Published in final edited form as:

J Pharm Biomed Anal. 2011 July 15; 55(5): 1127–1135. doi:10.1016/j.jpba.2011.03.043.

Buspirone, fexofenadine, and omeprazole: Quantification of probe drugs and their metabolites in human plasma

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Abstract

Probe drugs are critical tools for the measurement of drug metabolism and transport activities in human subjects. Often several probe drugs are administered simultaneously in a cocktail. This cocktail approach requires efficient analytical methods for the simultaneous quantitation of multiple analytes. We have developed and validated a liquid chromatography-tandem mass spectrometry method for the simultaneous determination of three probe drugs and their metabolites in human plasma. The analytes include omeprazole and its metabolites omeprazole sulfone and 5-hydroxyomeprazole; buspirone and its metabolite 1-[2-pyrimidyl]-piperazine (1PP); and fexofenadine. These analytes and the internal standard lansoprazole were extracted from plasma using protein precipitation with acetonitrile. Gradient reverse-phase chromatography was performed with 7.5 mM ammonium bicarbonate and acetonitrile, and the analytes were quantified in positive ion electrospray mode with multiple reaction monitoring. The method was validated to quantify the concentration ranges of 1.0–1000 ng/ml for omeprazole, omeprazole sulfone, 5-hydroxyomeprazole, and fexofenadine; 0.1–100 ng/ml for buspirone, and 1.0–100 ng/ml for 1PP. These linear ranges span the plasma concentrations for all of the analytes from probe drug studies. The intra-day precision was between 2.1–16.1%, and the accuracy ranged from 86–115% for all analytes. Inter-day precision and accuracy ranged from 0.3–14% and from 90–110%, respectively. The lower limits of quantification were 0.1 ng/ml for buspirone and 1 ng/ml for all other analytes. This method provides a fast, sensitive, and selective analytical tool for quantification of the six analytes in plasma necessary to support the use of this probe drug cocktail in clinical studies.

Keywords

Probe drug cocktail; Human plasma; Buspirone; Fexofenadine; Omeprazole

1. Introduction

Drug-drug and drug-diet interactions are a significant source of variability in response to both prescription and over-the-counter drugs. Often these interactions occur due to

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alterations in the functional activities of enzymes and transporters controlling the pharmacokinetics of affected drugs. Although a wide range of experimental systems, ranging from *in silico* to animal models, have been used to study these interactions, the ultimate test is to assess potential interactions in human subjects. [1–4] Probe drugs provide a powerful tool for detecting and characterizing such pharmacokinetic drug-drug and drug-diet interactions in humans. Probe drugs are well-tolerated drugs that may be administered to humans for the sole purpose of measuring functional activities of drug metabolizing enzymes or transporters. The pharmacokinetics of a validated probe drug is well characterized, and demonstrates that the disposition of the probe is primarily controlled by a specific enzyme or transporter. As a result, measurement of concentrations of the probe drug, or of specific metabolites, in plasma or urine provides a validated index of activity for an enzyme or transporter involved in drug disposition.

Combining multiple probe drugs into a cocktail greatly increases the efficiency of testing by allowing the measurement of multiple enzyme activities in a single dosing and sampling procedure. This cocktail approach, however, requires a corresponding efficiency in analytical procedures for the full advantage to be realized. The probe drug cocktail we have developed includes three drugs and their metabolites that must be quantified in serial plasma samples. Omeprazole and two of its metabolites are quantified as probes for CYP2C19 [5–8] and CYP3A4 activity [9, 10], and measurement of plasma concentrations of buspirone and its primary metabolite provide a probe for CYP3A4 activity [11–13]. The bioavailability of fexofenadine is controlled by OATPs and P-glycoprotein [14] and so measurement of fexofenadine concentrations in plasma provides a probe for transporter function. Structures of these analytes and of the internal standard, lansoprazole are shown in Figure 1.

Although individual validated analytical methods are available for each of these probe drugs and their metabolites [9, 15, 16], no method has been reported for the quantitation of all of these analytes in a single procedure. To provide this essential tool, an analytical method for the determination of these three substrates and their metabolites in human plasma has been developed. We describe here the development and validation of a single LC-MS/MS method for the concurrent examination of these three probe drugs and their metabolites in human plasma. This method provides the specificity, sensitivity, and the required efficiency of analysis to support the use of this probe drug cocktail for the characterization of drug metabolizing enzyme and transporter activities in humans.

2. Materials and Methods

2.1 Chemicals and reagents

Fexofenadine, omeprazole, buspirone, 1-[2-pyrimidyl]-piperazine (1PP), and lansoprazole were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Omeprazole sulfone and 5-hydroxy-omeprazole were generously provided by AstraZeneca (Molndal, Sweden). The structures of all analytes and the enzymes responsible for each metabolite are shown in Figure 1. Optima-grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA, USA). Pooled, heparinized, drug-free human plasma was purchased from Innovative Research, Inc. (Novi, MI, USA). All other reagents used were analytical grade and were purchased from Fisher Scientific, including ammonium hydroxide, ammonium bicarbonate, formic acid, and acetic acid. Water was prepared using a Millipore water purification system.

2.2 Calibration standards and quality control samples

The master stock solutions of each probe drug and metabolite, and of the internal standard lansoprazole, were prepared at 1.0 mg/ml in methanol. All stock solutions were stored at

4°C and remained stable for at least two weeks, based on consistent peak shape and peak area of the MRM chromatograms. The working solution of each analyte was prepared by diluting the stock solution with acetonitrile/water (25:75; v/v). Separate working solutions were prepared, with one used for preparation of calibration standards and a separate working solution for preparation of QC samples. Standard calibration samples and quality control (QC) samples were prepared by spiking blank human plasma with the working solution of the analytes. The specific concentration range for calibration of each analyte was based on the previously observed plasma concentrations for these probes in humans at the doses employed. The final internal standard concentration in plasma was 100 ng/ml.

2.3 Sample preparation

All calibration samples, QC samples, and clinical samples were processed using the same sample preparation procedure. The stock solutions of analytes and internal standard were combined into standard spiking solutions at appropriate concentrations, as to produce calibration and QC samples at proposed concentrations. These spiking solutions included a zero analyte solution, which contained only the internal standard. A 10 µl aliquot of the appropriate spiking solution was added to 0.5 ml of blank plasma. An acetonitrile protein precipitation was performed by addition of 2 ml ice-cold acetonitrile, followed by thorough vortex mixing. The sample was centrifuged for 20 min at 600 g at 0–4°C. The supernatant was transferred and the solvent was evaporated under vacuum at room temperature. The residue was reconstituted with 100 µl of acetonitrile/water (25:75; v/v) and centrifuged for 2 min, then filtered through a 0.2 µ filter. Plasma samples from the human subjects dosed with the probe drug cocktail were prepared and processed by the same protocol, except that the zero analyte spiking solution, containing only the internal standard, was used.

2.4 Liquid Chromatography-Mass Spectrometry

Chromatography was performed on a Phenomenex Luna C18 analytical column (50 × 2mm, 5 µ) with a Luna C18 4 × 2 mm guard cartridge (Phenomenex, Torrance, CA). Column temperature was maintained at 45°C. The mobile phase for the binary gradient consisted of acetonitrile and ammonium bicarbonate in water (pH 8; 7.5 mM) at a total flow rate of 0.5 ml/min. Elution employed a multiple stage linear gradient, with acetonitrile content changing from 20% to 25% between 0 and 4 min, and from 25% to 45% between 4 and 7 min. Acetonitrile content was maintained at 45% from 7 to 9 min, and then was decreased to 20% at 9 min over 0.25 min. The total run time was 12 min per sample. The injection volume was 10 µl.

A Waters Quattro Premier mass spectrometer with an electrospray ionization (ESI) probe was used (Waters, Milford, MA). The positive ionization mode was used for all analytes with the source and desolvation temperature at 120°C and 375°C, respectively. The capillary voltage remained at 1kV, with the cone and desolvation nitrogen gas flow rates set at 100 and 500 l/hr, respectively. Argon was used as the collision gas, with a collision cell pressure of approximately 3.5×10^{-3} mbar. Multiple Reaction Monitoring (MRM) was used to monitor the transitions of each analyte with a dwell time of 50 msec for each transition. MRM transitions and fragmentation conditions chosen for individual analytes are shown in Table 1. All data acquisition and analysis were carried out using MassLynx 4.1 and QuanLynx software.

2.5 Method validation

The method was validated using QC samples from each analyte's calibration curve. The validation was conducted with respect to linearity/sensitivity, sample preparation recovery, precision, accuracy, and matrix effects.

2.5.1 Linearity and sensitivity—Calibration samples included a zero concentration and 9 non-zero concentrations of each analyte in plasma, each containing 100 ng/ml lansoprazole as the internal standard. Non-zero concentrations for fexofenadine, omeprazole, and the two omeprazole metabolites were 1, 2, 10, 25, 50, 100, 250, 500, and 1000 ng/ml. Non-zero concentrations for buspirone and 1PP were 0.05, 0.10, 1, 5, 10, 25, 50, 75, and 100 ng/ml. The calibration curves for each analyte were constructed using the analyte/IS peak area ratio versus the analyte concentration, and were fitted by a linear least-squares regression with a $1/x^2$ weighting factor. The lower limit of quantification (LLOQ) was defined as the lowest tested concentration of analyte in plasma that was quantified with accuracy and precision within $\pm 20\%$ of the actual value. The LLOQs of the analytes in this assay are shown in Table 2, and are the lowest concentrations tested for the intra- and inter-day accuracy and precision (Tables 3 and 4).

2.5.2 Recovery—The protein precipitation recoveries for the plasma samples were determined by spiking an analyte and internal standard solution into 0.5 ml samples of mobile phase and of blank plasma. The plasma samples were processed by protein precipitation as described above. Mobile phase samples and plasma extracts were evaporated and the residues reconstituted in mobile phase as described. Recovery was defined as the ratio of analyte peak area from plasma to peak area from the corresponding sample from mobile phase. The recovery experiment was performed with each analyte at five different concentrations within the range of 5 ng/ml to 5000 ng/ml.

2.5.3 Precision and accuracy—For precision and accuracy, five replicates of each of four QC concentrations in pooled human plasma were processed and analyzed according to the method described above. The inter-day results shown are based on data from four different sample sets formulated and processed on separate days, each consisting of four non-zero analyte concentrations. Precision was calculated as the relative standard deviation (RSD). Accuracy was calculated from the % deviation = $[100 \times (\text{Measured} - \text{Actual}) / \text{Measured concentrations}]$. The acceptable precision and accuracy were both set at $\pm 15\%$ for all concentrations except the LLOQ, which was set at $\pm 20\%$.

2.5.4 Matrix effect—The matrix effect was evaluated by studying ionization suppression due to the components in the plasma. A 0.5 ml blank plasma sample was processed by the precipitation protocol described above, the supernatant was evaporated, and the residue was reconstituted with a 100 μ l of 25% acetonitrile containing analytes with the corresponding concentration to unextracted neat samples. The peak areas for analytes from plasma samples spiked post-extraction were divided by the corresponding peak areas from neat samples.

2.6 Clinical sample generation

All documents and procedures, including the informed consent document, were reviewed and approved by the Institutional Review Boards of the University of Kansas Medical Center. Healthy subjects, who had fasted overnight, had an intravenous line placed and blood was obtained for baseline analysis prior to the administration of probe drugs. Subjects then were administered probe drugs orally with 200 ml water. The probe drugs and doses were buspirone (10 mg), fexofenadine (60 mg), and omeprazole (20 mg). Fasting was continued for an additional two hrs after probe drug administration to allow for absorption. Blood was collected in heparinized tubes at 0.5, 1, 1.5, 2, 3, 4, 5, and 8 hrs after dosing for the determination of fexofenadine, buspirone, and omeprazole parent drug and metabolite concentrations. Plasma was prepared within 30 min of collection by centrifugation at 1000g at 0–4°C for 10 min, and was stored below -70°C .

3. Results

3.1 Mass spectrometry and chromatography

Each analyte was infused into the probe, a full-scan mass spectrum was obtained in positive ion mode using electrospray ionization, and the MS was tuned to optimize detection of the most abundant product ion. The transitions chosen and the optimized instrument parameters are presented in Table 1. We tested a range of different mobile phases in order to optimize chromatography and MS signal. Aqueous solvents were modified with formic or acetic acid, ammonium acetate, or ammonium bicarbonate, with resulting pH from 3 to 8, and organic solvents tested with each aqueous solvent were acetonitrile, methanol, and an acetonitrile-methanol mix (1:1, v/v). A mobile phase composed of 7.5 mM ammonium bicarbonate at pH 8 and acetonitrile was found to produce baseline resolution between analytes, acceptable peak shape, and the highest response factor for the analytes. Infusion of the analyte mixture into this flowing mobile phase as it entered the probe did not change the optimized instrument parameters listed in Table 1, that were obtained by infusion in the absence of mobile phase (data not shown). Figure 2 shows the ion chromatograms of a pooled blank human plasma sample spiked with all of the probe drug and metabolite analytes at a concentration of 10 ng/ml and the internal standard at 100 ng/ml.

3.2 Linearity and sensitivity

Calibration sets were run with nine non-zero concentrations of each probe drug analyte to determine the linearity of the assay. Fexofenadine, omeprazole, and the two omeprazole metabolites were tested over a range of 1–1000 ng/ml, whereas buspirone and its metabolite 1PP were tested over the concentration range of 0.05–100 ng/ml. These concentration ranges were chosen based on expected plasma concentrations for each analyte resulting from the oral doses employed in our probe drug cocktail. The lower limit of quantitation for each analyte was defined as the lowest concentration standard from spiked plasma that could be measured with accuracy of $\pm 20\%$ and precision within 20%. The LLOQ, indicating the sensitivity of the method, was 1 ng/ml for all analytes with the exception of buspirone, with a LLOQ of 0.1 ng/ml (Table 2). The calibration curve for each analyte was obtained by using a best-fit line with a weighting factor of $1/x^2$. Data from analyte concentrations below the LLOQ were excluded. Correlation coefficients were >0.98 for all analytes as shown in Table 2. Based on these data, the assay was defined as linear over the concentration range of 1–1000 ng/ml for fexofenadine, omeprazole, 5-hydroxyomeprazole and omeprazole sulfone, and for 0.1–100 ng/ml for buspirone and for 1PP.

3.3 Precision and accuracy

Intra-day precision and accuracy of the method were assessed by analysis of five replicate samples at each of five analyte concentrations. The concentrations of analytes chosen were the LLOQ and the upper limit of linearity, as determined from the linearity and sensitivity study, and three intermediate analyte concentrations. The precision and accuracy data derived from analysis of these replicate samples are shown in Table 3. The accuracy for non-LLOQ standards, presented as deviation from actual concentration, ranged from -14.2% – 7.4% . The precision, defined as relative standard deviation (RSD), ranged from 2.1% – 7.5% . The deviations in accuracy of LLOQ standards ranged from -5.4% – 15.3% , and the precision ranged from 5.0% – 16.1% .

The determination of inter-day precision and accuracy utilized four separate sets of spiked plasma samples, with each set prepared and analyzed on a separate day. The concentrations of standards were the same as for the determination of intra-day precision and accuracy, and were chosen to cover the range of analyte concentrations expected from actual study samples. These data (Table 4) demonstrated inter-day precision and accuracy for non-LLOQ

samples ranging from 0.3% – 13.6% and –8.9% – 8.3%, respectively. LLOQ sample precision and accuracy ranged from 4.0% – 14.% and from –10.% – 9.5%, respectively.

3.4 Recovery

Given the range of basic and zwitterionic analytes to be prepared, and the resulting challenges for liquid-liquid or solid-phase extraction approaches, a simple protein precipitation with organic solvent was employed for sample preparation. The acetonitrile protein precipitation method yielded a mean recovery of better than 90% for all analytes throughout the linear concentration range of the assay, except 1PP (Table 2). 1PP had a low mean recovery of 48%, however this recovery was reproducible. The accuracy and precision results for 1PP using this sample preparation method support the acceptability of this procedure for bioanalytical use.

3.5 Matrix effect

The ion suppression results ranged from mean values of –28% to –8% for all compounds except 1PP, which underwent signal enhancement of 29% by the plasma matrix. These results demonstrate that there is a minimal matrix effect on the ionization of analytes prepared from plasma samples (data not shown).

3.6 Application to probe drug study in human subjects

The specificity and sensitivity of this analytical method were demonstrated by the analysis of plasma samples from healthy human subjects enrolled in a study of drug-drug interactions. Representative chromatograms are shown from one subject (Figures 3 and 4). The chromatograms in Figure 3 show the results of processing and analyzing a 0.5 ml plasma sample obtained from a subject immediately prior to administration of the probe drug cocktail. The specificity of the method is apparent from the MRM chromatograms from that sample, which show a prominent peak from the internal standard, but no signal above background for any of the probe drug-derived analytes. The specificity and sensitivity of the method are demonstrated by the data obtained from a plasma sample collected 30 min after dosing, shown in Figure 4. Each MRM chromatogram shows a single peak, with the expected retention time for the selected probe drug or metabolite. Moreover, although this sample was obtained during the absorption phase for the probe drugs and before the time of maximum plasma concentrations, the signal intensity for all analytes was at least 100-fold higher than the background intensities from the pre-dose plasma of the same subject, shown in Figure 3.

The applicability of this method to actual probe drug cocktail studies is shown by the plasma concentration time profiles for the six analytes in Figure 5. We present profiles from a single representative subject who was administered the three probe drugs measured in plasma at the defined doses. The pre-dose plasma sample showed no detectable probe drugs or metabolites, and every measured value for each analyte over the 0.5 to 8 hr sampling period fell within the validated range for the analytes.

4. Discussion

The use of multiple probe drugs in a single dosing and sampling period, namely the —n in 1 approach, greatly increases the economy and efficiency of the clinical side of human studies of drug-drug or drug-diet interactions. This efficiency in dosing and sampling, however, must be matched by corresponding —n in 1 analytical approaches. The three probe drugs measured by the method presented here all have been used in human studies to characterize activities of specific drug metabolizing enzymes and transporters. Omeprazole and its metabolite 5'-hydroxyomeprazole provide a useful measure for CYP2C19 activity [8],

buspirone and its metabolite 1PP provide a measure of CYP3A4 activity [11, 16], and fexofenadine provides a broader measure of membrane transporter activity [14]. In addition, omeprazole sulfone has been proposed as an additional probe for CYP3A4 activity [10]. The use of multiple probes for CYP3A4 was suggested in 2003 as a response to problems correlating results obtained from different individual probes. [17] The potential use of both buspirone and omeprazole as CYP3A4 probes would address this issue, but will only be feasible if these two drugs do exhibit altered disposition *in vivo*. This potential interaction between buspirone and omeprazole is the focus of the first study we are performing using the analytical method reported here.

Buspirone, fexofenadine, omeprazole, and their metabolites must be measured in plasma. Although single time-point metabolite ratios may serve as a validated endpoint for omeprazole as a probe drug [8], buspirone and fexofenadine indices are based on parent and metabolite AUCs [18, 19]. This necessitates analyzing plasma concentrations in at least six serial samples collected over eight hours or more for these two probe drugs. Robust, validated analytical methods exist for each of these probe drugs and their metabolites, using either conventional HPLC [9, 15, 16] or LC-MS/MS [20–22], but each employs a separate and distinct sample preparation and analytical procedure. Previously, we used an HPLC-UV method for detection and quantitation of omeprazole and its metabolites [23], a fluorescence-HPLC method for fexofenadine [15], and an LC-MS/MS for buspirone and 1PP quantification [20]. This required three separate plasma samples for each time point, and a different sample preparation and analytical procedure for each drug. This multiplicity of samples and methods unnecessarily complicated both the clinical and the analytical aspects of the probe drug approach. We have taken advantage of the selectivity and sensitivity of tandem mass spectrometry to develop an —n in 1 analytical method for these three sets of analytes. This validated method decreases the required plasma volume by 80% from that required for the three separate assays, and decreases sample preparation and analysis time by 85% as well.

Focusing the power of LC-MS/MS-based —n in 1 analytical approaches to facilitate probe drug cocktail studies is not novel. Scott et al. reported an LC-MS/MS method for six probe drugs and their metabolites in both plasma and urine [24], Yin et al. validated a similar approach for five drugs and their metabolites [25], Zhang et al. developed an assay for four parent probe drugs in human plasma [26], and Videau et al. presented a UPLC-MS/MS assay for ten probe drugs and ten metabolites in human plasma [27]. The overarching goal in each of these studies was the same as for the current work — to accelerate and simplify the processing and analysis of samples for probe drug studies, such that the efficiency of subject treatment and sampling is matched by the efficiency of the analytical process. Although the rationale was identical for those previous studies and for the current work, they differ from our method in the specific analytes addressed. Three of the methods include omeprazole as one of the probe drugs [25–27], and two of those methods also quantify at least one omeprazole metabolite [25, 27]. None of these methods, however, include the probe drugs buspirone and fexofenadine, and the buspirone metabolite 1PP that are essential analytes for our cocktail.

In addition to the dramatic increase in efficiency provided by this LC-MS/MS method, we also benefitted from the improved specificity of this approach relative to the conventional HPLC methods. We found previously that when analyzing human plasma samples for fexofenadine using the HPLC-fluorescence method [15], about 7% of our subjects did not yield useful data. Those subjects had an interfering compound or compounds in all of their plasma samples, including pre-dose samples that fluoresced strongly and nearly co-eluted with fexofenadine under the chromatographic conditions employed (unpublished observation). This interfering compound either completely masked or at least prevented the

accurate integration of the actual fexofenadine peak. These —analytical dropouts required the recruitment and processing of additional subjects, and thus increased both the cost and the time required for completion of a study. To date we have processed and analyzed plasma samples from over 100 subjects using the LC-MS/MS method we report here, and have not encountered any analytical problems from these samples.

Our data clearly demonstrate that this analytical method reproducibly isolates and quantifies these three probe drugs and their metabolites from human plasma, and provides the sensitivity required for the analysis of samples from clinical studies. The analyte recoveries, linearity of response, accuracy, and precision of the method using spiked pooled human plasma all support the use of this method, and the preliminary results presented from pre-dose and 60 min post-dose plasma samples from a human subject further establish the utility of the approach. This assay provides the required analytical component to support the efficient use of our probe drug cocktail. Moreover, given the specificity of tandem mass spectrometry for detection, this method may serve as an effective platform for modification to accommodate the analysis of additional probe drugs and their metabolites in plasma, expanding the —n of this —n in 1 approach.

Acknowledgments

Supported by an NCI contract (N01 CN-35008-M3) and by grants from NCCAM (R21 AT002907) and from NCRR (P20 RR021940).

Abbreviations

1PP	1-[2-pyrimidyl]-piperazine
OM-S	Omeprazole sulfone
5-OH-OM	5'-Hydroxyomeprazole

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Figure 1.
Structures of analytes: probe drugs, metabolites, and pathways.

Figure 2. Analysis of standards from spiked human plasma

Probe drugs and their metabolites were added to 0.5 ml of blank human plasma at 10 ng/ml, and the IS at 100 ng/ml. Samples were processed and analyzed as described in Materials and Methods.

Figure 3. Analysis of baseline human plasma sample

Analysis of a 0.5 ml plasma sample from a human subject, taken at the time of oral administration of 60 mg Fexofenadine, 20 mg Omeprazole, and 10 mg Buspirone. Plasma was processed and analyzed as described in Materials and Methods.

Figure 4. Analysis of human plasma from t=30 min

Analysis of a 0.5 ml plasma sample from the same human subject as shown in Figure 3, but from a blood sample taken 30 min after the oral administration of 60 mg Fexofenadine, 20 mg Omeprazole, and 10 mg Buspirone. Plasma was processed and analyzed as described in Materials and Methods.

Figure 5. Plasma concentrations of probe drugs and their metabolites, 0-8 hrs

Analysis of 0.5 ml plasma samples from a human subject, taken pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, and 8 hrs after the oral administration of 60 mg fexofenadine, 20 mg omeprazole, and 10 mg buspirone. Plasma was processed and analyzed as described in Materials and Methods.

Table 1

Analytes, Transitions, and MS Conditions

Analyte	Parent	Product	Cone Voltage (V)	Collision Energy (eV)
Fexofenadine	502.2	466.3	40	27
Omeprazole	346.	136.	20	35
Omeprazole Sulfone	362.	150.1	30	23
5-Hydroxyomeprazole	362.	214.	20	10
Buspirone	386.1	122.	30	50
1PP	165.	122.	30	15
Lansoprazole	370.	252.	20	12

Table 2

Validated Range, Linearity, and Analyte Recoveries

Analyte	Calibration range	r ²	Recovery %	LLOQ
Fexofenadine	1–1000 ng/ml	.9918	104. ± 5.	1 ng/ml
Omeprazole	1–1000	.9888	96. ± 8.	1
Omeprazole Sulfone	1–1000	.9902	102. ± 6.	1
5-Hydroxyomeprazole	1–1000	.9956	95. ± 5.	1
Bupirone	0.1–100	.9950	90. ± 10.	0.1
1PP	1–100	.9985	48. ± 16.	1
Lansoprazole	100		94. ± 8.	

Table 3**Intra-Day Accuracy and Precision**

Analyte	Actual Concentration	Measured Concentration	Accuracy (% deviation)	Precision (% RSD)
Fexofenadine	1 ng/ml	1.15 ± 0.06 ng/ml	15.3	5.0
	3	3.1 ± 0.1	3.8	3.6
	100	98. ± 5.	-2.3	5.4
	1000	947. ± 37.	3.8	4.0
Omeprazole	1	0.97 ± 0.15	-3.0	15.5
	3	3.11 ± 0.06	3.8	2.1
	100	98. ± 4.	-1.7	4.0
	1000	1011. ± 31.	1.1	3.1
OM-S	1	0.97 ± 0.16	-3.0	16.1
	3	2.97 ± 0.13	-0.9	4.3
	100	103. ± 5.	3.1	4.4
	1000	1030. ± 33.	3.0	3.2
5-OH-OM	1	0.98 ± 0.06	-2.2	6.0
	3	2.85 ± 0.08	-4.9	2.7
	100	99.7 ± 4.4	-0.3	4.5
	1000	993. ± 24.	-0.6	2.4
Buspirone	0.1	0.098 ± 0.007	-1.9	7.1
	0.3	0.32 ± 0.02	6.9	5.6
	10	10.2 ± 0.2	1.9	2.5
	100	107. ± 4.	7.4	4.3
1PP	1	0.95 ± 0.12	-5.4	10.6
	3	3.0 ± 0.2	-1.2	7.5
	10	8.6 ± 0.4	-14.2	4.6
	100	86. ± 4.	-13.7	5.0

Table 4**Inter-Day Accuracy and Precision**

Analyte	Actual Concentration	Measured Concentration	Accuracy (% deviation)	Precision (% RSD)
Fexofenadine	1 ng/ml	1.09 ± 0.07 ng/ml	9.5	6.6
	3	3.0 ± 0.3	0.4	11.3
	100	108. ± 11.	7.5	10.5
	1000	986. ± 44.	-1.4	4.5
Omeprazole	1	1.08 ± 0.07	8.2	6.8
	3	3.12 ± 0.12	4.1	4.0
	100	100. ± 9.	0.1	8.8
	1000	1005. ± 28.	0.55	2.8
OM-S	1	0.98 ± 0.12	-1.57	12.1
	3	3.1 ± 0.2	3.0	5.6
	100	103. ± 2.	3.2	2.2
	1000	1029. ± 4.	2.9	0.3
5-OH-OM	1	0.96 ± 0.04	-4.1	4.0
	3	3.0 ± 0.3	1.2	10.6
	100	97. ± 8.	-3.4	8.5
	1000	990. ± 9.	-1.0	1.0
Buspirone	0.1	0.10 ± 0.01	2.3	14.0
	0.3	0.27 ± 0.02	-8.9	8.7
	10	9.6 ± 0.9	-3.8	9.3
	100	108. ± 10.	8.3	9.8
1PP	1	0.90 ± 0.10	-10.0	11.5
	3	2.9 ± 0.3	-2.9	10.3
	10	9.8 ± 1.3	-2.2	13.6
	100	105. ± 5.	5.1	5.0