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Determination of domperidone in human serum and human breast milk by high-performance liquid chromatography–electrospray mass spectrometry

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Abstract

A sensitive and selective method for the determination of domperidone in human breast milk and serum has been developed. The same method may be successfully applied to both matrices to a lower limit of quantitation of 0.5 ng/ml. Samples are processed by a liquid–liquid extraction, and analyzed by LC–ESI-MS in positive ion mode. There was no interference, on the domperidone quantitation, from over 30 drugs. Samples from patients, at various times post-dose, were analyzed and a large number showed significant levels of domperidone in the breast milk as well as in the serum. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Domperidone

1. Introduction

Domperidone (DOM), or 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 potent gastrokinetic and antinauseant drug that is apparently devoid of central sedative and autonomic effects [1]. Metabolism and excretion in dog, rat and man has been studied with high-performance liquid chromatography (HPLC) as the dominant analytical technique [1]. The pharmacokinetics and bioavailability in man have been studied with domperidone levels measured by an radioimmunoassay (RIA) method using antibodies raised in rabbits against domperidone [2]. The effect of domperidone on prolactin levels was studied with the plasma levels of domperidone measured by an HPLC technique [3]. The excretion of drugs into breast milk has been studied [4] and it has been proposed that many drugs transfer into breast milk by passive diffusion [5–10].

This study is an effort to determine if domperidone is present in the breast milk of lactating patients who are under treatment with domperidone. The antibodies used successfully in previous studies were not available, so the study of breast milk levels of domperidone would require a sensitive and selective instrumental technique. From the above references it was expected that the breast milk con-

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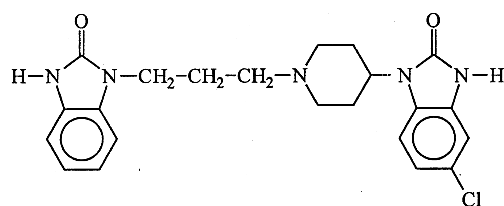
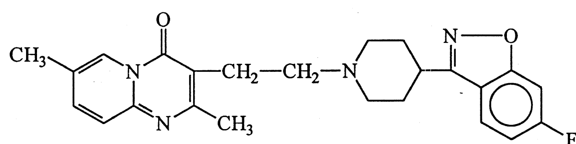
centrations could be expected to be between 1 and 10 ng/ml. Given the complex nature of both serum and breast milk the detection method would have to be highly selective. HPLC with electrospray ionization mass spectrometry (ESI-MS) would provide the ability to quickly develop a technique adequate for the requirements of the study. The use of atmospheric pressure ionization LC–MS in drug disposition studies has been recently discussed [11].

This work presents an HPLC–ESI-MS technique suitable for the determination of domperidone in either breast milk or serum matrix, with lower limits of quantitation (LLOQs) to 0.5 ng/ml matrix. The between-day precision and accuracy are largely within a few percent throughout the range. Over 35 different drugs were tested and were found not to interfere with the determination of domperidone under the analysis conditions.



DOMPERIDONE

$C_{22}H_{24}N_5O_2Cl$ M.W. 425



R68808

INTERNAL STANDARD

$C_{24}H_{25}N_4O_2F$ M.W. 420

Fig. 1. (a) Structure of domperidone. (b) Structure of R6088 internal standard.

2. Experimental

2.1. Reagents and standards preparation

Stock solutions were made by weighing the reference standard domperidone (Fig. 1a) or, the compound used as the internal standard, R68808 (Fig. 1b) into volumetric glassware and making to volume with methanol. Janssen Pharmaceutica (Beerse, Belgium), kindly provided all the standards. The stock solutions were made to a concentration of 0.1 mg/ml of methanol and were stored at −15°C.

Drug-free serum was prepared by mixing 3 g of Norit-A (Fisher, Toronto, Canada) with ca. 500 ml serum pooled from HIV negative donors, on a rotating mixer at 4°C overnight. The mixture was allowed to stand for 4 h and then centrifuged for 40 min at 1700 *g*. The centrifugate was filtered by suction through a Whatman No. 1 filter. A sample of this serum was checked and found to be free of interferences by the method described in this paper. The balance of this blank serum was used to prepare the plasma standards.

A high-level serum standard was prepared by transferring 0.5 ml domperidone stock solution to a 50-ml volumetric flask and making to volume with drug free blank serum described above. The resultant solution was mixed on a rotating mixer for 1 h at 4°C. This serum standard had a concentration of 1000 ng/ml, and was used to prepare the serum standards used in the calibration curve. The serum standards used in the calibration curve were prepared by suitable dilution, of high-level serum standard, with drug-free blank serum. Serum standards were prepared at the following concentrations: 0.00, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 10.0, 20.0 ng/ml.

Drug-free breast milk was prepared by pooling frozen breast milk collected from nursing mothers with no reported use of the medication. A sample of the pooled, drug-free breast milk was checked and found to be free of interferences by the method herein described. The balance of the pooled drug-free breast milk was used for the preparation of breast milk standards.

A high-level breast milk standard was prepared by transferring 0.5 ml domperidone stock solution to a 50-ml volumetric flask and making to volume with drug-free blank breast milk described above. The resultant solution was mixed on a rotating mixer for 1 h at 4°C. This breast milk standard had a concentration of 1000 ng/ml, and was used to prepare

the breast milk standards used in the calibration curve. The breast milk standards used in the calibration curve were prepared by suitable dilution, of high-level breast milk standard, with drug-free blank breast milk. Breast milk standards were prepared at the following concentrations: 0.00, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 10.0, 20.0 ng/ml.

The internal standard (I.S.) working solution was prepared by transferring 0.5 ml R68808 to a 50-ml volumetric flask and making to volume with methanol. A further 1:50 dilution of this solution was made in methanol to arrive at the final concentration (20 ng/ml) of the I.S. working solution.

The extraction solvent was composed of 49.5% isooctane (HPLC grade, J.T Baker, Phillipsburg, NJ, USA), 49.5% dichloromethane (Omnisolve EM Science supplied by VWR-Canlab, Canada), and 1% isoamyl alcohol (Fisher Scientific, Toronto, Canada). This extraction solvent was dispensed by a Repipet (Labindustries, Fisher).

Prior to extraction, the pH adjustment of the serum and breast milk samples was performed by the addition of a pH 9.0 borate buffer made in the following manner: boric acid (Fisher Scientific) 61.8 g and potassium chloride (Fisher Scientific) 74.6 g was added to a 1-l volumetric flask, made to volume with deionized water, and then mixed. From this solution, a 630-ml aliquot was transferred to a 2-l beaker. An aqueous solution of 106 g/l sodium carbonate monohydrate (Fisher Scientific) was made and 370 ml of this solution was added to the beaker. The resulting solution was adjusted to pH 9.0.

The water used in this analysis was deionized and polished to 16 MΩ/cm resistivity by a Milli-RO 30 Plus/Milli-Q water purification system, with final filtration through a 0.22-μm filter. The aqueous component of the mobile phase was a 5 m*M* solution of ammonium formate (Analar grade BDH, supplied by VWR-Canlab) adjusted to pH 3.0 with 90% formic acid (Analar grade BDH, supplied by VWR-Canlab). The final mixture was filtered through a 0.45-μm filter by vacuum and degassed under vacuum for 15 min. This component was passed through a channel of the in-line degasser and subsequently pumped by pump A of the binary HPLC system. The acetonitrile was filtered through a 0.45-μm filter by vacuum and degassed under vacuum for 15 min. The acetonitrile was passed through a channel of the in-line degasser and subsequently pumped by pump B of the binary HPLC system.

2.2. Extraction from serum

Serum was collected by venipuncture, separated by centrifugation and aliquoted into labeled 75 mm×12 mm polypropylene tubes. The tubes were then capped and stored at −72°C. Breast milk was collected and stored at −72°C until analysis. The specimens were allowed to thaw at room temperature and mixed by inversion prior to analysis.

The domperidone levels in both serum and breast milk were determined by the same extraction procedure. Matrix standards and unknown samples were treated identically. To 1.0 ml of either breast milk or serum sample in a 100×16 mm culture tube was added: 20 μl of internal standard working solution, 500 μl of borate buffer and 5.0 ml of extraction solvent. The tubes were capped and mixed on a multi-tube vortex mixer (SMI 2600, Fisher Scientific) for 10 min. The organic layer was separated by centrifugation at 1500 *g* for 10 min. The centrifugate was transferred to clean tapered centrifuge tubes in preparation for the back-extraction step. To these tubes were added 100 μl of MeOH–0.1 *M* HCl (40:60) followed by capping and mixing on a multi-tube mixer for 10 min. The layers were separated by centrifugation and the upper organic layer was aspirated to waste. The acidic lower layer (ca. 100 μl after the back-extraction step) was further extracted to remove residual lipid by the addition of 100 μl of hexane and mixing on the multi-tube vortex mixer for a further 5 min. The layers were separated by centrifugation, once again, and the upper organic layer was removed by suction. The remaining acid lower layer was transferred to polypropylene microvials and capped with PTFE-lined seals.

2.3. LC–MS

The LC–MS experiments were conducted with a HP 1100 LC–diode array detection (DAD)–MS system (Hewlett-Packard, Palo Alto, CA, USA). The system components included a binary pump, mobile phase vacuum degassing unit, autosampler, temperature controlled column compartment, UV–Visible diode array detector, and HP 1100 mass spectromet-

ric detector (LC–MSD). A single HP Vectra 150/PC under the Windows NT operating system using HP LC–MSD ChemStation software performed the system control and data acquisition, for both DAD and MSD. The source was a nebulizer assisted electrospray unit incorporating a proprietary orthogonal spraying configuration. The nitrogen supply was provided by a Jun-Air 1000-25M oil-less air compressor, which was, connected to a Whatman 75-726 nitrogen generator. The system was installed and performance verified by Hewlett-Packard Canada.

The column was a 50 mm×2.1 mm I.D., packed with LUNA C_{18} Series 2 (3 μm particle size) marketed as a LUNA LC–MS column, part No. 00B-4248-B0, serial No. 222577 (Phenomenex, Torrance, CA, USA). The column was maintained at 40°C. A 2-μm particle filter (Model A-315, Upchurch Scientific, supplied by SPE, Canada) was installed between the injector's needle seat and the six-port injector valve. This particle filter was installed prior to running any samples on the instrument, and served to capture particles, from extracted samples, that may score the valve rotor. The mobile phase was 5 m*M* ammonium acetate–acetonitrile (82.5:17.5). The pump was operated in isocratic mode at a flow of 0.6 ml/min with the 5 m*M* ammonium acetate in pump A, and acetonitrile delivered by pump B. The DAD instrument was set to monitor 280 nm (20 nm bandwidth) in the UV with a non-absorbing reference wavelength at 450 nm (30 nm bandwidth) in the UV. The reference wavelength reduces baseline fluctuations caused by refractive index effects and produces resultant signals that display less baseline drift in gradient elution runs. The DAD system was used during development but the UV response at 280 nm did not appear sufficiently selective to be the basis of a reliable HPLC bioanalytical technique for this compound. The injection volume was 5 μl and represented no more than 10% of the total sample available for injection. Signal intensity could be increased by injecting more of the final extract.

The mass spectrometer was operated in positive ion mode and tuned for optimum abundance and unit mass resolution (0.65 m/z peak width at half-height) over the mass range utilized in these experiments, by HP's proprietary AutoTune program and Mass Axis Calibration Reagent mixture. The exact source conditions were: drying gas temperature 350°C, drying gas flow 10 l/min, nebulizer pressure 50 p.s.i.g. (1 p.s.i.=6894.76 Pa), quadrupole temperature 100°C, and capillary voltage 4000 V. Nitrogen was used exclusively as drying gas. In the single ion monitoring (SIM) experiments, the ion for domperidone was m/z 426.1 and that for the internal standard R68808 was m/z 421.3, both ions had a dwell time of 500 ms per ion.

After acquisition the resultant SIM chromatograms were integrated by the HP ChemStation software. The integration parameters were determined on low-level plasma standards and were subsequently used without modification. The baselines were set automatically and were not manually altered.

3. Results and discussion

3.1. Mass spectrometry

Fig. 2a and b show the mass/charge region of interest for domperidone and R68808, respectively, as produced by ESI-MS under the conditions described above The protonated molecule for domperidone is observed at m/z 426.1 and that for the internal standard R68808 at m/z 421.3. No fragmentation of either molecule was observed under these conditions and the majority of the total ionization was seen as the protonated molecule for both compounds. The spectrum illustrates the mass resolution typically expected in this experiment. The spectrum is dominated by the protonated molecule $[M+H]^+$ for both molecules. There is also some minor contribution to the spectrum from the sodium adduct $[M+Na]^+$ or the potassium adduct $[M+K]^+$. There are no other observed artifacts in the mass spectrum under these conditions.

3.2. Extraction and chromatography

The extraction gave a clean final extract free of suspensions or precipitates. The serum samples could have dispensed with the hexane wash step, but the breast milk samples contained too much lipid to be injected. The hexane wash further cleaned the breast milk samples to the point that the final step gave a clear solution.

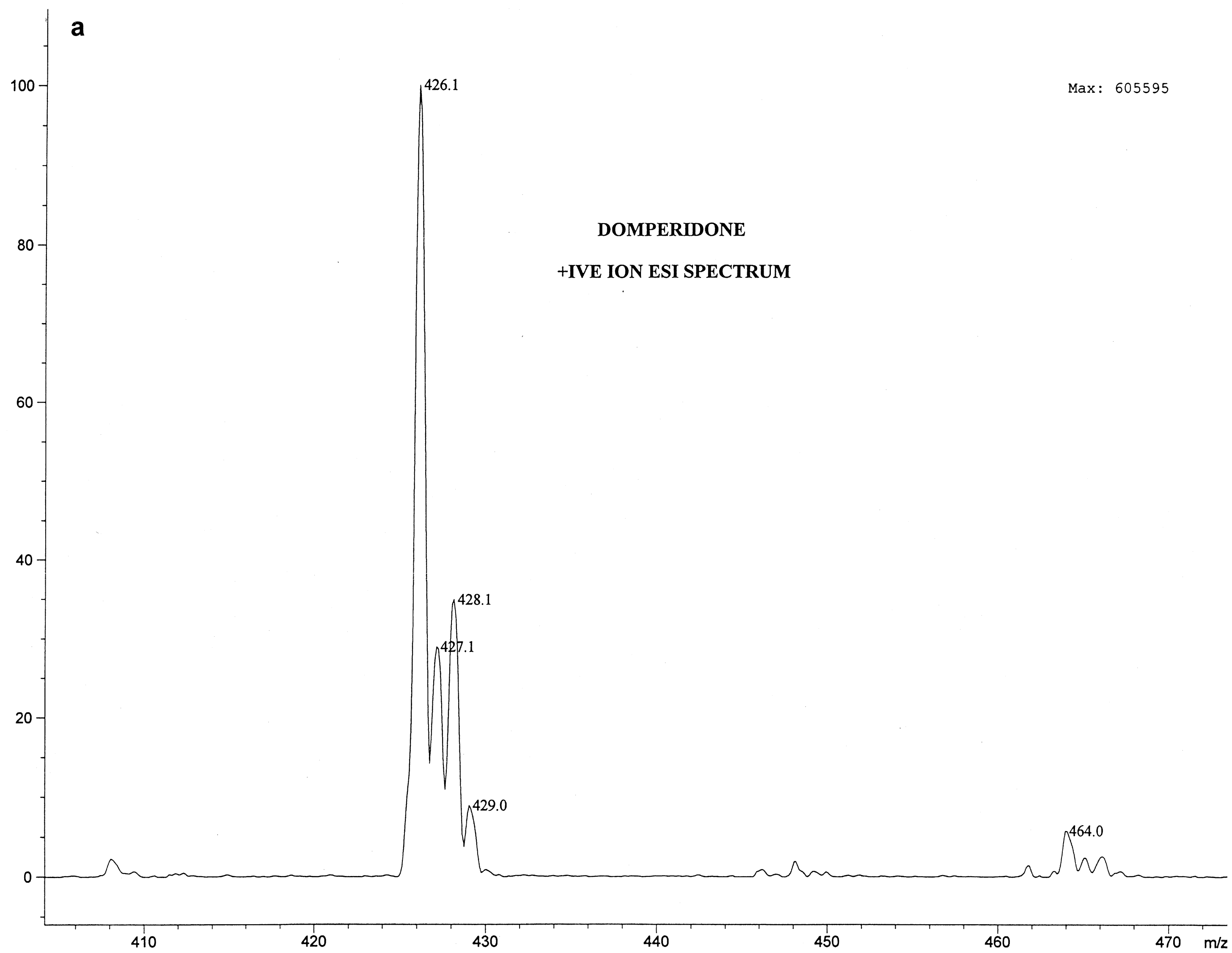


Fig. 2. (a) Positive ion ESI spectrum of domperidone. (b) Positive ion ESI spectrum of R68808 used as internal standard.

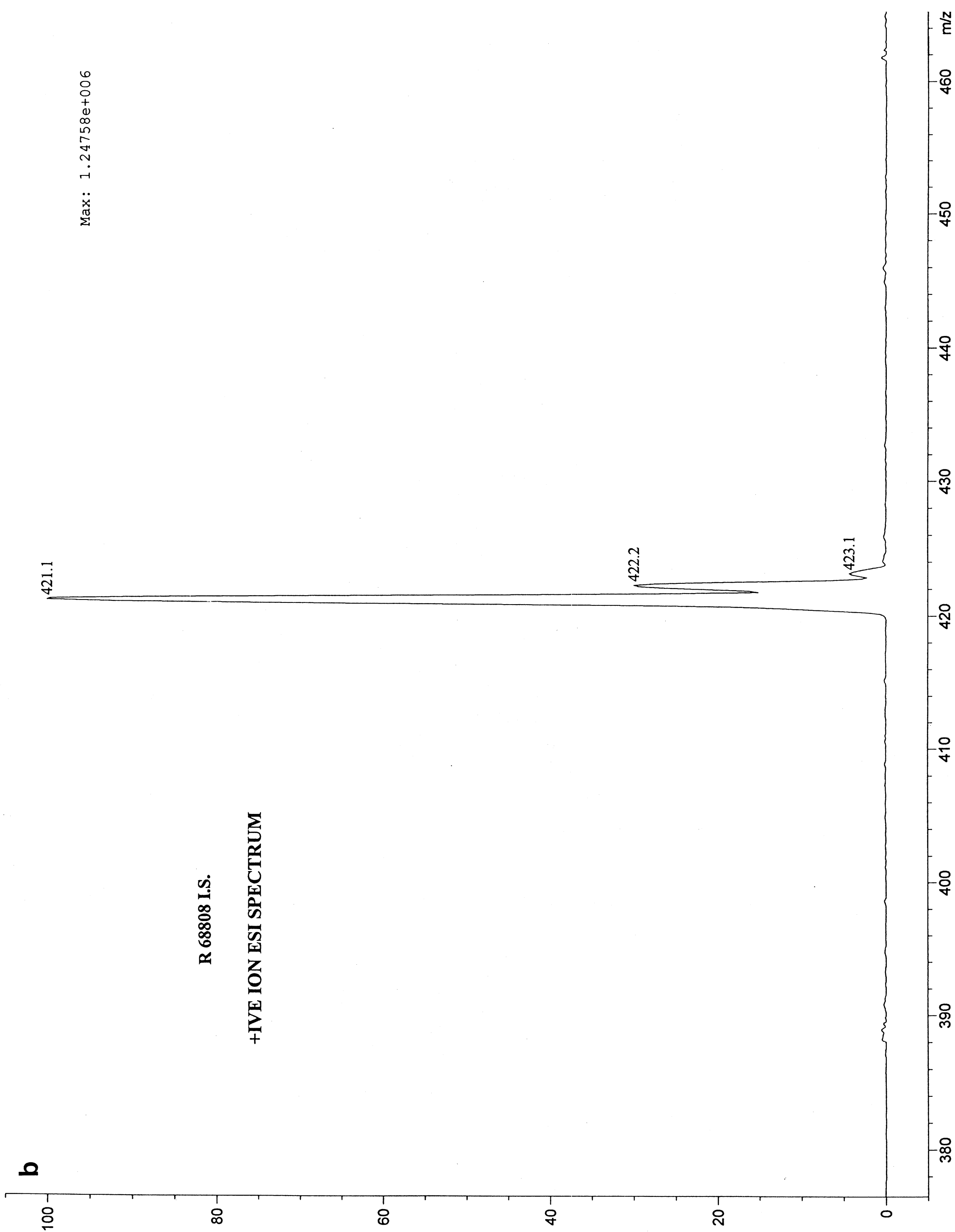


Fig. 2. (*continued*)

Fig. 3a and b show typical chromatograms attained with the method described above. The separation in time, between compounds, is adequate. In the LC–MS method and under these conditions there is no interference at the m/z for the domperidone protonated molecule by the internal standard and it does not interfere with the determination of domperidone even at the 0.50 ng/ml level. As can be seen from Fig. 3b, the sensitivity is more than adequate for both the calibrated range and the concentrations present in vivo over the expected therapeutic range. In addition the mass sensitivity, or the sensitivity based on the mass of domperidone injected, is greater than that provided by an HPLC technique based on UV detection (Fig. 4a). An example of a 100 pg/ml human breast milk standard is shown for comparison (Fig. 4b). The SIM chromatograms of patient samples from both matrices showed good peak shape and adequate intensity, even at the LOQ of 0.5 ng/ml (Fig. 4a). It must be borne in mind that this signal results from less than 10% of the injectable sample. Given the selectivity of this detection, larger injections could increase the available signal at all levels without decreasing chromatographic integrity. However a level of 0.5 ng/ml was deemed adequate as LOQ for the goals of the study in both matrices.

Table 4 details a sample of the patient data. The clinical significance of the data as well as a full discussion of clinical study will be published elsewhere. The data clearly shows that this method could be applied successfully to in vivo samples. Chromatograms from both serum and breast milk (Fig. 5a and b) are typical for the results of this method. There is little chromatographic difference, at this concentration level, between the in vitro matrix standards (Fig. 4a and 4b) and the in vivo patient samples (Fig. 5a and 5b) samples in the case of the LC–MS technique. The same comparison shows that, at the same concentration, the LC–UV technique displays considerable differences between the in vitro and in vivo samples. While the breast milk standards appear to be promising, the patient samples are too complex to be usable. The LC–UV technique, due to insufficient selectivity, would require additional clean-up steps to arrive at a usable technique.

No previous instrumental methods describing the determination of domperidone in human breast milk could be found. A HPLC method for the compound in rat plasma has been detailed; however the method was used in metabolic studies and the concentrations found in animals were higher than those expected in man [1]. Antibody based methods have given excellent results for this compound in plasma, but suitable antibodies were not available for this study.

3.3. Statistics

Single replicates of either breast milk or serum standards were processed, and the area under the chromatographic peak was determined for both domperidone and the internal standard R68808. The area ratio of domperidone to R68808 was determined for each concentration level and then plotted against concentration. The data was best fitted by the relationship $y=ax^2+bx+c$ and weighted 1/concentration, where y is the area ratio and x is the concentration. The correlation coefficient for the regression was 0.99964 for the breast milk curve and 0.99954 for the serum curve. The data is summarized in Table 1. All back calculated data points for both serum and breast milk matrices were within $\pm 10\%$ of the value predicted by the regression line. The area ratio versus concentration relationship, for both matrices, was predictable and sufficiently accurate for the requirements of the study.

The within run precision and accuracy was determined for the breast milk matrix by running replicates of samples at known concentrations against a calibration curve. Five identical sets of standards, at four concentrations in the calibration range, were run along with the calibration curve and these standards were treated as samples. This exercise was performed two times. The data from all replicates at all sample levels for each of the two trials is shown in Table 2. The within-run precision and accuracy was not performed for the serum matrix, but the between-day precision and accuracy indicate that the within-day performance was would be satisfactory. The accuracy for all concentrations on both days was better than 4% relative to the expected concentration, with the exception of the 1.00 ng/ml sample on the first day, which was better than 9%. Reproducibility was excellent, for all concentrations, with relative standard deviation

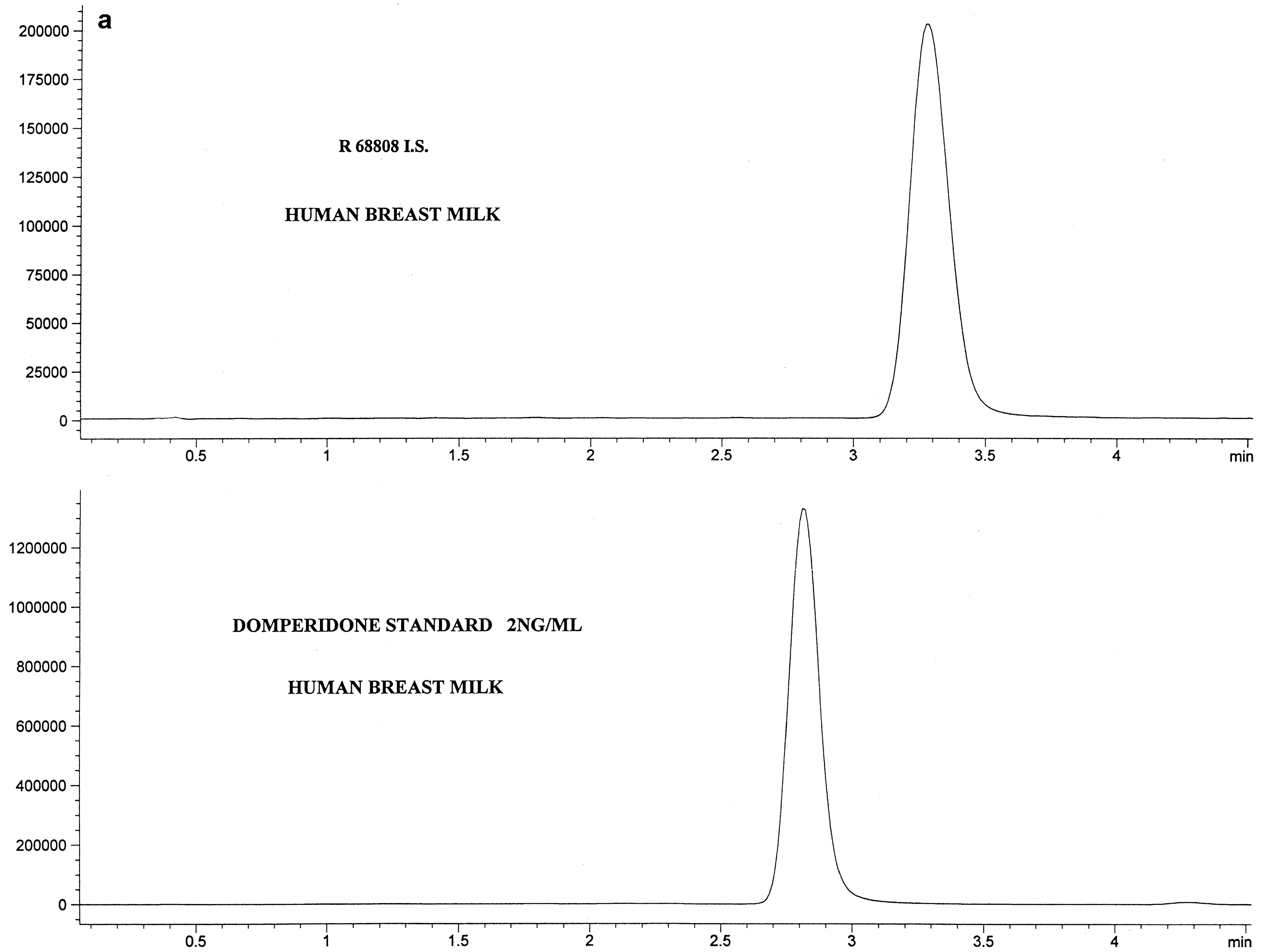


Fig. 3. (a) Chromatogram of breast milk standard at 2 ng/ml. Domperidone determined at nominal m/z 426 and internal standard determined at nominal m/z 421. (b) Chromatogram of serum standard at 2 ng/ml. Domperidone determined at nominal m/z 426 and internal standard determined at nominal m/z 421.

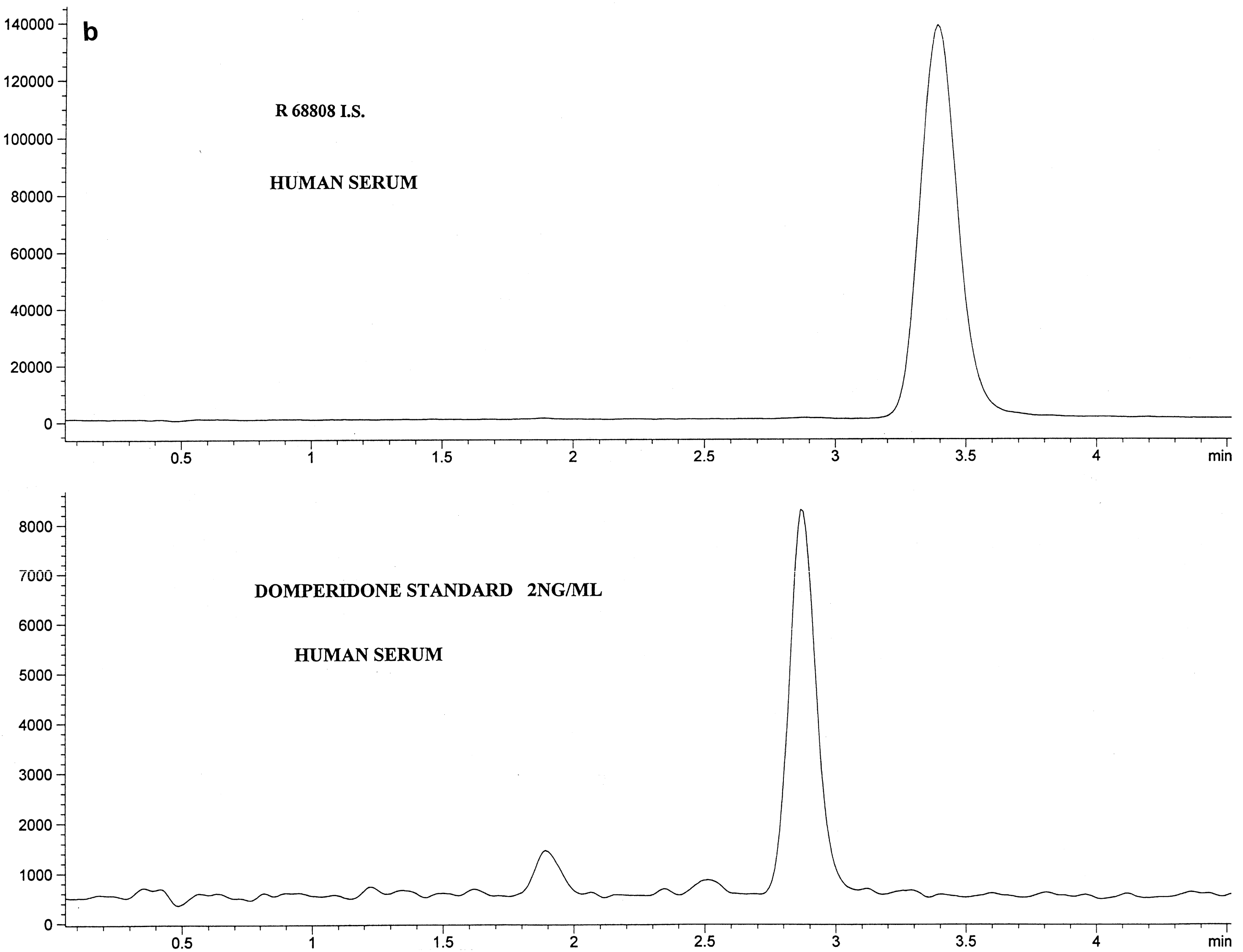


Fig. 3. (*continued*)

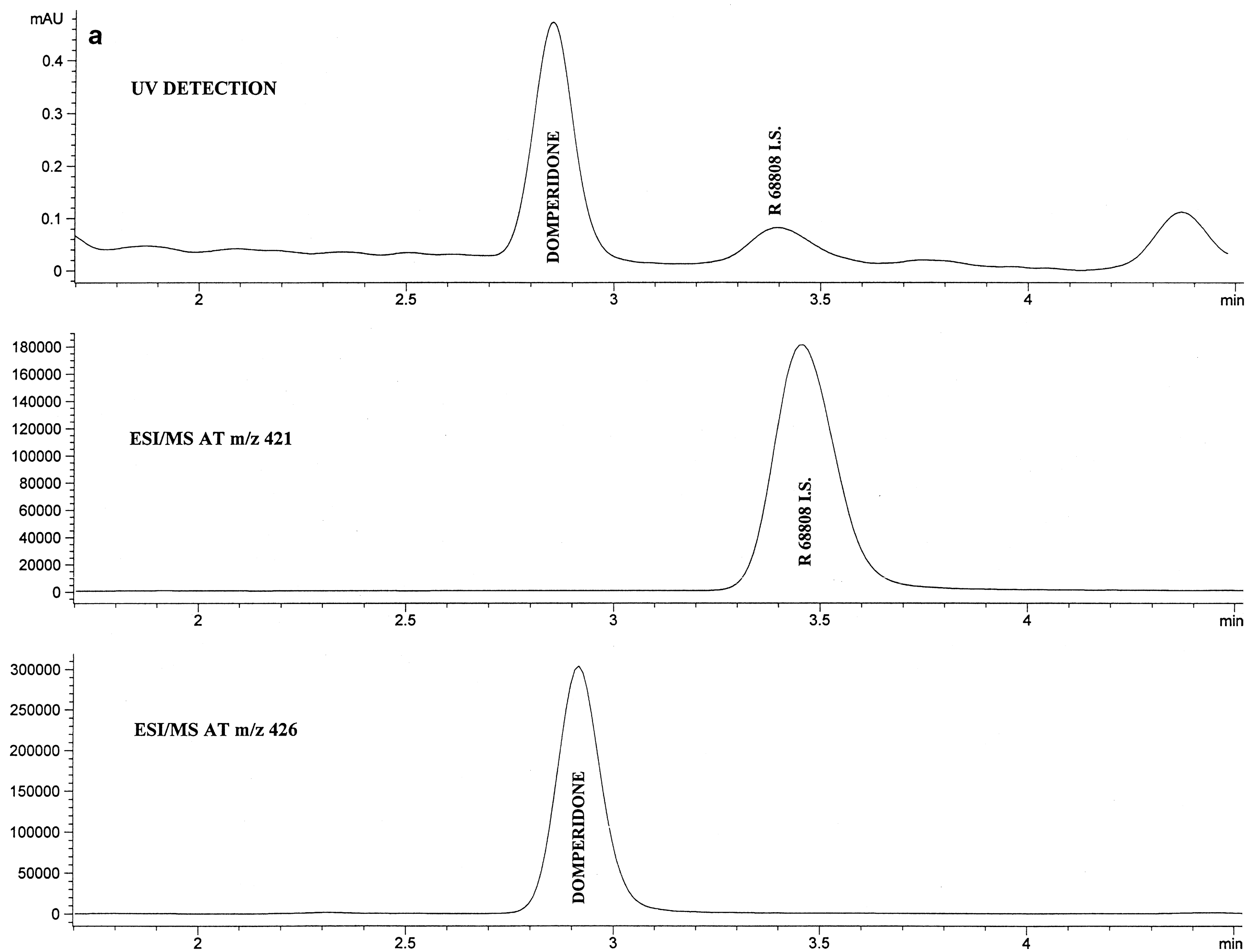


Fig. 4. (a) Comparison of HPLC–DAD and LC–ESI-MS signals for a breast milk standard at 0.5 ng/ml. (b) Example of a 100 pg/ml human breast milk standard. This level is five-times lower than the lowest concentration range used in the calibration curves.

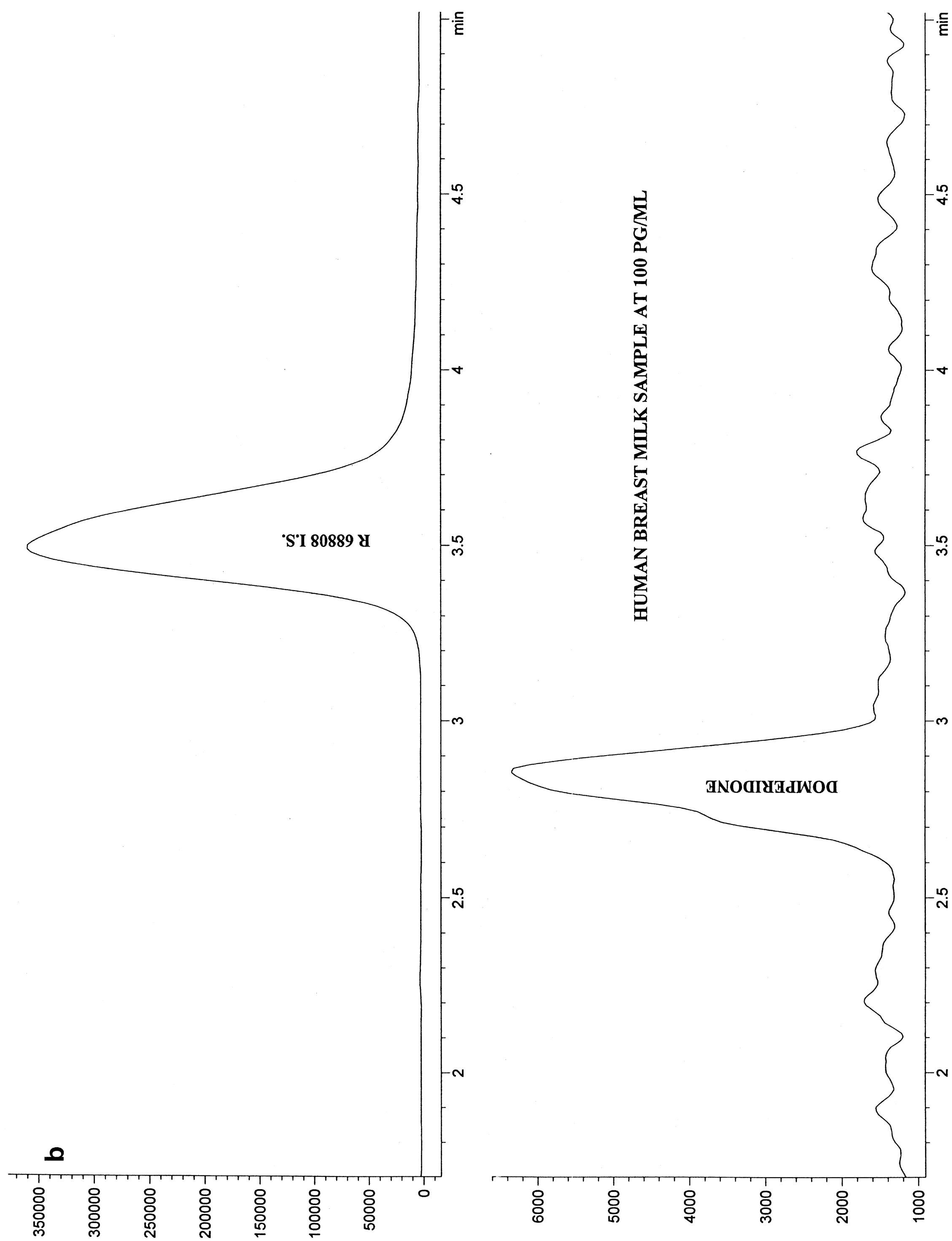


Fig. 4. (*continued*)

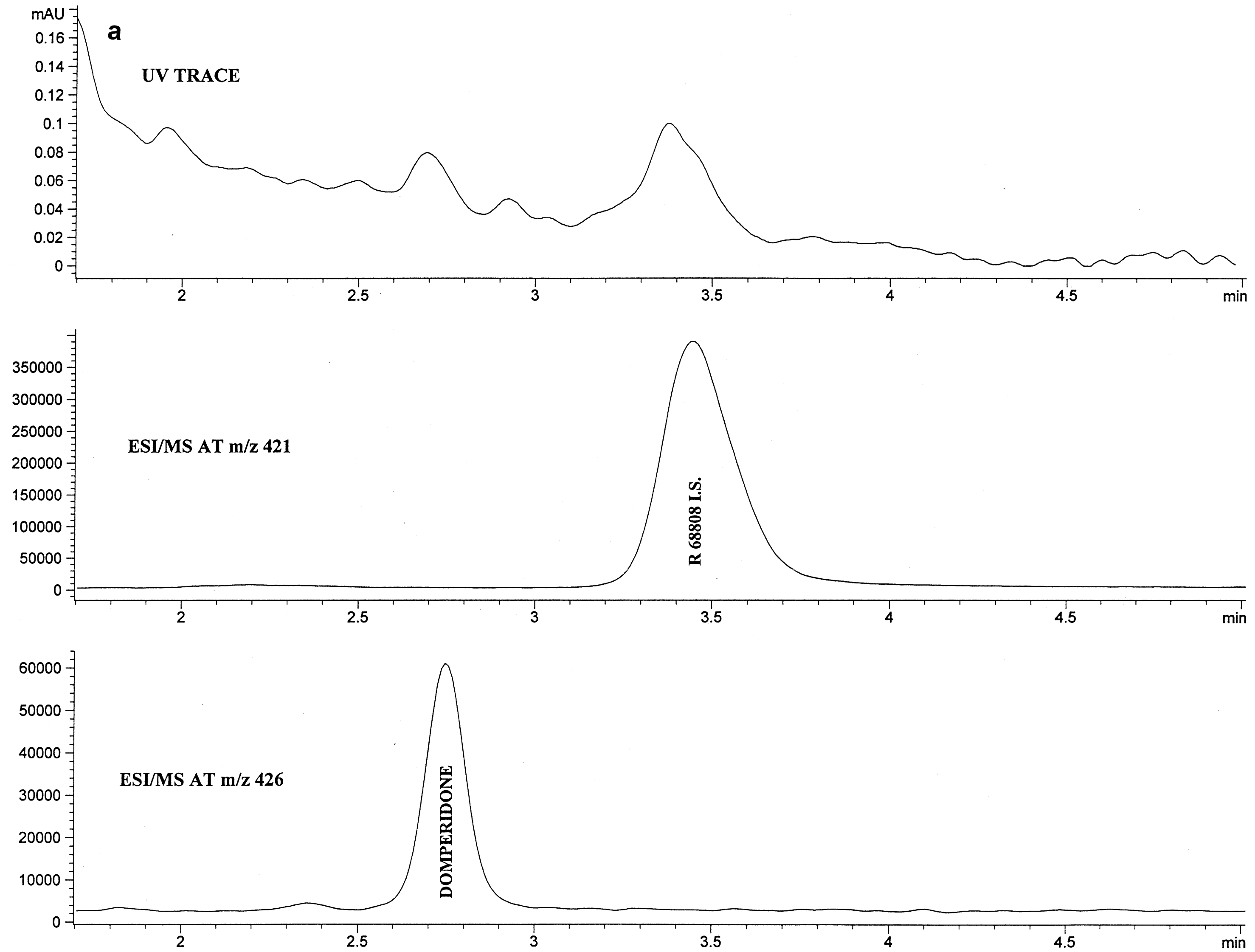


Fig. 5. (a) Typical chromatogram of a breast milk sample for subject T52023 determined to be 1.2 ng/ml. Domperidone determined at nominal m/z 426 and internal standard determined at nominal m/z 421. (b) Typical chromatogram of a serum sample for subject T52023 determined to be 2.4 ng/ml. Domperidone determined at nominal m/z 426 and internal standard determined at nominal m/z 421.

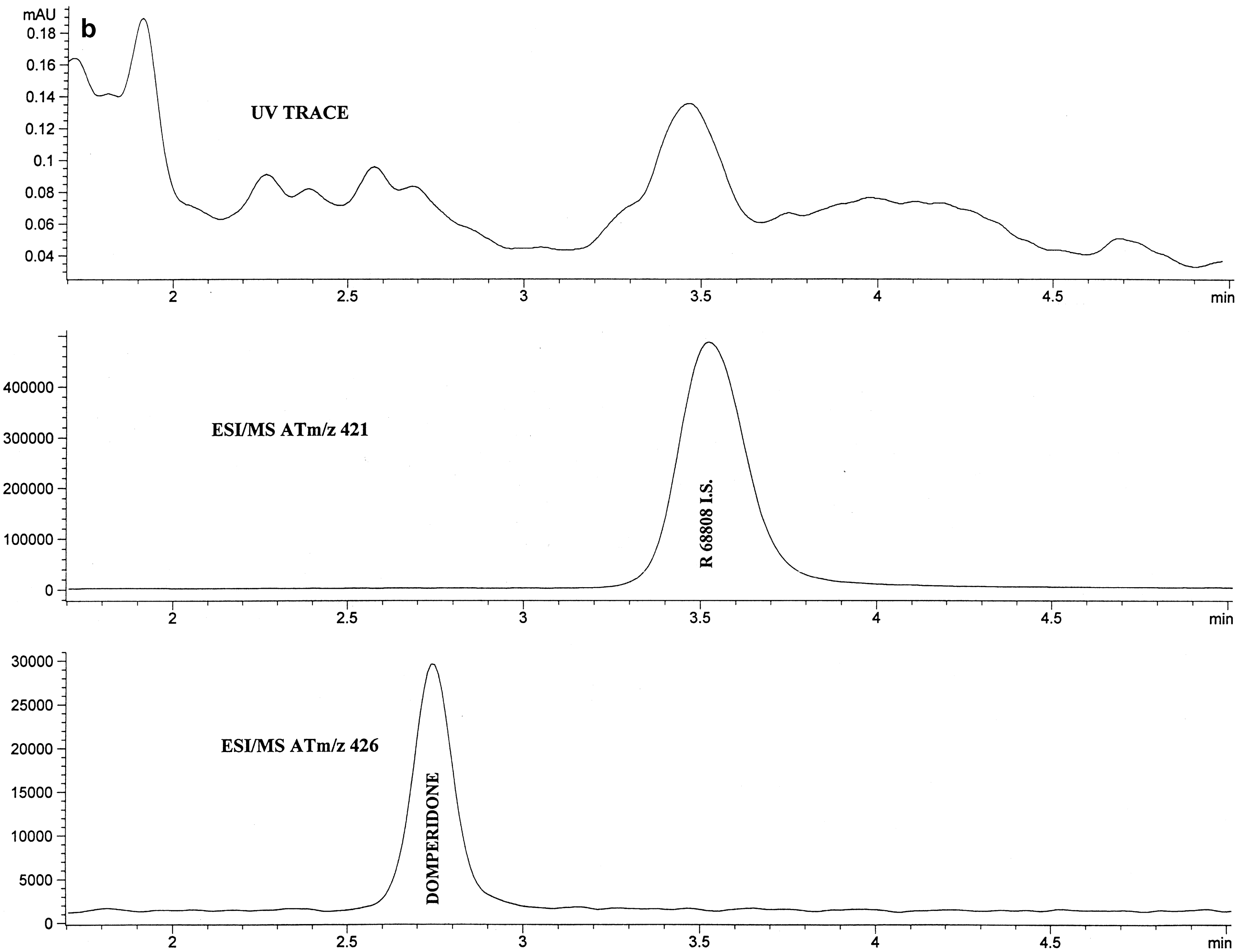


Fig. 5. (*continued*)

Table 1
Difference of measured to back calculated data

Amount DOM (ng/ml)	% Difference from back calculated data	
	Serum[a]	Breast milk[b]
0.5	6.3	−3.4
1.0	−7.3	1.5
2.0	−0.9	5.0
4.0	−0.6	−3.0
6.0	0.4	−0.3
8.0	2.9	−0.2
10.0	−1.8	0.6

[a] See serum curve.
[b] See breast milk curve.

(RSD) values better than 5%. The statistics indicate that the method would provide adequate data given the purposes of the study

The between-run precision and accuracy was determined by running duplicate curves on each of five days. One of the curves was used to calibrate the instrument and the standards in the other curve were treated as samples. The data from each of the five days is detailed in Table 3 and shows the comparison between the amount of domperidone added to the sample and that found by the method. The RSD on a determination at any level for either matrix was under 10%. The error in the determination, for both matrices, was found to be under 7% at any concentration in the range save one. The error in the lowest level serum standard was calculated at 29.2% and was roughly three times larger than any other point. The integration was largely responsible for the discrepancy and greater accuracy could have been available if manual integration would have been used for the lowest level serum standards. Since the breast milk levels were the primary target of the study, an error of 30% was considered adequate at the 0.5 ng/ml level in serum. Moreover, there were very few patient serum samples that were determined between 0.5 and 1.0 ng/ml so the potential impact of greater error at the LOQ was minimal.

3.4. Interference study

The method observed no interferences for either domperidone or R68808, in the SIM chromatograms, from the following compounds: acetominophen, alprazolam, amikacin, caffeine, carbamazepine, chloramphenicol, clomiprimine, codeine, desmethylclomiprimine, digoxin, disopryamide, ethosuximide, fentanyl, flecanide, fluvoxamine, hydroxyamoxapine, hydroxyloxapine, gentamicin, lidocaine, loxapine, methotrexate, morphine, *N*-acetylprocainamide, netilmicin, pentyl-PPX, phenobarbital, phentoin, primadone, procainamide, propanolol, quinidine, respiridone, 9-hydroxyrespiridone, salicylate, theophylline, tobramycin, triazolam, valproic acid, vancomycin.

None of these compounds gave a response at either of the two mass-to-charge ratios monitored in the method, at a retention time that could interfere with domperidone or the internal standard. This is

Table 2
Domperidone within-run precision and accuracy for breast milk on (a) Day 1 and (b) Day 2

Amount added (ng/ml)	Amount found Day 1 (ng/ml)							
	Replicate					Mean	Error (%)	RSD (%)
	1	2	3	4	5			
0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.7	4.8
1.0	0.9	0.9	0.9	0.9	0.9	0.9	8.6	2.8
4.0	3.9	4.0	4.0	4.0	4.0	4.0	0.4	0.8
10.0	9.9	9.8	9.6	9.8	9.8	9.8	2.4	1.2
	Amount found Day 2 (ng/ml)							
0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.3	3.3
1.0	0.9	1.0	1.0	0.9	1.0	1.0	−3.8	4.4
4.0	4.2	4.2	4.1	4.1	4.2	4.1	3.6	1.2
10.0	9.8	10.0	10.1	10.0	9.8	9.9	−0.7	1.4

Table 3
Domperidone between-run accuracy and precision for (a) serum and (b) breast milk

Amount added (ng/ml)	Amount found (ng/ml) Day 1	2	3	4	5	Mean	Error (%)	RSD (%)
Serum								
0.5	0.7	0.7	0.6	0.6	0.6	0.6	3.6	29.2
1.0	1.0	1.2	1.0	1.0	1.0	1.0	9.3	4.0
2.0	2.0	1.9	2.2	2.1	2.1	2.0	4.6	1.3
4.0	3.8	3.7	3.8	4.0	3.5	3.7	4.3	−6.4
6.0	5.8	6.0	5.9	5.7	6.2	5.9	2.7	−1.4
8.0	8.5	8.3	8.3	8.4	8.5	8.4	1.0	5.0
10.0	9.8	9.8	9.9	9.9	9.8	9.8	0.5	−1.8
Breast milk								
0.5	0.5	0.5	0.5	0.4	0.5	0.5	2.0	0.0
1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.2	0.8
2.0	2.1	2.1	2.0	2.1	2.2	2.1	2.0	5.0
4.0	3.8	3.8	3.7	3.7	3.9	3.8	2.0	−4.9
6.0	5.9	5.7	5.9	6.1	5.9	5.9	2.4	−1.5
8.0	8.0	8.1	7.9	8.2	8.2	8.1	1.6	1.0
10.0	10.3	10.4	10.5	9.9	9.9	10.2	2.8	1.9

not surprising considering that, in the method described above, there is extensive clean-up followed by a chromatographic separation of significant resolving power where the target compounds are well retained, well separated from the void volume, selectively ionized, and detected by a very selective detector. The sample is not exposed to the artifact formation or to the ionization irregularities that could be present if introduced without a column in line.

The role of these compounds, or other matrix components, in either suppressing or enhancing the response of domperidone and the internal standard was not investigated. There may be a concern that given the selectivity of LC–MS, a co-eluting compound can exert influence on the target molecule without itself being detected in a SIM run. The calibration curve is not injected from pure solutions, but from standards made in drug-free matrix, and extracted by the same method as the patient samples (see Table 4 for patient data). Under this scheme, the matrix calibration curve will suffer the same effects as the sample and the resultant determination will be accurate. The only concern remaining is that the matrix that was used for the calibration standards mirrors the suppression or enhancement effects of the samples. The method described gave excellent results given the limited circumstances of the study. The response suppression or enhancement by matrix components between patients, will be further studied in future work in preparation for a more definitive study

3.5. Speed of method development

The most impressive aspect of the entire study was the speed with which a method of such performance was developed. The total time taken, from the uncrating of the instrument to the injection of the

Table 4
Patient data

Patient ID	Serum	Breast milk Replicate 1	2	3	Mean
T51970	1.3	1.3	1.3	1.3	1.3
T52000	4.6	1.5	1.6	1.5	1.5
T51977	<0.5	<0.5	<0.5	<0.5	<0.5
T52040	2.6	7.6	7.3	7.1	7.3
T51997	<0.5	<0.5	<0.5	<0.5	<0.5
T52018	<0.5	<0.5	<0.5	<0.5	<0.5
T52023	3.7	1.2	1.2	1.5	1.3
T52026	16.4	1.3	1.2	1.3	1.3

first extracted samples, was about 72 h. Initially, drug-free human breast milk was not available, so the first samples were made in commercial bovine whole milk from the hospital cafeteria. The method from bovine milk was essentially complete within the first 72h. Human breast milk gave a residue with considerably more lipid in the extract, and lead to the inclusion of the hexane extraction as the final extraction step. The final extract, as described in the procedure, was deemed clean enough for trouble free injection. In all, the development effort was effectively complete in less than a week from the uncrating of the instrument. Moreover, methods for other compounds were completed during the same time period.

4. Conclusions

The method described above performed reliably over the duration of the study without problems. The column lifetime was excellent and the sensitivity was maintained for the duration of the study. Data quality exceeded expectations, as did the speed of development and speed of project completion. The greater selectivity of LC–MS as compared to LC–UV and the far greater sensitivity of the SIM detection mode as compared to most other HPLC techniques, contributed to the speed of method development and to data quality.

Future efforts could center about a time course of the domperidone levels in breast milk following a therapeutic dose. Such an effort may require slight modifications to the method to utilize larger injection volumes thereby increasing the sensitivity of the measurement.

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