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Quantification of a cytochrome P450 3A4 substrate, buspirone, in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

A sensitive HPLC-tandem mass spectrometry method was developed for determination of buspirone levels in human plasma. After solid phase extraction and reversed phase HPLC separation, detection of buspirone and the internal standard (prazosin) was performed using eletrospray ionization and selected reaction monitoring in the positive ion mode. Linear calibration curves were established over a concentration range of 0.025–2.5 ng/ml when 0.5 ml aliquots of plasma were used. Satisfactory results of within-day precision (RSD of 1.9–7.7%) and accuracy (% difference of 0.5–6.6%) and between-day precision (RSD of 3.7–11.1%) and accuracy (% difference of 2.2–6.8%) were obtained. The assay has been successfully applied to the analysis of buspirone levels in more than 500 human plasma samples collected from a drug interaction study.

Keywords: Buspirone; Human plasma; Liquid chromatography; Tandem mass spectrometry; Electrospray ionization

1. Introduction

Cytochrome P450 (CYP) 3A4 is an important drug metabolizing enzyme that is responsible for the metabolism of a majority of commonly used medications. Because CYP 3A4 activity is highly variable among individuals and can be affected by the usage of medications and other dietary and environmental variables, clinical assessment of CYP3A4 activity is important to predict drug response/toxicity and potential drug–drug and drug–nutrient interactions. Current CYP3A4 probe substrates used clinically are less than ideal due to lack of specificity or good clinical safety profiles. Buspirone is an anti-anxiety drug that acts as a partial agonist at the 5-HT_{1A} receptor. Although buspirone is almost completely absorbed after oral administration, its bioavailability is less than 5% because of extensive first-pass metabolism [1]. Clinical and preclinical studies have shown that buspirone is primarily metabolized by human CYP3A4 [2–6]. Buspirone has been recommended as one of the preferred *in vivo* sensitive probe substrates for the evaluation of CYP3A4 interaction by the U.S. Food and Drug Administration [7]. However, its usage as a clinical probe substrate has been limited by the requirement of a sensitive assay for quantification of low buspirone plasma concentrations, especially in the presence of a CYP3A4 inducer. Cho et al. has recently published a manuscript reporting a sensitive liquid chromatography–tandem mass spectrometry assay for quantification of buspirone in human plasma [8]. This assay has been validated and applied to a small set of clinical samples. Similarly, we have developed a liquid chromatography–tandem mass spectrometry method that also employs electrospray ionization and selective reaction monitoring in the positive ion mode. We have established a similar level of limit of quantification. The advantages of our method include utilization of a solid phase extraction procedure which could be automated for sample processing and demonstration of the assay ruggedness with its application to more than 500 clinical samples.

2. Experimental

2.1. Materials

Buspirone hydrochloride, prazosin hydrochloride (internal standard) and ACS grade ammonium hydroxide were purchased from Sigma Chemicals (St. Louis, MO, USA). Reagent grade sodium hydroxide, HPLC grade acetonitrile, methanol and trifluoroacetic acid were obtained from J.T. Baker (Phillipsburg,

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NJ, USA). An extraction buffer (0.05 M potassium dihydrogen phosphate) was prepared by dissolving 6.805 g potassium dihydrogen phosphate in 1 l water, and pH adjusted to 7.2 with 30% (w/v) sodium hydroxide. An elution solvent was prepared with acetonitrile and ammonium hydroxide (99:1, v/v). Mobile phase A consisted of 0.0175% (v/v) trifluoroacetic acid in water and mobile phase B consisted of 0.0175% trifluoroacetic acid (v/v) in acetonitrile.

2.2. Plasma calibration standards and quality control (QC) standards

Buspirone and prazosin stock solutions with concentrations of 1 mg/ml were prepared in methanol and stored at −20 °C before use. The buspirone stock solution was serially diluted in methanol to working solutions in concentrations of 0.25 to 25 ng/ml. Prazosin stock solution was diluted in methanol to a working solution of 200 ng/ml. When stored at −20 °C, stock and working solutions were found to be stable for at least six months. Plasma calibration standards were prepared fresh daily by spiking buspirone working standard solutions to blank human plasma to the following concentrations: 0.025, 0.05, 0.1, 0.5, 1.25, and 2.5 ng/ml. Quality control standards were prepared by spiking buspirone working solutions into blank human plasma to concentrations of 0.05, 0.5, and 2.5 ng/ml. Quality control standards were aliquoted and stored at −20 °C throughout the validation period.

2.3. Sample extraction procedure

Analytes were extracted from the plasma using a published solid phase extraction procedure [9] with minor modifications. Briefly, 500 μl of plasma standards or samples were mixed with 10 μl of the internal standard working solution and 1 ml of 0.05 M potassium dihydrogen phosphate (pH 7.2). The sample mixture was applied to solid phase extraction cartridges (Bakerbond SPE C18, 100 mg, JT Baker, Phillipsburg, NJ, USA) preconditioned with 2 ml of methanol and 2 ml of 0.05 M potassium dihydrogen phosphate (pH 7.2). Following sample application, the cartridges were consecutively washed with 2 ml of 0.05 M potassium dihydrogen phosphate (pH 7.2) and 0.5 ml of 50% methanol, and allowed to be vacuum dried completely. Buspirone and prazosin were eluted with 2 ml of an elution solvent (acetonitrile–ammonium hydroxide, 99:1, v/v). The eluates were evaporated to dryness in a centrifugal evaporator (SPD SpeedVac, Thermo Electron Corp., Milford, MA). The dry residues were reconstituted with 200 μl of 20% acetonitrile and 15 μl were injected onto the HPLC-MS system.

2.4. HPLC-mass spectrometric conditions

The HPLC-mass spectrometry system consisted of a Surveyor HPLC system and a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron, San Jose, CA, USA). Chromatographic separation of buspirone and the internal standard was achieved on a Luna C_{18} column (50 mm L × 2.00 mm ID, 5 μ, Phenomenex, Torrance, CA, USA) with a Luna C_{18} guard column (4 mm L × 2.00 mm ID, Phenomenex) and a gradient of two mobile phases. Mobile phase A consisted 0.0175% TFA in water, and mobile phase B consisted 0.0175% TFA in acetonitrile. The analytes were eluted using a linear gradient from 90% A to 90% B in 10 min. The column was re-equilibrated with 90% A for 5 m before the next injection. The flow rate was maintained at 0.3 ml/min. Column effluent was diverted to waste from 0 to 2.4 min and during re-equilibration. Sample vials were maintained at 5 °C in the autosampler tray.

The mass spectrometric analysis was performed with the electrospray ionization interface operated in positive ion mode with a spray voltage of 4000 V. Ion transfer capillary temperature was set at 350 °C. Nitrogen was used as sheath gas at a pressure of 25 arbitrary units and auxillary gas at a pressure of 5 arbitrary units. The analyte and the internal standard were measured by selected reaction monitoring. The most abundant ion transition for the analyte was selected for identification and quantification of the analyte. Argon was used as the Q2 collision gas and maintained at a constant pressure of 0.8 mTorr. The selected reaction monitoring transition and collision energies selected were: m/z 386 → 122 (32 eV) for buspirone and m/z 385 → 247 (34 eV) for the internal standard. Both Q1 and Q3 mass analyzers were operated under unit resolution. The described conditions were optimized to achieve the best sensitivity for the analyte. Xcalibur (Version 1.3; ThermoFinnigan) was used to control the HPLC/TSQ Quantum system and to acquire and process data.

2.5. Assay validation

The calibration curve consisted of a blank sample (blank human plasma), a zero sample (blank human plasma spiked with the internal standard), and blank human plasma spiked with different buspirone concentrations and a fixed concentration of the internal standard. Calibration standards were prepared on each analysis day from a single batch of buspirone and prazosin working solutions. The linearity of the method was evaluated with single determination of blank, zero, and each of the six different buspirone concentration standards.

The analytical method was also evaluated to assess within-day and between-day variations at buspirone concentrations of 0.05, 0.5, and 2.5 ng/ml. Five determinations were performed for each concentration within an assay validation batch for within-day assay variation determination. The analysis was repeated over five different assay days for between-day assay variation assessment. The concentrations of the quality control standards were determined from the calibration curve prepared for each assay day. The RSD of the concentration measured within a run (five replicates) and among five different runs was used to determine the within-day and between-day precision of the assay, respectively, and was determined as RSD = (standard deviation)/(mean measured concentration) × 100.

The percent difference between measured and theoretical concentrations determined within a run and among five different runs was used to determine the within-day and between-day accuracy of the assay, respectively, and was deter-

mined as % difference = [(measured − theoretical concentration)/(theoretical concentration)] × 100.

The extraction recovery of buspirone was determined by comparing the peak area of the extracted quality control standards with unextracted standards that represent 100% recovery.

Studies were performed to evaluate the effect of the plasma matrix on the signal intensities of the analytes. An extracted blank plasma sample was injected under the chromatographic conditions described above when a solution containing buspirone and prazosin (200 ng/ml each) was infused into the column effluent via a T-valve at a flow rate of 10 μl/min. Signal intensities of the analytes were monitored using the mass spectrometric conditions described above.

2.6. Application of the analytical method to a clinical study

The developed method was applied to determine buspirone plasma concentrations in clinical samples collected up to 8 h after oral administration of 10 mg buspirone. Buspirone administration and sample collection were carried out in 41 healthy individuals before and four weeks after daily green tea catechin administration at a daily dose that contained 800 mg epigallocatechin gallate.

3. Results and discussion

3.1. Chromatography and mass spectrometry

The feasibility of electrospray versus atmosphere pressure chemical ionization sources under positive and negative ion detection modes were evaluated during the early stage of assay development. It was found that electrospray ionization with positive ion detection mode provided the best signal-to-noise response

The chromatographic conditions were also optimized during the early stage of assay development. Addition of an acidic modifier (trifluoroacetic acid) in the mobile phase improved assay sensitivity by promoting the formation of protonated molecules. Fig. 1 illustrates representative HPLC chromatograms of blank human plasma (1A), a medium calibration standard (1B) and a plasma sample (1C). Buspirone and prazosin are chromatographically separated with complete baseline resolution. The retention times of buspirone and prazosin are 4.3 and 3.9 min, respectively. No interfering peaks were observed at the retention times of buspirone and the internal standard.

3.2. Assay validation

Plasma calibration curves were constructed with the peak area ratios of buspirone to the internal standard versus buspirone concentrations. Linear least-squared regression with a weighting factor of 1/*y* was used. The calibration curve was found to be linear over the concentration range of 0.025–2.5 ng/ml. The correlation coefficients (r^2) ranged between 0.9953 and 0.9989. The lower limit of quantification (LLOQ) was determined as the lowest concentration on the calibration curve that showed a peak response at least five times the response compared to blank response and yielded a RSD of less than 20% and an accuracy of 80–120% of nominal concentration. Based on these criteria, the LLOQ was determined to be 0.025 ng/ml when 0.5 ml of plasma sample was used (1 pg on column mass). This is a significant improvement over previous HPLC methods with reported LLOQs of 0.1–5 ng/ml with 1 ml of plasma [9–11] and is similar to a recently reported liquid chromatography–mass spectrometry method with LLOQ of 0.02 ng/ml with 0.5 ml of plasma [8]. The reported and our liquid chromatography–mass spectrometry methods allow determination of low concentrations of buspirone in human plasma, especially in the case of drug interaction studies that involve induction of CYP3A4 enzyme which would lead to further reduction of plasma buspirone concentrations.

Table 1 summarizes the assay accuracy, precision, and extraction recovery. Within-day analysis assesses assay accuracy and precision during a single analytical run. Five replicates were analyzed for each quality control standard. The concentrations of these quality control standards were determined using a calibration curve prepared for the batch. The between-day accuracy and precision were determined from five separate analytical runs. Three replicates of each quality control standard were analyzed for each analytical run. The concentrations of these quality control samples were determined using a calibration curve prepared for each run. The within-day % RSD and % difference ranged from 1.9 to 7.7% and from 0.5 to 6.6%, respectively. The between-day % RSD and % difference ranged from 3.7 to 11.1% and from 2.2 to 6.8%, respectively. Extraction recoveries for buspirone were greater than 86% at all tested concentrations. The extraction recovery for the internal standard was 89%.

Ion suppression from sample matrix is an important issue that can adversely affect the quantitative performance of mass spectrometry with electrospray ionization. Therefore, it is preferable to use a stable isotope of the analyte as the internal standard to correct for any potential matrix associated ion suppression. Fig. 2 illustrates the effect of plasma matrix on the signal intensities

Table 1
Assay accuracy, precision, and extraction recovery

	Theoretical buspirone concentrations (ng/ml)		
	0.05	0.5	2.5
Within-day			
Mean measured concentration ($n = 5$)	0.052	0.53	2.51
Standard deviation	0.003	0.01	0.20
Precision[a] (% RSD)	4.8%	1.9%	7.7%
Accuracy[b] (% difference)	3.0%	6.6%	0.5%
Between-day			
Mean measured concentration ($n = 15$)	0.049	0.53	2.65
Standard deviation	0.006	0.03	0.10
Precision[a] (% RSD)	11.1%	4.7%	3.7%
Accuracy[b] (% difference)	2.2%	6.8%	6.0%
Extraction recovery (%, $n = 5$)	100	103	86

[a] Precision is expressed as % RSD: (standard deviation)/(mean measured concentration) × 100.

[b] Accuracy is expressed as % difference: [(measured − theoretical concentration)/theoretical concentration] × 100.

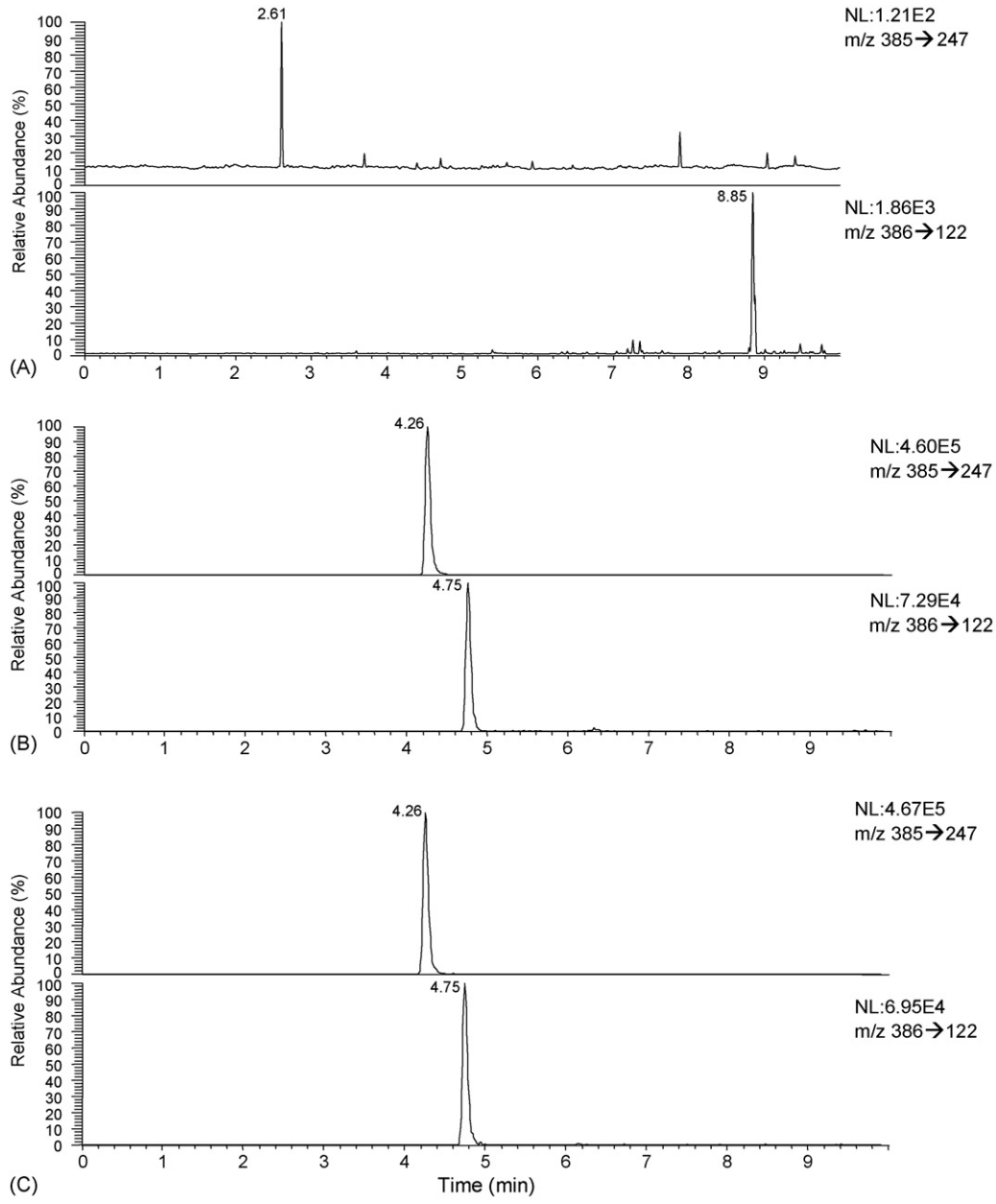

Fig. 1. Representative chromatograms of buspirone and internal standard. (A) A blank human plasma sample; (B) a blank human plasma sample spiked with buspirone and internal standard; (C) a plasma sample collected from a participant 4 hrs after oral administration of 10 mg buspirone. Retention times of internal standard and buspirone were 4.26 and 4.75 m, respectively.

of buspirone and prazosin. There appeared to be no significant matrix effects in the timeframes relevant for the detection of the analytes.

3.3. Assay application

The assay was successfully applied to an interaction study aiming to determine the effect of repeated green tea catechin administration on human cytochrome P450 activity. Buspirone plasma concentration–time data in 41 healthy individuals (more than 500 plasma samples) were determined after oral administration of 10 mg buspirone before and after repeated green tea catechin administration. Fig. 3 illustrates the mean plasma buspirone concentration versus time data. A 20% increase in the mean area under the plasma buspirone concentration–time curve (AUC) was observed after repeated green tea catechin administration (AUC increased from 136.7 ± 115.7 to 166.0 ± 137.7 min × ng/ml).

3.4. Summary

In summary, we developed a sensitive LC–tandem mass spectrometry assay for quantification of buspirone concentrations in human plasma. This assay has been successfully applied to more than 500 human plasma samples collected from a drug interaction study.

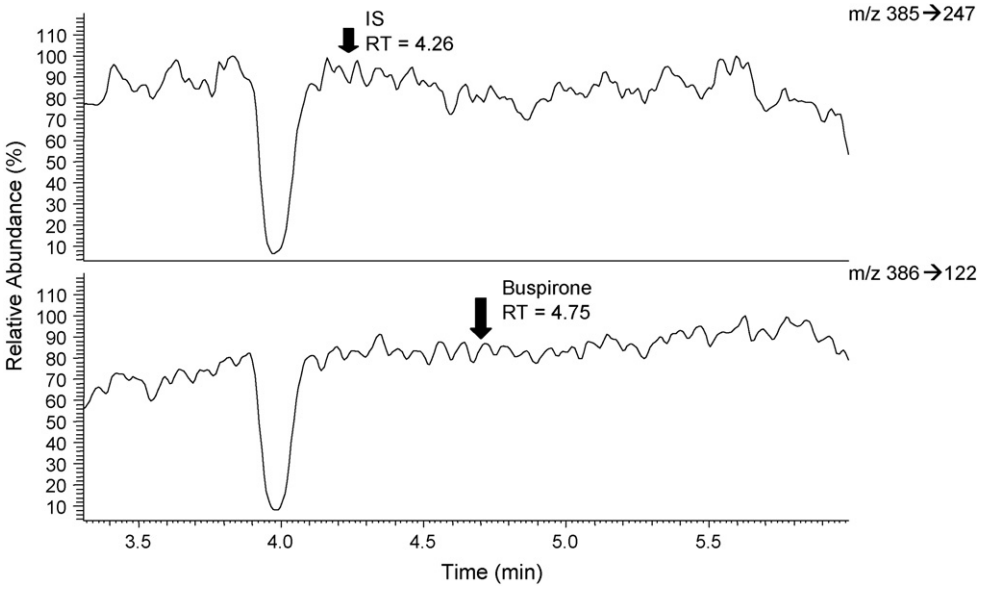

Fig. 2. Effect of the plasma matrix on the signal intensities of buspirone and internal standard. Arrows indicate the retention times of buspirone and internal standard.

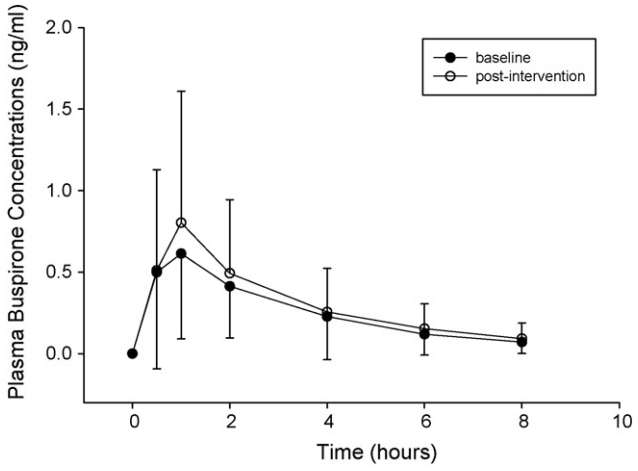

Fig. 3. Mean plasma concentration–time profile of buspirone after oral administration of 10 mg buspirone to 41 healthy volunteers before and after four weeks of green tea catechin intervention. Each point represents the mean ±SD.

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