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Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of domperidone in human plasma

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Abstract

A rapid and sensitive method for the determination of domperidone in plasma was developed, using high-performance liquid chromatographic separation with tandem mass spectrometry detection. The samples were rendered basic with 1 *M* Na_2CO_3 and the domperidone extracted using *tert.*-butyl methyl ether, followed by back-extraction into formic acid (2% in water). Chromatography was performed on a Phenomenex Luna C_8 (2), 5 μm, 150×2 mm column with a mobile phase consisting of acetonitrile–0.02% formic acid (300:700, v/v), delivered at 0.2 ml/min. Detection was performed using an Applied Biosystems Sciex API 2000 mass spectrometer set at unit resolution in the multiple reaction monitoring mode. TurboIonSpray ionisation was used for ion production. The mean recovery of domperidone was ±100%, with a lower limit of quantification set at 0.189 ng/ml. This assay method makes use of the increased sensitivity and selectivity of tandem mass spectrometric detection resulting in a rapid (extraction and chromatography) and sensitive method for the determination of domperidone in human plasma, which is more sensitive than previously described methods.

Keywords: Domperidone; Melperone

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 which does not penetrate fully into the central nervous system. The effects are confined to the periphery and therefore has a lower propensity to cause extrapyramidal side effects than other dopamine antagonists. Although domperidone is rapidly absorbed after oral administration, its bioavailability is only 5% resulting in most of the drug being excreted in the faeces. The elimination half-time of domperidone in plasma is about 7–8 h [3]. Although domperidone is widely used as an antiemetic, very little information regarding the pharmacokinetics of domperidone is available, due to the lack of sensitive quantitation methods described [10]. HPLC methods with fluorescence detection have been described for domperidone in plasma with lower limits of detection ranging from 1 to 10 ng/ml [2,10]. High-performance thin-layer chromatography has also been used for quantitation, but only in pharmaceutical preparations [11]. Spectrophotometric methods, using 3-methyl-2-benzothiazolinone hydrazone hydrochloride as reagent and ceric ammonium sulphate as oxidant, have also been described for the determination of domperidone in drug formulations [1,7]. Radioimmunoassay (RIA) methods that use

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antibodies against domperidone were also described, with a limit of quantitation of 0.2 ng/ml and 85% binding specificity [4,5]. The mass spectrometry methods described made use of laser microprobe mass spectrometry (LMMS), but only for structural characterisation of the organic molecule, and an electrospray LC–MS method in human serum and breast milk [9,12]. However most of these methods require laborious extraction procedures and long chromatographic run times (6–10 min). This lowers sample throughput and it was therefore decided to develop a faster and more sensitive method using the increased selectivity and sensitivity of a mass-selective detector and a liquid–liquid extraction.

This report describes a sensitive LC–MS–MS method for the determination of domperidone in plasma using a simple liquid–liquid and back-extraction procedure with a total chromatography time of 3.5 min, allowing the assay of up to 250 samples per day. The method was sensitive [lower limit of quantification (LLOQ) of 0.189 ng/ml] enough to determine plasma concentrations of up to 72 h following a single 10-mg dose of domperidone, making it not only fast, but highly sensitive.

2. Experimental

2.1. Materials and chemicals

A Phenomenex Luna C_8 (2) 5 μm, 150×2.1 mm column (Phenomenex, Torrance, CA, USA) was used for separation at a flow-rate of 0.20 ml/min after injecting 18 μl of the extract onto the column. A Hewlett-Packard Series 1100 gradient pump (Hewlett-Packard, Palo Alto, CA, USA) delivered mobile phase, and the samples were injected by a Perkin-Elmer Series 200 autosampler. Detection was performed by an Applied Biosystems Sciex API-2000 detector (Applied Biosystems Sciex, Ontario, Canada) using TurboIonSpray for ion production.

tert.-Butyl methyl ether (99.8%) was obtained from Fluka (Buchs, Switzerland); formic acid (high purity) from BDH (Poole, UK); acetonitrile (Burdick and Jackson, High Purity) from Baxter (USA) and sodium carbonate (puriss analytical-reagent grade) from Fluka. All chemicals were used as received. Water was purified by RO 20SA reverse osmosis and Milli-Q polishing system (Millipore, Bedford, MA, USA).

Domperidone, $C_{22}H_{24}ClN_5O_2$, and melperone (internal standard), $C_{16}H_{22}FNO$, were supplied by Cheminor Drug (Pydibhimavaram, India) and Schweizerhall Pharma (Hamburg, Germany), respectively (Fig. 1).

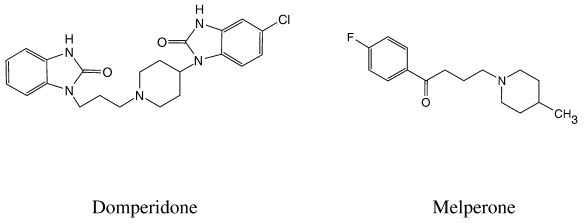
2.2. Preparation of calibration standards and quality controls

Domperidone stock solutions were prepared in methanol and used to spike plasma immediately. Calibration standards and quality control standards were prepared in normal human plasma by spiking a pool of normal plasma which was then serially diluted with normal blank plasma to attain the desired concentrations (0.189–48.3 ng/ml). The calibration standards and quality control standards were aliquoted into tubes and stored under the same conditions as the trial samples, at −20 °C.

2.3. Sample preparation

To 0.8-ml plasma in a 10-ml amber glass ampoule was added 50 μl internal standard solution (346 ng/ml melperone in water), 200 μl sodium carbonate (1.0 *M*, pH 9.0) and 4 ml *tert*.-butyl methyl ether. The sample was vortexed for 1 min and centrifuged at 1300 *g* for 3 min at 8 °C.

The aqueous phase was frozen at −30 °C on a Fryka Polar cooling plate (Kältetechnik, Esslingen, Germany) and the organic phase decanted into a clean 5-ml amber glass ampoule containing 200 μl of a formic acid solution (2% in water). After vortexing for 1 min and centrifuging at 1300 *g* for 3 min at 8 °C, the aqueous phase was again frozen at −30 °C and the organic phase discarded. The aque-



Domperidone Melperone

Fig. 1. Structures of domperidone and melperone.

ous phase was thawed at room temperature, and any residual organic phase was evaporated under a gentle stream of nitrogen for 1 min at 45 °C. The residue was transferred to an autosampler vial insert and 18 μl injected onto the HPLC column.

2.4. Liquid chromatography

Chromatography was performed at ambient temperature with a mobile phase (sparged with helium) consisting of acetonitrile–0.02% aqueous formic acid (300:700, v/v) at a flow-rate of 0.20 ml/min.

2.5. Mass spectrometry

Electrospray ionisation was performed in the positive ion mode with nitrogen as the nebulizing, turbo spray and curtain gas with the optimum values set at 70, 70 and 50 units, respectively. The TurboIonSpray temperature was set at 400 °C. The instrument response was optimised for domperidone and melperone by infusing a constant flow of a solution of the analyte and internal standard dissolved in mobile phase into the stream of mobile phase eluting from the column. The pause time was set at 5 ms and the dwell time at 150 ms.

The Applied Biosystems Sciex API 2000 LC–MS–MS detector was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions m/z 426.2 and 264.1 to the product ions m/z 175.1 and 165.0 for domperidone and melperone, respectively. Fig. 2 shows the single parent (m/z 426.2) to product ion (m/z 175.1) full MS–MS spectrum of domperidone and Fig. 3 the single parent (m/z 264.1) to product ion (m/z 165.0) full MS–MS spectrum of the internal standard (melperone). TurboIonSpray ionisation (ESI) was used for ion production and the collision gas (N_2) set at 3 units. Collision energies of 39 and 27 eV were used for the domperidone and internal standard, respectively. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.1 software.

2.6. Validation

The method was validated [8] by analysing plasma quality control samples ($n=6$) at seven different concentrations (39.3, 19.7, 4.91, 1.80, 0.901, 0.450 and 0.225 ng/ml) to determine the accuracy and precision of the method. The quality control values were calculated from a standard regression curve containing nine different calibration standards spanning the concentration range 48.3–0.189 ng/ml. Calibration graphs were constructed using a weighted linear regression (1/concentration) of the drug–internal standard peak–area ratio of the product ions for domperidone and the internal standard, versus nominal drug concentration. The effect of endogenous matrix compounds on ionisation was investigated

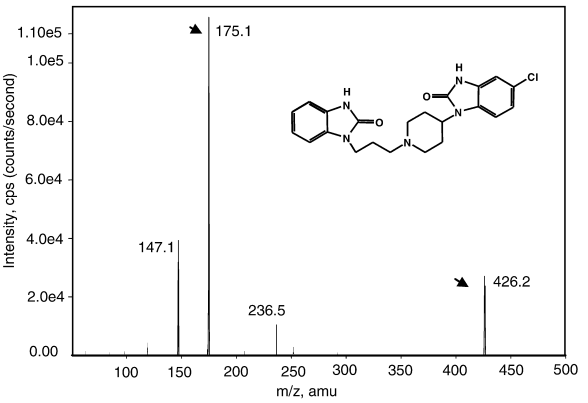


Fig. 2. Full mass spectrum of the protonated domperidone molecular ion (m/z 426.2, molecular structure given) and the possible product ions formed after collision (MS–MS).

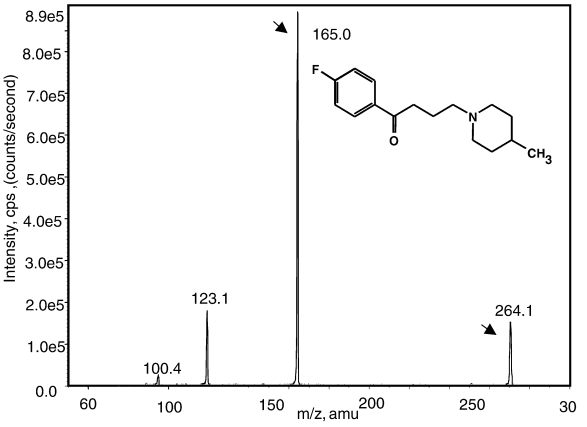


Fig. 3. Full mass spectrum of the protonated melperone molecular ion (m/z 264.1, molecular structure given) and the possible product ions formed after collision (MS–MS).

by extracting ''blank'' plasma from 10 different sources, reconstituting the final extract in injection solvent containing a known amount of the analyte, analysing the reconstituted extracts and then comparing the peak areas of the analyte [6].

Absolute recoveries of the analyte were determined in triplicate in normal plasma by extracting drug-free plasma samples spiked with domperidone at high, medium and low concentrations. Recoveries were calculated by comparison of the analyte peak–areas of the extracted samples with those of the unextracted system performance verification standard mixtures (prepared in the injection vehicle) representing 100% recovery.

3. Results and discussion

3.1. Recovery

The mean absolute recovery of domperidone determined in triplicate at 19.7, 4.91 and 0.45 ng/ml was ±100%.

3.2. Matrix effects

No matrix effects for domperidone were observed for the 10 different plasma pools tested. The peak areas of the 10 reconstituted samples had an RSD of 2.92% indicating that the extracts were ''clean'' with no undetected co-eluting compounds influencing the ionisation of the analytes.

3.3. Sensitivity

The LLOQ is defined as that concentration of domperidone which can still be determined with acceptable precision (RSD%<20%) and accuracy (bias<20%) and was found to be 0.189 ng/ml, the concentration of lowest non-zero calibration standard.

3.4. Validation results

Results from the intra-day validation assays indicate a valid calibration range of 0.189–48.3 ng/ml. The intra- and inter-day assay method performance statistics are presented in Tables 1 and 2. The inter-day assay method performance statistics include data of the intra-day validation and two inter-day validations, assayed on consecutive days.

Table 1
Intra-day quality control results of domperidone

Nominal concentration (ng/ml)	Mean concentration found (ng/ml)	RSD (%)	% Nominal
39.3	39.1	2.0	99.4
19.7	19.6	2.1	99.2
9.83	9.49	3.0	96.5
4.91	4.89	3.6	99.6
1.80	1.81	3.0	100.4
0.901	0.924	3.1	102.6
0.450	0.465	5.3	103.3
0.225	0.242	3.1	105.3

3.5. On-instrument and matrix stability

On-instrument stability was inferred from stability samples that were prepared and included in the validation batch. No significant degradation could be detected in the cooled samples (4 °C) left on the autosampler for at least 27 h. Plasma samples stored at −20 °C for 2 months were analysed and showed no significant degradation (2.8–5.3%) at high, medium and low concentrations indicating that domperidone is stable in plasma (at −20 °C) for at least 2 months.

3.6. Specificity

Due to the high specificity of MS–MS detection, no interfering or late eluting peaks were found when blank plasma extracts from six different sources were analysed.

3.7. Extraction

Several extraction procedures were tested to develop for a rapid extraction procedure; then included solid-phase (96-well plates) and liquid–liquid extraction methods using various organic solvents and buffers. The liquid–liquid back-extraction procedure of the analyte from *tert*.-butyl methyl ether into a 2% formic acid solution resulting in clean extracts with high recovery, proved to be the most successful.

Table 2
Inter-day quality control results of domperidone

	Nominal concentration (ng/ml)				
	0.225	0.450	4.91	9.83	19.7
Mean	0.234	0.466	4.887	9.584	19.572
RSD (%)	8.5	5.3	3.3	4.2	2.8
% Nominal	104.1	103.4	99.5	97.5	99.4
n	18	18	18	18	18

Table 3 summarises the recovery achieved with different organic solvents. Retention times were 2.36 and 2.38 min for domperidone and the internal standard (melperone), respectively. Melperone was chosen as internal standard because, with the liquid chromatography conditions used, domperidone and the internal standard eluted almost simultaneously, limiting potential differential matrix effects. The sample turn-around time was 3.5 min. Fig. 4 shows representative chromatograms obtained with the lowest concentration calibration standard (0.189 ng/ml) and of a study sample close to the limit of quantification in the elimination phase of the drug concentration versus time profile.

The method was employed to assay samples obtained in human plasma after a single 10-mg dose of domperidone was administered to 24 healthy volunteers. Concentration versus time profiles were constructed up to 72 h (Fig. 5) and the C_{max} plasma concentration varied between 3.291 and 26.928 ng/ml, with a mean C_{max} of 9.629 ng/ml.

In this method we made use of the increased sensitivity and selectivity of MS–MS detection to

Table 3
Extraction recovery of domperidone with different organic solvents during extraction

Organic solvent use for extraction at pH 9	Recovery (%)
Heptane–dichloromethane–iso-amyl alcohol (49:49:2)	27.0
Hexane–iso-amyl alcohol (98:2)	23.0
Hexane–dichloromethane (1:1)	13.8
Ethyl acetate	27.7
Isopropyl ether	36.7
Diethyl ether	92.9
tert.-Butyl methyl ether–dichloromethane (1:1)	88.0
tert.-Butyl methyl ether	100

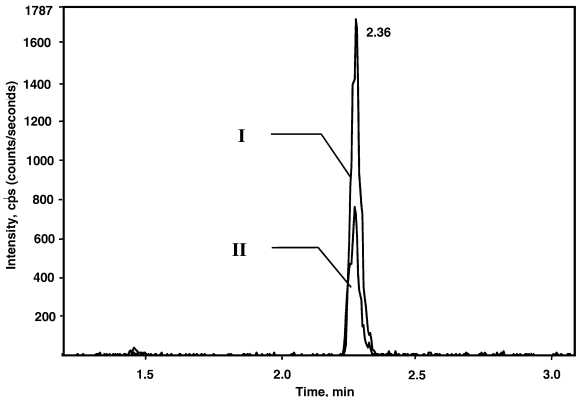


Fig. 4. High-performance liquid chromatograms of the calibration standard (I) at the lower limit of quantification (LLOQ) containing 0.189 ng/ml domperidone and of a study sample (II) at the terminal phase of the pharmacokinetic profile, containing 0.567 ng/ml domperidone (48 h).

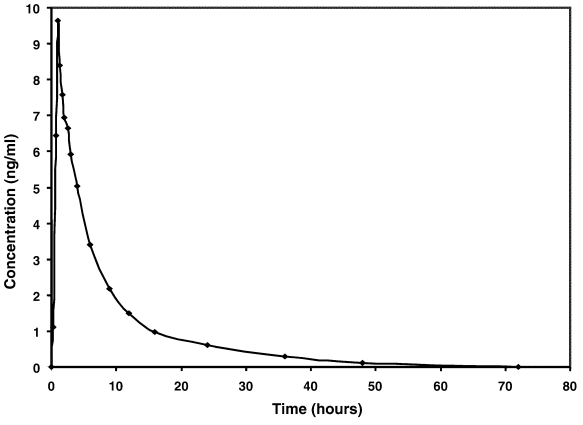


Fig. 5. Representative domperidone mean plasma concentrations versus time profile obtained after a single 10-mg oral dose of domperidone over 72 h (28 subjects).

decrease the sample preparation and chromatography time, enabling us to assay 250 samples per day.

4. Conclusion

A highly sensitive and selective method for the determination of domperidone in human plasma was developed, using high-performance liquid chromatographic separation with tandem mass spectrometric detection. The sensitivity obtained with this method (LLOQ of 0.189 ng/ml, with a signal-to-noise ratio of 50) was high enough to track the concentrations in human plasma samples for most of the 72-h period, making this method suitable for use in pharmacokinetic studies. The method is more sensitive than previously described methods and allows increased sample throughput (200–250 samples/day) due to the short chromatography time (3.5 min) and relatively simple sample preparation procedure.

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