol or ondansetron, notably in pharmacokinetics studies when many samples with low amount of plasma have to be analyzed, as shown in our clinical study.

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