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Short communication

High-performance liquid chromatographic analysis for the determination of domperidone in human plasma

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Abstract

A specific and sensitive high-performance liquid chromatographic method for the determination of domperidone in human plasma is described. Domperidone was isolated by solid-phase extraction using nitrile SPE cartridges. The drug was eluted with a mixture consisting of methanol–triethylamine–acetic acid, separated on a reversed-phase column, and measured by fluorimetric detection after post-column photoderivatization. The absolute extraction recovery from plasma samples was 83%. The limit of quantitation was established as 1 ng/ml. The relative standard deviation of the determination of plasma levels by this method over the standard curve concentration range was less than 10%, expect with the concentration of 1 ng/ml. The suitability of the method is shown for pharmacokinetic studies. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Domperidone

1. Introduction

Domperidone (5-chloro-1-{1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one) is a dopamine antagonist with antiemetic properties similar to metoclopramide and certain neuroleptic drugs. Unlike these drugs, however, domperidone does not readily cross the blood–brain barrier and seldom causes extrapyramidal side-effects [1,2].

There is little information available on the pharmacokinetics of domperidone in humans, mainly because of the lack of a convenient assay. In early studies, the pharmacokinetics of domperidone were determined in human volunteers by a radioimmunoassay (RIA) method using antibodies raised in rabbits against domperidone [3,4]. Two recently published assay methods describe the quantitative analysis of domperidone in biological specimens [5,6]. The assays provided sufficient sensitivity and selectivity for the determination of domperidone in rat plasma and in human serum, using high-performance liquid chromatography (HPLC) with fluorescence and electrospray ionization mass spectrometric detection. However, these two chromatographic methods both use time-consuming liquid–liquid sample preparation procedures of two steps or more. This paper describes a HPLC method using a relatively simple solid-phase extraction (SPE) procedure for sample preparation prior to analysis of domperidone in human plasma. In addition, unlike liquid–liquid extraction schemes, the present extraction can be easily automated. The method employed reversed-phase chromatography with fluores-

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cence detection after post-column photoderivatization. This method has been shown to be sensitive, accurate, reproducible and suitable for analyzing a large number of samples to support bioavailability and pharmacokinetic studies.

2. Experimental

2.1. Chemicals

Domperidone maleate was supplied by International Pharmaceutical Chemicals (Wormerveer, The Netherlands). Cisapride was supplied by the Pharmacy Department of the Pharmaceutical Research Institute (Warsaw, Poland). HPLC-grade methanol, acetone and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other reagents were of analytical grade. SPE CN cartridges (200 mg, 3 ml) were obtained from Waters (Milford, MA, USA).

2.2. Apparatus

The HPLC system consisted of a Model LC-10A pump coupled to a Model SIL-10A autosampler, a Model CTO-10A oven, a Model RF-551 fluorescence detector operated at 282 nm for excitation and 326 nm for emission wavelength, a Model SCL-10A system controller (Shimadzu Europa, Duisburg, Germany) and a Model A1026 post-column photoreactor (Knauer, Berlin, Germany). The apparatus was connected to a personal computer with Class-LC10 Chromatography Data System software (Shimadzu).

The post-column photoreactor consisted of a knitted PTFE reactor coil (6 m×0.3 mm I.D.) and a low-pressure UV lamp, 1.2 watt UV capacity, 85% at 254 nm.

SPE was performed using a Chromabond 12-column vacuum manifold (Macherey–Nagel, Düren, Germany).

2.3. Chromatographic conditions

The chromatographic separation was performed using a Supelcosil LC-8 analytical column (150×4.6 mm I.D., 5 μm; Supelco, Bellefonte, PA, USA), preceded by a 20×4.6 mm I.D. LC-8 Supelguard column. The column was heated at 35°C. The mobile phase consisted of methanol–water–triethylamine–acetic acid (60:40:0.02:0.3, v/v) and was delivered at a flow-rate of 1.4 ml/min. The solution was filtered using a 0.45-μm nylon membrane (Supelco) and ultrasonically degassed prior to use.

2.4. Plasma samples

Venous blood samples (8 ml) were withdrawn into heparinized tubes. Blood samples were centrifuged at 1000 *g* for 10 min at 4°C and the plasma obtained was stored at −70°C until analysis. Stability was determined in samples comprising 10 ng/ml of domperidone in human blank plasma stored at −70°C for 2 months.

2.5. Extraction procedure

To 1 ml of plasma in a glass tube were added 20 μl of methanol, 50 μl of an aqueous internal standard solution of cisapride (4 μg/ml) and 0.1 ml of 0.1 *M* HCl. The plasma was mixed for 10 s with a hand vortex mixer and centrifuged at 2000 *g* for 10 min. Then, the sample was applied to a 3 ml SPE CN cartridge which had been previously activated by washing with 2 ml of water and 1 ml of 0.1 *M* HCl. The solution was passed slowly through the column by mild suction ($<$1 ml/min). Then, the column was washed with 2 ml of water and 1 ml of acetone and was dried under vacuum for 10 min. An aliquot of 1 ml mixture consisting of methanol (100 ml), triethylamine (0.03 ml), and acetic acid (0.3 ml) was applied to the column. The liquid was allowed to pass through the column by gravity and finally drained completely by centrifugation at 2000 *g* for 1 min. The eluent was evaporated to dryness under an air stream at 50°C. The residue was reconstituted in 200 μl of mobile phase and an aliquot of this solution (20 μl) was injected onto the HPLC system for analysis. Samples having a concentration of domperidone higher than 20 ng/ml were diluted with control plasma.

2.6. Standard solutions

Stock solutions of domperidone maleate (1 mg/ml calculated as free base) and internal standard cisap-

ride (1 mg/ml) were prepared in methanol and stored at −20°C.

2.7. Calibration and recovery

Calibration was performed by adding known amounts of domperidone to blank human plasma to yield concentrations over the range 1–20 ng/ml. These standards were than extracted according to the procedure described in Section 2.5.

The absolute recovery of domperidone from human plasma was calculated by comparing the peak height obtained from extracts of spiked plasma samples and the peak height obtained from direct injection of known amounts of standard solutions of domperidone.

The precision of analysis was assessed by six replicate analyses of human plasma spiked with domperidone to give concentrations of 2, 10 and 20 ng/ml, and then the within-day variations were calculated. Between-day variations were also calculated for above concentrations.

Quality control (QC) samples prepared at the same concentrations were placed at random among volunteer samples in each analytical batch. Revalidation was assessed from the standard curves made on days when volunteers' samples were analyzed. The limit of detection (LOD) was defined as the sample concentration of domperidone resulting in a peak height of 3 times the noise level. The limit of quantitation (LOQ) was the lowest point on the calibration curve which can be detected with variation below 15%.

2.8. Application

The method was applied to the plasma from 32 healthy volunteers (male), who participated in pharmacokinetic studies of domperidone. The study was performed according to the ethical guidelines of the revised Declaration of Helsinki. All subjects gave written informed consent and the study protocol was approved by the Ethical Committee of the Rzeszów Hospital. The subjects received Motilium tablets (Janssen, Copenhagen, Denmark) 10 mg orally in a 20-mg dose (two tablets). Blood samples were taken from a forearm vein into heparinized tubes immediately before (time zero) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 9, 12, 15 and 24 h, after drug administration.

2.9. Analysis of data

The pharmacokinetic parameters for domperidone were determined from the plasma concentration–time data with the aid of the Pharm/PCS program [7]. The maximum plasma concentration (C_{max}) and the time to peak (T_{max}) for domperidone were taken directly from the experimental data. The elimination rate constant (k_{el}) was estimated by least-square regression analysis from the data of the last 3–4 points of each plasma concentration–time curve. The terminal elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/k_{el}$. The area under the concentration versus time curve (AUC_{0-t}) was calculated using the trapezoidal rule and then extrapolated to infinity.

3. Results and discussion

UV irradiation of domperidone increased its native fluorescence about three times. No changes were observed either in excitation or emission spectra before and after photoreaction. The 6 m long reactor coil of 0.3 mm I.D. gave an irradiation time of 24 s at a flow-rate of 1.4 ml/min. In Fig. 1 chromato-

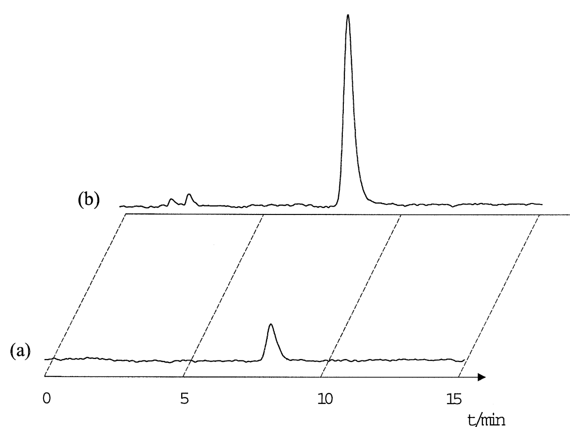

Fig. 1. Comparison between chromatograms of domperidone (20 ng in 200 μl of mobile phase) obtained without (a) and with (b) post-column photochemical reaction. See Section 2.3 for chromatographic conditions.

grams obtained with and without photochemical irradiation are compared.

The chromatogram of the plasma from drug-free volunteers (Fig. 2A) did not show any interfering compound extracted from the sample. A typical chromatogram of a drug-free human plasma sample spiked with domperidone (20 ng/ml) and internal standard (0.2 μg/ml) is shown in Fig. 2B. The chromatogram of the extract of plasma sample from a volunteer receiving Motilium tablets 10 mg orally in a 20-mg dose is shown in Fig. 2C. The retention times of domperidone and the internal standard (I.S.) were 7.6 min and 14.9 min, respectively.

The standard curve for domperidone was linear over the range 1–20 ng/ml. The standard curve was calculated by the linear regression method: $y=ax+b$, where y is the peak height ratio of drug to internal standard, a and b are constants, and x is the domperidone concentration (ng/ml). Typical values for the regression parameters a (slope), b (y-intercept) and correlation coefficient were calculated to be 0.0509364, 0.0209281 and 0.9998, respectively ($n=6$).

The minimum detectable concentration of domperidone (LOD) was determined to be 0.2 ng/ml, whereas the quantitative limit (LOQ) was 1 ng/ml and the relative standard deviation (RSD) of replicate determinations was 13.05% ($n=6$).

The within-day assay variations were determined by analyzing six 1-ml aliquots of spiked plasma samples containing 2, 10 and 20 ng/ml of domperidone. The between-day assay variations were determined by analyzing 1-ml aliquots of spiked plasma samples containing 2, 10 and 20 ng/ml of domperidone in duplicates on three separate days. In both cases, the RSD was less than 10% at all the concentrations studied (Table 1). Accuracy was within the range 94–105% for all concentrations investigated. For quality control samples precision ranged from 3% at 20 ng/ml to 7.4% at 2 ng/ml (Table 2). Accuracy was within the range 99–101% for all concentrations studied. In the range of calibration standards, the absolute recovery of domperidone from human plasma using this method was 83.70±2.17%. The mean recovery for internal standard at a concentration of 0.2 μg/ml was

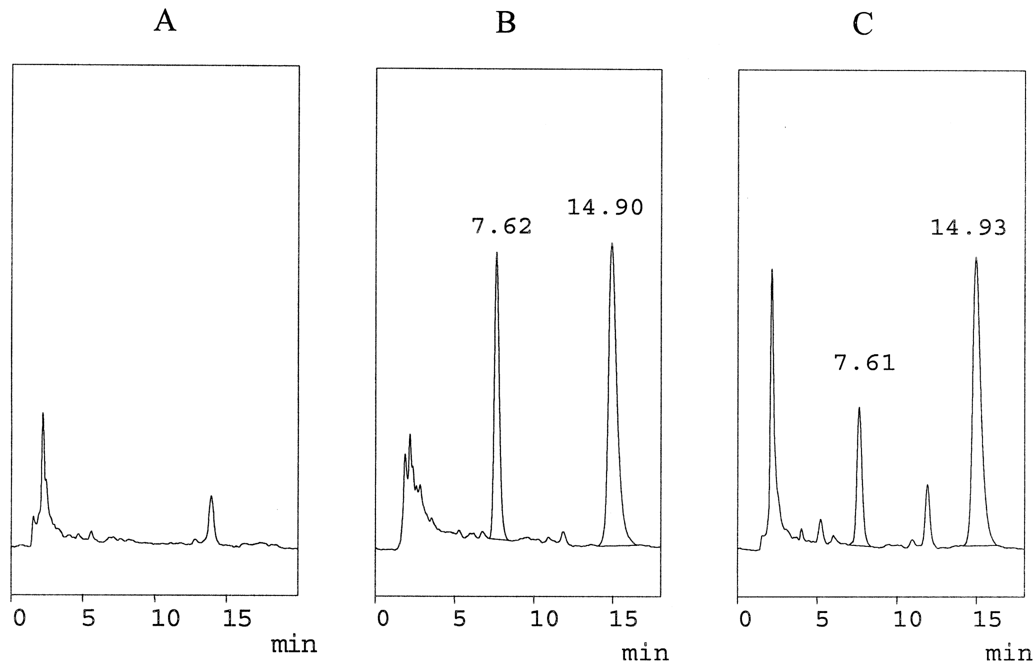

Fig. 2. Chromatograms of (A) blank human plasma, (B) spiked human plasma with 20 ng of domperidone and 0.2 μg of internal standard and (C) a plasma sample from a volunteer at 2 h after drug administration. The retention times for domperidone and internal standard are 7.6 and 14.9 min, respectively. See Section 2.3 for chromatographic conditions.

Table 1
Validation of analytical method

Nominal concentration added (ng/ml)	Concentration found (ng/ml)±SD	RSD (%)	Accuracy (%)
Intra-assay (n=6)			
2	1.9±0.1	6.6	95.6
10	10.6±0.4	3.7	105.6
20	20.6±1.5	7.2	103.2
Inter-assay (n=6)			
2	1.9±0.2	9.7	94.7
10	10.4±0.6	5.5	103.8
20	19.5±0.6	3.1	97.6

Table 2
Results of quality control

Nominal concentration added (ng/ml)	Concentration found (ng/ml)±SD	RSD (%)	Accuracy (%)	n
2	2.0±0.2	7.4	99.6	54
10	10.1±0.4	4.1	101.3	54
20	19.8±0.6	3.2	99.1	54

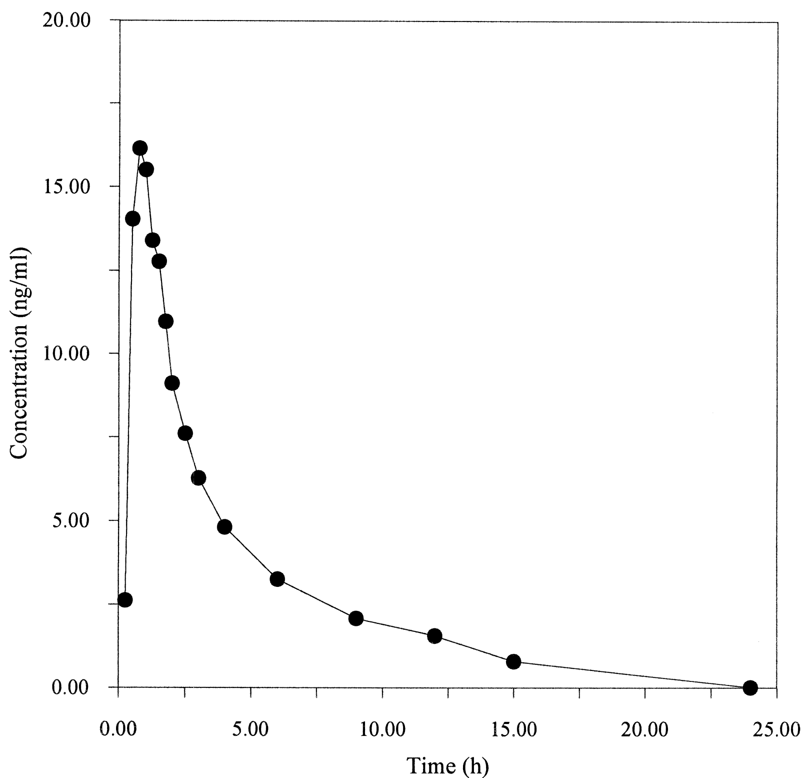

Fig. 3. Mean plasma levels of domperidone in 32 healthy volunteers following a single oral dose of 20 mg Motilium tablets.

97.55±4.38% (n=6). The stability study shows that domperidone was stable in human plasma stored at −70°C at least for 2 months.

The applicability of the assay procedure is illustrated in Fig. 3, which shows an average plasma concentration–time curve of domperidone after administration of Motilium tablets to 32 volunteers. Table 3 summarizes some of the mean pharmacokinetic parameters and their standard deviations.

In summary, the described method for the determination of domperidone in human plasma is sensitive, specific, rapid and reproducible. This method would allow pharmacokinetic studies for domperidone after oral administration to be conducted.

Table 3
Pharmacokinetic parameters of domperidone following a single oral administration of 20 mg Motilium tablets to 32 healthy volunteers

Pharmacokinetic parameter	Mean value±SD (n=32)
k_{el} (h^{-1})	0.11±0.05
$t_{1/2}$ (h)	7.70±3.07
C_{max} (ng/ml)	20.67±5.32
T_{max} (h)	0.84±0.36
$AUC_{0-\infty}$ (ng·h/ml)	75.45±24.33

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