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**Research Article**

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# Liquid Chromatography Tandem Mass Spectrometry Method for Determination of Domperidone in Human Plasma

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## ABSTRACT

*A highly sensitive, accurate and rapid analytical method based on reversed-phase liquid hromatography/ electrospray ionization tandem mass spectrometry (RP-LC-ESI-MS/MS) has been developed and validated for the determination of Domperidone in human plasma using Atorvastatin as an internal standard (IS). The method was validated over a linear range of 0.101 – 30.300 ng/ml, $R^2$ 0.9983. After addition of IS, analytes were extracted from the plasma samples by liquid-liquid extraction using tert-butylmethyl ether. Chromatographic separations were achieved reversed phase column (ACE $C_{18}$ (50 x 4.6) mm, 3 µm particle size) using a mobile phase consisting of formic acid in water (0.1%) and acetonitrile (25: 75, v/v (%)), flow rate 0.50 (mL/min.). Detection utilized a tandem MS/MS, the analytes were ionized using an ESI source in the positive ion mode prior to detection by Multiple Reaction Monitoring (MRM) mode. The analytes were monitored at the following transitions (m/z) 426.2 → 175.1 for Domperidone and (m/z) 559.5 → 440.4 for Atorvastatin respectively. The proposed method was fully validated in terms of linearity, accuracy, precision, specificity, sensitivity, recovery and stability, giving results within the acceptable range.*

**Keywords**: Domperidone, HPLC-MS/MS, ESI source, positive ion mode, MRM mode

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## INTRODUCTION

Domperidone, 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)propyl]piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one. It looks almost white powder, shown in the structure below is a dopamine antagonist with antiemetic properties similar to metoclopramide and certain narcoleptic drugs. Unlike these drugs, domperidone does not readily cross the blood-brain barrier and seldom cause extrapyramidal side effects [1-3].

In one-third of mothers, domperidone did not increase milk production. In the remainder, milk production increased at both domperidone doses, and there was a trend for a dose-response relationship. The amount of domperidone that transfers into milk was extremely low, and infant exposure via breastfeeding was not considered to be significant [4].

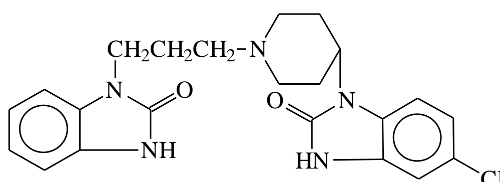


**Figure 1: Chemical Structure of Domperidone**

Until recently, there was little information available on the pharmacokinetics of domperidone in humans, mainly because of the lack of a convenient assay. Earlier studies relied primarily on radioimmunoassay (RIA) method using antibodies raised in rabbits against domperidone. According to literature study so many analytical methods are reported viz, UV spectrophotometry [5-8]. More recently, the published assay methods described the determination of domperidone in biological fluids using sufficiently selective and sensitive methods. These methods utilized high performance liquid chromatography-coupled with UV spectrophotometry and fluorescence detection [9-11].

Some published methods described the determination of domperidone in human plasma using sufficiently selective and sensitive methods using HPLC with electrospray ionization mass spectrometric detection in all methods time consuming liquid-liquid extraction methods have been used for sample preparation. [12-14] Furthermore solid phase extraction was also utilized prior to HPLC- fluorescence detection although the method was sensitive, accurate and reproducible however, it was necessary to introduce a post-column photochemical reaction to enhance the quantum efficiency of domperidone [15].

Until to date, only one UPLC-MS/MS method have been published for the determination of domperidone in human plasma [16]

Atorvastatin, (3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid used as internal standard in this study. [17]

The present work reports a selective, simple and sensitive HPLC-MS/MS bioanalytical method for the determination of Domperidone in human plasma.

## EXPERIMENTAL SECTION

### 2.1 Chemicals and reagents

Domperidone (assay 99.37%) and Atorvastatin (assay 99.40 %) were provided by pharma international (Amman – Jordan). Formic acid, methanol, tert-butylmethyl ether, acetonitrile and deionized water (HPLC grade solvent) were purchased from Scharlau (Darmstadt-Germany). Reagents were used without further purification. Blank human plasma samples have been obtained from the plasma of participants who volunteered in blood bank (five units).

### 2.2 Instrumentation

The LC-MS/MS system consisted of a HPLC (Agilent 1200 series, Agilent Technologies, Germany) coupled with a, Sciex Triple Quadrupole Mass Spectrometer (API 5000, MDS, Sciex, Ontario-Canada) equipped with an electrospray ionization (ESI) (Applied Biosystems). Data acquisition and processing were controlled by Applied Biosystems/ MSD SCIEX Analyst software (version 1.4.2).

### 2.3 Chromatographic Conditions and MS/MS instrumental settings

Chromatographic separations were performed using ACE $C_{18}$ (50 × 4.6) mm, 3 µm particle size. The mobile phase was an formic acid in water (0.1%) and acetonitrile (25: 75, v/v (%)). The separation was performed under isocratic conditions set at a constant flow rate of 0.50 (mL/min.). The injection volume was fixed at 20µL.

LC-MS/MS experimental conditions utilized the Multiple Reaction Monitoring (MRM) for both Domperidone and the internal standard (Atorvastatin). The detection was performed in the positive ESI mode for each of the analytes respective ions $[M+H]^{+}$. Instrument settings of the MS/MS are summarized in the following table (**Table 1**).

**Table 1: Experimental setting for the tandem mass-spectrometer during the analysis of Domperidone and Atorvastatin (IS)**

| Parameter | unit | Domperidone |
|---|---|---|
| Source temperature | °C | 500.0 |
| Nebulizer gas | psi | 20 |
| Turbolon gas | psi | 40 |
| Curtain gas | psi | 20 |
| Collision gas | psi | 12 |
| Ion spray voltage | V | 5500 |
| Dwell time per transition | ms | 400 |

| Dwell time per transition | ms | **Domperidone** | **Atorvastatin (IS)** |
|---|---|---|---|
| Entrance potential | V | 5 | 10 |
| MRM transition | amu | 426.2 → 175.1 | 559.5 → 440.4 |
| Collision energy | V | 20 | 17 |
| Declustering potential | V | 90 | 90 |
| Collision cell exist potential | V | 15 | 12 |

### 2.4 Preparation of stock and working standard Solutions

A Domperidone stock solution was prepared by dissolving of 12.10 mg Domperidone (99.37%) in 100.0 mL mobile phase to make up the concentration of 120.238 (µg / mL), one working standard solutions concentration of 505.000 (ng/mL) was also prepared. The internal standard (Atorvastatin) stock solution was prepared by dissolving of 2.70 mg Atorvastatin (assay 99.40 %) in a 50.0 mL methanol to make up the concentration 47.019 (µg / mL), one working standard solutions concentration of 1.001 (µg /mL) was also prepared.

### 2.5 Preparation of matrix based calibrators and quality control samples

Domperidone matrix based calibrators were prepared from the above working standard solutions of Domperidone in 25.0 mL plasma to produce the following calibrators: 0.101, 0.202, 1.010, 2.222, 4.040, 8.080, 12.120, 20.200, and 30.300 (ng Domperidone/ mL). Additionally 50.0 mL standard solutions of each of the quality control (QC) samples were prepared in blank plasma to make up the concentrations of: 0.303, 15.150, and 24.240 (ng/mL). Prior to analysis each sample was spiked with a 1.001 µg aliquot of Atorvastatin (25 µL).

### 2.6 Sample Preparation

Aliquots of 500 µL plasma were pipetted into 6.-mL polypropylene tubes. The samples were extracted with 4.0 mL tert-butylmethyl ether by shaking for 20 min. After centrifugation for 5 min at 4000 rpm, the upper organic layers were transferred to clean tubes and evaporated under a stream of nitrogen at room temperature. The samples were then reconstituted with 500 µL mobile phase. A 20 µL volume was injected onto the equilibrated LC-MS/MS system.

## RESULTS AND DISCUSSION

### 3.1 Method Validation

The developed method was fully validated with respect to the following parameters: selectivity, stability, linearity, limit of detection and as well as of lower and upper limits of quantitation, sensitivity, recovery, accuracy, and precision studies following USFDA guidelines [18].

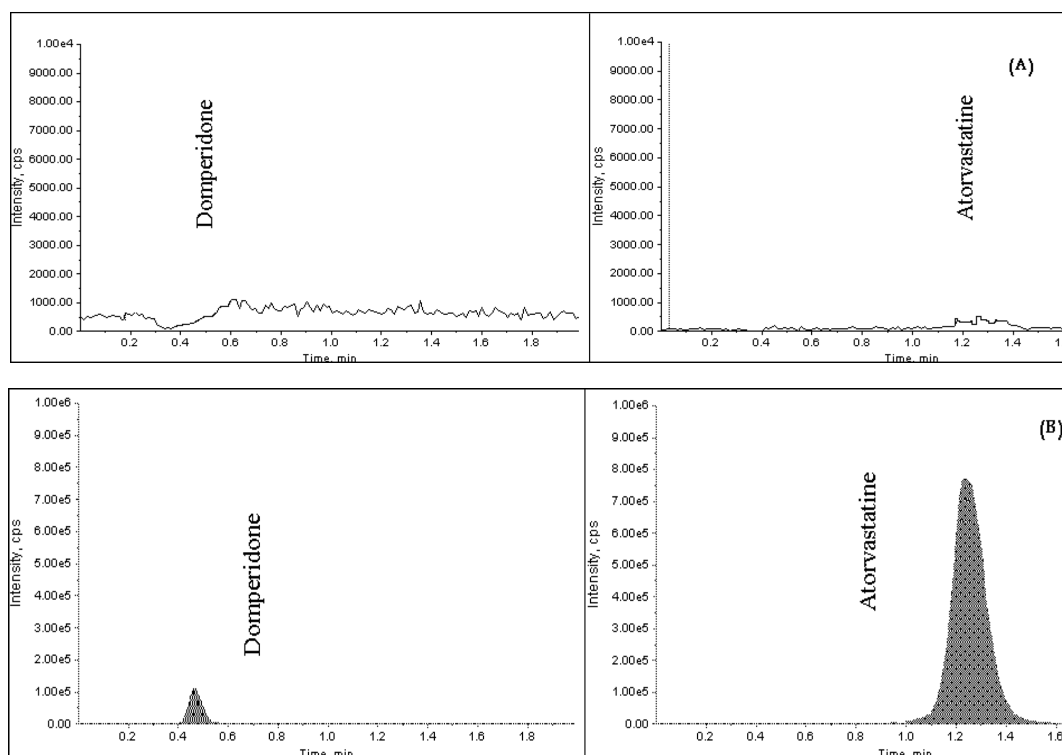


**Figure 2: Representative chromatograms for (A) an extract of blank plasma Sample. (B) An extract of spiked plasma sample containing (0.303 ng/mL) at the (Law QC) Domperidone and Atorvastatin (IS) (25.025 ng/mL)**

#### 3.1.1 Selectivity

All samples were extracted and analyzed using the developed and optimized method, no interferences were observed at the retention times of Domperidone or the IS. Analytical signals from blank plasma sample extracts and from

spiked samples at the lower limit of quantitation (0.101 ng/mL) are illustrated in the **Figure 2**. In addition, chromatograms obtained from law and high quality control samples after of Domperidone are illustrated in **Figure 3**. A high selectivity with no matrix effects were observed, this is attributed to the high dilution factor (20) of the protein precipitated matrix. The use of a highly sensitive MS/MS (API 5000) facilitated the recording of a high signal. Consequently a simple, highly selective and highly sensitive method was concluded.

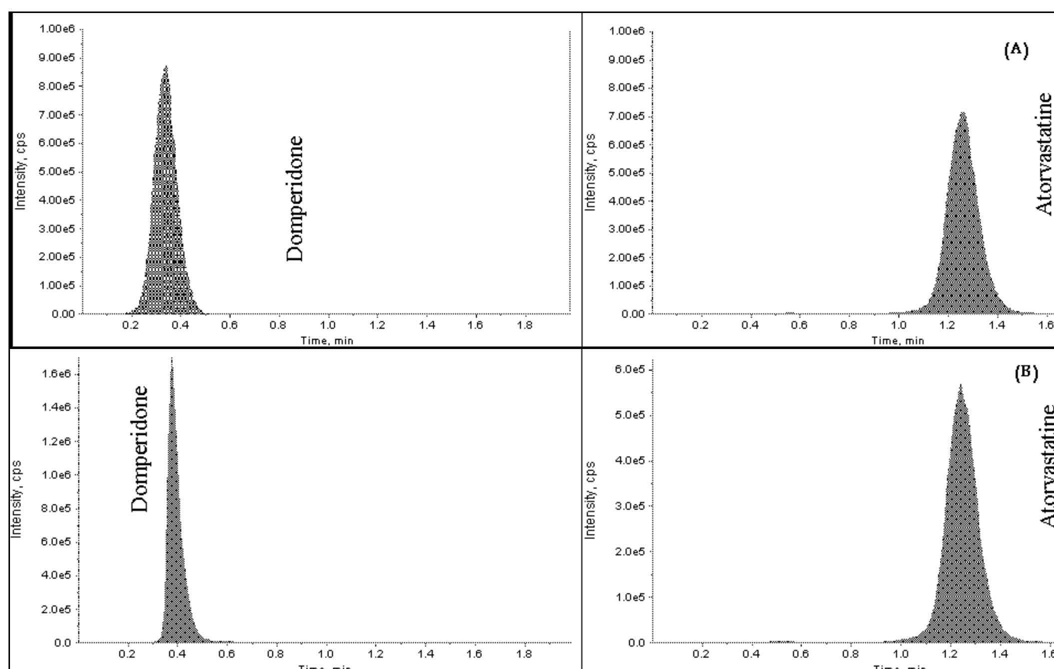


**Figure 3: Representative chromatograms for (A) An extract of plasma containing Domperidone (15.150 ng/mL) (Medium QC) and Atorvastatin (IS) (25.025 ng/mL). (B) An extract of plasma containing Domperidone (24.240 ng/mL) (High QC sample) and Atorvastatin (IS) (25.025 ng/mL)**

### 3.1.2 Linearity, linear working range and calibration model

Response function of Domperidone was plotted against corresponding concentration level in the dynamic ranges: 0.101 to 30.300 ng /mL. Linearity for six calibration curves, was demonstrated by visual inspection and by calculating the correlation coefficient, of 0.9983 using the weighted regression model with a statistical weight of $1/\chi^2$, the calibration equations were Y= 0.1048X-0.0066, where y represents peak area ratio of analyte to IS and X represents the analyte concentration. The lower limit of quantitation for Domperidone was found to be 0.101 ng/mL.

### 3.1.3 Precision and accuracy

A replicates measurements of each quality control containing $QC_L$ 0.303 (ng/mL), $QC_M$ 15.150 (ng/mL) and $QC_H$ 24.240 (ng/mL) matrix based standards of Domperidone were chromatographed to evaluate instrument precision, as well as method, inter-day, and intra-day precision and accuracy. The results summarized in **Table 2, Table 3, Table 4, Table 5** and **Table 6**. Results indicates that the bioanalytical method id precise, CV (%) not exceeding 15% for all matrix.

### 3.1.4 Stability

Stability during sampling, sample storage, processing and analysis was investigated. Stability data was evaluated with respect to analytical signals obtained from freshly prepared QC samples, compared to those samples measured after stressed conditions. Stability experiments extended throughout the analysis duration until assay of the last harvested sample. For short term stability studies, quality control samples in plasma including $QC_L$ (0.303 ng/mL), $QC_M$ (15.150 ng/mL) and $QC_H$ (24.240 ng/mL) were thawed and kept un-treated, at room temperature for 6h. Autosampler stability was evaluated over 65h. Freeze and thaw stabilities covered five cycles of freeze and thaw cycles. Long term matrix based solution stability was investigated under prolonged storage condition (14 days, -80°C). **Table 7, Table 8, Table 9** and **Table 10** which summarizes stability data demonstrate that Domperidone was stable under the investigated experimental conditions.

### 3.2 Method application

The validated bioanalytical method is recommended to apply and evaluate the comparative bioavailability (Bioequivalence) of a test and reference drug products for Domperidone (1 mg Domperidone / 10 mL Suspension), a paediatric suspension) in healthy volunteers in the fasted state.

**Table 2: Summary of instrument precision data (standard solution)**

| | Area ratio ($A_{DRUG}/A_{IS}$) | | |
|---|---|---|---|
| | **Low Concentration (0.303 ng/mL)** | **Medium Concentration (15.150 ng/mL)** | **High Concentration (24.240 ng/mL)** |
| Average (N=10) | 0.0347 | 1.4884 | 2.5315 |
| Std. Dev. | 0.0013 | 0.0267 | 0.0715 |
| CV (%) | 3.75 | 1.79 | 2.82 |

**Table 3: Summary of method precision data (spiked plasma)**

| | Measured conc. (ng/mL) | | |
|---|---|---|---|
| | **Low Concentration (0.303 ng/mL)** | **Medium Concentration (15.150 ng/mL)** | **High Concentration (24.240 ng/mL)** |
| Average (N=6) | 0.286 | 14.147 | 23.658 |
| Std. Dev. | 0.013 | 0.751 | 1.005 |
| CV (%) | 4.55 | 5.31 | 4.25 |

**Table 4: Summary of method accuracy data (spiked plasma)**

| | | Measured conc. (ng/mL) | | |
|---|---|---|---|---|
| | | **Low Concentration (0.303 ng/mL)** | **Medium Concentration (15.150 ng/mL)** | **High Concentration (24.240 ng/mL)** |
| | Average N=6 | 0.322 | 14.061 | 23.554 |
| Accuracy | Error (Bias) | 0.019 | -1.089 | -7.19 |
| | Relative Error (%) | 6.22 | -0.686 | -2.83 |

**Table 5: Summary of intra-day precision data (spiked plasma)**

| Theoretical concentration (ng/mL) | Day 1 | | | Day 2 | | | Day 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Mean recovered conc. N=6 (ng/mL)** | **Std. Dev.** | **CV (%)** | **Mean recovered conc. N=6 (ng/mL)** | **Std. Dev.** | **CV (%)** | **Mean recovered conc. N=6 (ng/mL)** | **Std. Dev.** | **CV (%)** |
| 0.303 | 0.319 | 0.017 | 5.33 | 0.329 | 0.013 | 3.95 | 0.310 | 0.015 | 4.84 |
| 15.150 | 15.374 | 0.914 | 5.95 | 15.108 | 0.958 | 6.34 | 15.548 | 0.928 | 5.97 |
| 24.240 | 25.601 | 1.443 | 5.64 | 25.611 | 1.042 | 4.07 | 26.045 | 1.390 | 5.34 |

**Table 6: Summary of inter-day precision data (spiked plasma)**

| | Measured conc. (ng/mL) | | |
|---|---|---|---|
| | **Low Concentration (0.303 ng/mL)** | **Medium Concentration (15.150 ng/mL)** | **High Concentration (24.240 ng/mL)** |
| Mean Conc. (14 Days) | 0.307 | 15.209 | 24.114 |
| Std. Dev. | 0.023 | 1.111 | 1.680 |
| CV% | 7.49 | 7.30 | 6.97 |

**Table 7: Summary of stability data, short term temperature stability 9spiked plasma)**

| | Low Concentration (0.303 ng/mL) | | High Concentration (24.240 ng/mL) | |
|---|---|---|---|---|
| | **Peak Area 0 hr** | **Peak Area 6 hr** | **Peak Area 0 hr** | **Peak Area 6 hr** |
| Mean (N=5) | 100311 | 95964 | 8448972 | 8163745 |
| Stability (%) | 100.00 | 95.67 | 100.00 | 96.62 |

**Table 8: Summary of stability, long term stability data (spiked plasma)**

| | Low Concentration (0.303 ng/mL) | | Medium Concentration (15.150 ng/mL) | | High Concentration (24.240 ng/mL) | |
|---|---|---|---|---|---|---|
| | **Measured conc. (*ng*/mL )** | **Recovery (%)** | **Measured conc. (*ng*/mL )** | **Recovery (%)** | **Measured conc. (*ng*/mL )** | **Recovery (%)** |
| Mean (16 Days) | 0.317 | 104.455 | 15.711 | 103.705 | 23.929 | 98.718 |
| Std. Dev. | 0.011 | 3.466 | 0.946 | 6.247 | 1.855 | 7.65 |
| C.V % | 3.47 | 3.32 | 6.02 | 6.02 | 7.75 | 7.75 |

**Table 9: Summary of stability data, auto sampler stability (spiked plasma)**

| | Measured conc. (ng/mL) | |
|---|---|---|
| | Low Concentration (0.303 ng/mL) | High Concentration (24.240 ng/mL) |
| | 24 hr | 24 hr |
| Mean (N=3) | 0.308 | 23.698 |
| Stability (%) | 101.65 | 97.76 |

**Table 10: Summary of stability data, freeze thaw cycle (spiked plasma)**

| | Low Concentration (0.303 ng/mL) | | Medium Concentration (15.150 ng/mL) | | High Concentration (24.240 ng/mL) | |
|---|---|---|---|---|---|---|
| | Average conc. measured N=5 (ng/mL) | Recovery (%) | Average conc. Measured N=5 (ng/mL) | Recovery (%) | Average conc. Measured N=5 (ng/mL) | Recovery (%) |
| Zero time | 0.304 | 100.33 | 15.300 | 100.99 | 24.225 | 99.94 |
| Cycle three | 0.323 | 106.60 | 15.657 | 103.35 | 22.181 | 91.51 |
| Cycle five | 0.299 | 98.68 | 15.360 | 101.39 | 22.878 | 94.38 |

## CONCLUSION

A highly sensitive, simple and fast RP-LC-ESI-MS/MS method for the determination of Domperidone in human plasma was developed and fully validated according to the current FDA guidance. This method involves a single step liquid-liquid extraction, using Atorvastatin, a commercially available substance, as internal standard. The short run time of 2.0-min and the relatively low flow rate (0.50 mL/min) allows the analysis of a large number of samples with less mobile phase consumption. Validation results show that the optimized RP-LC-ESI-MS/MS method possesses specificity, accuracy, precision, sensitivity, linearity, recovery and stability over the entire range of significant therapeutic plasma concentrations. The proposed method is recommended for the analysis of a large number of authentic plasma samples withdrawn from normal volunteers participating in a bioequivalence study.

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