BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **16**: 187–190 (2002)
DOI: 10.1002/bmc.125

ORIGINAL RESEARCH

Simultaneous determination of ondansetron and tropisetron in human plasma using HPLC with UV detection

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Received 16 July 2001; revised 1 August 2001; accepted 20 August 2001

ABSTRACT: A rapid and sensitve HPLC method for the simultaneous quantitation of ondansetron and tropisetron, two serotonin (5-HT) receptor antagonists frequently used in treatment and prevention of nausea and emesis, is described. The procedure involves liqid–liquid extraction of human plasma with dichloromethane coupled with reversed-phase HPLC and UV detection. The lower limits of quantification (LOQ) were 0.62 ng/mL for ondansetron and 1.25 ng/mL or tropisetron. Intra- and inter-assay coefficients of variation ranged from 1.5 to 7.5% and 5.3 to 13.7%, respectively. The sensitivity and precision were sufficient for determination of plasma concentrations after therapeutic administration of both drugs and the method can be used for the estimation of pharmacokinetic parameters. Copyright © 2002 John Wiley & Sons, Ltd.

INTRODUCTION

The serotonin (5-hydroxy-tryptamine; 5-HT) type 3 receptor (5-HT_3) antagonists ondansetron and tropisetron (Fig. 1) have become first line therapy for the treatment of postoperative nausea and emesis as well as of emetogenic side effects of cancer chemotherapy (Gregory and Ettinger, 1998; Hesketh, 2000; Loewen *et al.*, 2000; Oge *et al.*, 2000). The biotransformation of these drugs is mediated by multiple cytochrome P-450 enzymes, among them the polymorphic CYP2D6 and CYP1A2 (Fischer *et al.*, 1994; Dixon *et al.*, 1995) resulting in a high interindividual variability in plasma concentrations and effectiveness. The control of emetogenic side effects is of major importance for the success of cancer chemotherapy. Pharmacogenetic studies and monitoring of drug levels serve as tools to determine the optimal dosage for each patient, thus generating a demand for fast, simple and sensitive quantitation methods for ondansetron and tropisetron in human plasma under the conditions of drug treatment.

Previously described methods for ondansetron (Liu and Stewart, 1997) focused on the separation of enantiomers and the validated concentration range of 15–750 ng/mL was not suitable for the measurement of ondansetron trough levels. Colthup *et al.* (Colthup and Palmer, 1989; Colthup *et al.*, 1991) presented a method

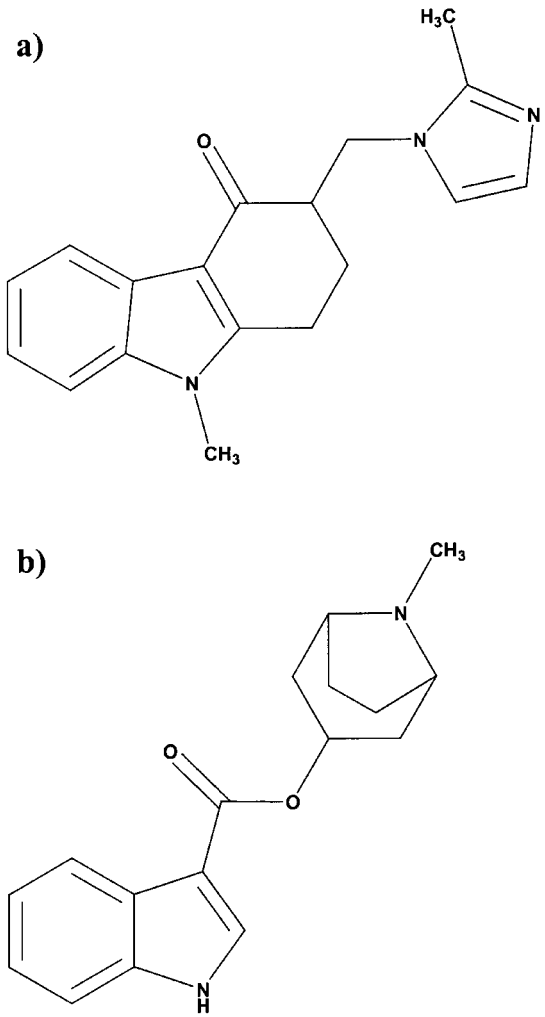

Figure 1. Structures of the 5-HT_3 antagonists ondansetron (a) and tropisetron (b).

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Abbreviations used: 5-HT, serotonin; LOD, limit of detection; LOQ, limit of quantitation.

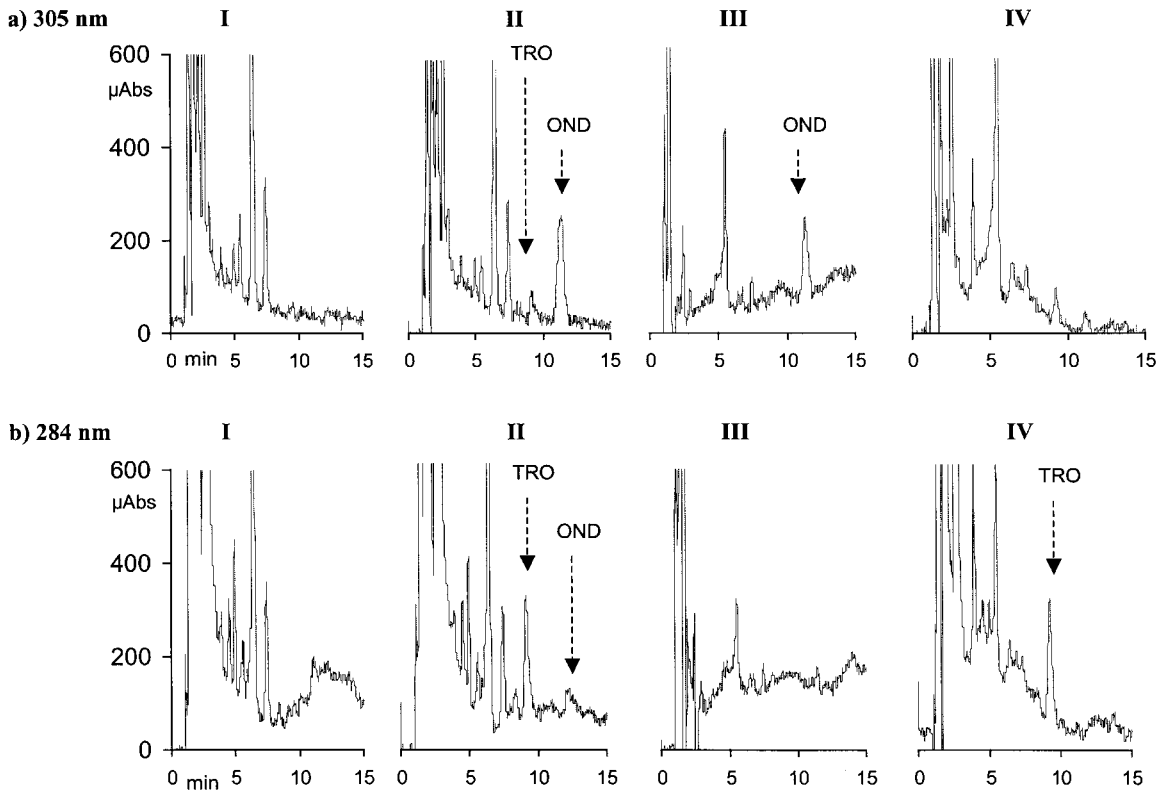

Figure 2. HPLC traces of ondansetron (OND) and tropisetron (TRO) using UV detection at 305 nm (a) and 284 nm (b), respectively. I, blank plasma sample; II, calibration standard containing 5 ng/mL ondansetron and 5 ng/mL tropisetron; III, plasma sample 6 h after oral administration of 8 mg ondansetron (3.1 ng/mL); IV, plasma sample 3 h after oral administration of 5 mg tropisetron (4.7 ng/mL).

for the quantitation of ondansetron with a working range of 1–20 mg/mL, which involved a solid phase extraction with multiple steps for column conditioning, washing and sample extraction. Tropisetron has been determined in rat blood by Huang *et al.* (1999) at high concentrations (1–5 µg/mL) only; to our knowledge this is the first method described for the quantitation of tropisetron in human plasma. The simple and sensitive method for the determination of ondansetron described by Depot *et al.* (1997) was adapted to allow quantitation of both analytes under identical conditions using UV detection at two different wavelengths.

EXPERIMENTAL

Chemicals and Reagents

Ondansetron hydrochloride was obtained from Glaxo SmithKline (Bad Oldesloe, Germany) and tropisetron hydrochloride was provided by Novartis Pharma (Basel, Switzerland). Sodium carbonate, sodium hydrogen phosphate, dichlormethane, methanol and acetonitrile were of HPLC or analytical grade and were purchased from Merck (Darmstadt, Germany). Blank human plasma was obtained from the blood bank of the University Hospital Charité, Humblodt-University Berlin.

Chromatography

Ondansetron and tropisetron were quantified using a Shimadzu HPLC system (Duisburg, Germany) consisting of a pump (LC 10AS), an automatic sampler (SIL 10A), and a dual wavelength UV detector (SPD 10AVP). The Class LC10 software Version 1.6 (Shimadzu) was used for data analysis and processing. The compounds were separated at room temperature on a Phenomenex Luna $C_{18}(2)$ column (5 µm, 250 × 4.6 mm i.d.; Phenomenex, Aschaffenburg, Germany) with a Phenomenex Luna C_{18} (2) guard column (5 µm, 4 × 4.6 mm i.d.) and quantified by UV detection at 305 nm for ondansetron and 284 nm for tropisetron. The mobile phase consisted of 20% of acetonitrile and 80% of 0.05 M sodium hydrogen phosphate buffer (pH 5.0) and was delivered at a flow rate of 1.5 mL/min. The analytes were quantified using peak height.

Sample preparation

In a 10 mL glass tube 1 mL plasma was mixed with 50 µL of saturated sodium carbonate solution and 5 mL of dichloromethane and extracted with a rotary mixer for 15 min at room temperature.

Table 1. Limits of detection (LOD) and limits of quantitation (LOQ) for ondansetron and tropisetron in human plasma

		LOQ ($n = 18$)		
	LOD (pg)	pg/mL	Inter-assay CV	Accuracy
Ondansetron	100	620	13.2%	110.9%
Tropisetron	200	1250	16.4%	107.0%

Table 2. Assay linearity for the quantitation of ondansetron and tropisetron in human plasma ($n = 10$)

		Mean	SD	Range
Ondansetron	Slope	52.1	3.8	46.7–57.2
	y-axis intercept	16.1	28.8	−13.1–71.5
	r^2	0.9987	0.0016	0.9959–0.9996
Tropisetron	Slope	51.9	4.7	44.5–58.7
	y-axis intercept	95.6	92.9	−16.9–226.99
	r^2	0.9974	0.005	0.9950–0.9989

The mixture was centrifuged for 5 min at 5000***g*** and 4.5 mL of the organic phase were transferred into a new tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 100 μL of mobile phase and a volume of 50–75 μL was injected into the HPLC for analysis.

Preparation of stock solutions, calibration standards and quality control samples

Stock solutions (100 ng/μL) were prepared by dissolving 5.64 mg of tropisetron hydrochloride or 5.62 mg of ondansetron hydrochloride in methanol in a 50 mL volumetric flask. The solutions were stored at −20°C.

For preparation of calibration standards 4 μL of both stock solutions were measured using a 10 μL Hamilton syringe, evaporated to dryness and reconstituted in 20 mL of blank human plasma yielding the highest calibration standard with a concentration of 20 ng/mL ondansetron and tropisetron, which was subsequently used to generate standards with final concentrations of 0.62, 1.25, 2.5, 5.0 and 10.0 ng/mL by serial dilution with blank plasma.

For preparation of quality control samples appropriate aliquots of the stock solutions were evaporated to dryness and reconstituted in blank human plasma. The final ondansetron and tropisetron quality control concentrations were 1.9, 3.8, 7.5 and 15.0 ng/mL. Calibration standards and quality control samples were stored in aliquots of 1.1 mL at −20°C until analysis.

RESULTS

Separation and specificity

Ondansetron and tropisetron were well separated under the HPLC conditions applied. Retention times were 8.9 min for tropisetron and 11.3 min for ondansetron. No interferences were observed in blank plasma samples and in samples from patients undergoing treatment with various chemotherapeutic agents. Figure 2 shows the HPLC chromatograms of a blank plasma sample (I), a calibration standard (II), and plasma samples after administration of ondansetron (III) and tropisetron (IV) recorded simultaneously at 305 and 284 nm.

Limit of detection and limit of quantitation

The limit of detection (LOD) was determined as the amount of drug corresponding to a signal-to-noise ratio of 3:1. The limit of quantitation (LOQ) was determined as the lowest concentration of the analyte in plasma that could be quantified with an inter-assay coefficient of variation (CV) of <20% and an accuracy between 80% and 120%. The respective values for the two analytes are reported in Table 1.

Linearity

Assay linearity was evaluated up to concentrations of 20 ng/mL ondansetron and tropisetron. The mean slopes and r^2 values with SD and range are reported in Table 2.

Intra-assay and inter-assay variation

The intra-assay CV for the four quality control samples ranged from 1.5 to 17.5% and the inter-assay CV ranged from 5.3 to 11.6% (Table 3).

Accuracy

The accuracy of the measurements was determined using four quality control samples for each compound in every run and the results are reported in Table 3.

Table 3. Intra-assay and inter-assay variability and accuracy for the quantitation of ondansetron and tropisetron in human plasma

		Intra-assay ($n = 10$) Measured		Inter-assay ($n = 15$) Measured	
	Nominal (ng/mL)	Mean (ng/mL)	CV	Mean (ng/mL)	CV
Ondansetron	1.9	2.02	3.6%	1.86	9.4%
	3.8	3.68	2.1%	3.60	4.8%
	7.5	7.39	3.3%	7.45	5.3%
	15.0	14.20	2.4%	14.77	5.7%
Tropisetron	1.9	1.80	7.5%	1.86	11.6%
	3.8	3.88	5.4%	3.57	13.7%
	7.5	7.38	4.5%	7.63	8.9%
	15.0	12.88	1.5%	14.62	8.5%

DISCUSSION

We introduced a method for the simultaneous determination of ondansetron and tropisetron in human plasma combining a simple liquid–liquid extraction procedure with highly sensitive isocratic reversed-phase HPLC analysis and UV detection at two different wavelengths. This is the first method describing the analysis of tropisetron in human plasma and was designed to determine both compounds under identical conditions thus facilitating rapid sample processing and quantitation. Previously published methods were available for ondansetron only and lacked sufficient sensitivity (Kelly *et al.*, 1993; Liu and Stewart, 1997) or involved a multistep solid phase extraction (Colthup and Palmer, 1989). Limits of quantitation of 0.62 ng/mL for ondansetron and 1.25 ng/mL for tropisetron were sufficient for the determination of trough levels of both 5-HT_3-antagonists observed during antiemetic treatment of patients undergoing cancer chemotherapy.

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