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Buprenorphine assay and plasma concentration monitoring in HIV-infected substance users

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Abstract

The availability of buprenorphine (BUP) provides an alternative approach to the treatment of opioid addiction with methadone, an agent that has many drug–drug interactions when combined with antiretroviral therapy (ART). However, due to limited long-term pharmacokinetic studies in HIV-infected patients, the clinical use of BUP, a CYP450-3A4 substrate, will require that studies be conducted to examine safety, tolerability and pharmacokinetics when these drugs are taken for chronic treatment. One clinical approach could include plasma concentration monitoring to avoid under- or overdosing BUP secondary to drug interactions with ART. The measurement of BUP and its active metabolite, norbuprenorphine (NBUP) facilitates the addition of BUP to ART in an attempt to avoid drug toxicity as described in a recent report by Bruce et al. Therefore, our objective was to validate a BUP assay and integrate its application into an ongoing antiretroviral (ARV) plasma concentration monitoring program.

A chromatographic method for monitoring BUP and its active metabolite, NBUP was investigated. An assay was developed that would facilitate BUP and ARV measurement from a single 3 mL blood sample (0.75 mL plasma required) in conjunction with a previously validated multiple ARV HPLC method. The method measures BUP and NBUP over the range from 0.25 to 50 ng/mL with mass spectrometry detection. Inter- and intra-assay variation was ≤11%, across the concentration range. The method quantitates BUP and NBUP plasma concentrations within the range of expected values from current BUP dosing guidelines. Use of this combined BUP and ARV plasma concentration monitoring approach for a representative patient receiving BUP, atazanavir and efavirenz demonstrated its clinical application.

Keywords: Plasma concentration monitoring; Antiretrovirals; Buprenorphine; HIV; Substance abuse treatment

1. Introduction

The availability of buprenorphine (BUP) provides an alternative approach to the treatment of opioid addiction with an agent that may have reduced drug–drug interactions with ARVs in ART regimens [1–8]. Promising results have been reported in non-HIV-infected patients and HIV-infected patients using BUP, a CYP450-3A4 substrate, for opioid addiction. Pharmacokinetic studies in non-HIV-infected patients have reported initial pharmacodynamic findings (e.g., lack of withdrawal) with favorable pharmacokinetic data during short-term studies (2 weeks) [7,9]. The prolonged receptor binding properties of BUP require that long-term administration be thoroughly investigated in the context of HIV disease modulation, medication adherence and adverse effects. However, the use of BUP in HIV-infected patients will require that long-term studies be conducted to examine safety, tolerability and pharmacokinetics when these

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drugs are taken together in the presence of HIV infection with nonnucleoside reverse transcriptase inhibitors (NNRTIs) and/or protease inhibitors (PIs) [8,10–12].

Pharmacotherapeutics for HIV-infected patients with concurrent opioid addiction has primarily been based on the use of methadone therapy. While the development of potent antiretroviral therapy (ART) has led to extended survival in patients with both HIV infection and opioid addiction, the use of methadone has been associated with numerous drug–drug interactions [13]. For example, withdrawal symptoms have been well described with the introduction of NNRTIs such as nevirapine- or efavirenz-containing ART regimens [14,15]. Additional complexities arise in patients with multiple ART regimens who require ART regimens that include drug metabolism inhibitors such as delavirdine or HIV-1 PIs with ritonavir pharmacokinetic enhancement. These complexities can lead to unpredictable effects on methadone dosing requirements [4,16–21]. Lastly, since most ART regimens include dual nucleoside reverse transcriptase inhibitors (NRTIs), the influence of methadone on NRTI pharmacokinetics presents another complicating factor to achieve successful viral suppression as additional pharmacokinetic interactions are introduced [22–24].

Therefore, our objectives were to implement a plasma concentration monitoring program to compare ARV concentrations before and after BUP was added and to compare BUP and norbuprenorphine (NBUP) plasma concentrations after ARVs were added, such that strategies to optimize both treatments could be developed. Since our laboratory has previously described a validated HPLC method for the determination of HIV-1 protease inhibitors and efavirenz [25], a method to accurately quantitate low concentrations of BUP and NBUP was developed, validated and implemented. These assays support pharmacokinetic drug interactions research and guide clinical dosing in patients with HIV infection who initiate ART or BUP in addition to their ongoing treatment regimens.

2. Materials and methods

2.1. Clinical protocol for collecting plasma samples for BUP assay

Specimens were collected during an open-label trial in patients with HIV-1 infection receiving ART with and without active substance use that were also receiving regimens with complex drug–drug interactions. Patients were instructed on medication adherence and blood samples for pharmacokinetic analysis were obtained at entry (random), a trough sample 1–2 weeks later, and then repeated sampling during directly observed therapy (DOT). This protocol was approved by the Institutional Review Boards at all participating sites as well as at the principal site sponsor's.

To facilitate this protocol The University at Buffalo Pharmacotherapy Research Center Core Analytical Laboratory developed a secure, interactive website area on the HIV ePharmacotherapy Website titled HIV Drug Interactions and TDM Registry (www.tdm.buffalo.edu). Each sample collected had a unique accession number and all data resulting from that number were matched to only that number. The Core Analytical Laboratory did not receive any personally identifiable patient information as described by Health Insurance Portability and Accountability Act of 1996 (HIPAA). During each study visit, the previous three doses of all ARVs were recorded on the study forms, as well as the previous three doses of all interacting concomitant drugs of interest. All concurrent prescription medications, non-prescription medications and herbals were also recorded. Sodium heparin and potassium EDTA collection tubes were utilized for venous blood samples. The blood was processed for plasma within 1 h of collection and aliquotted into multiple tubes per study form instructions. Samples were frozen and stored at −70 °C until shipped on dry ice to the Core Analytical Laboratory for analysis. EDTA plasma was utilized for the analysis of BUP, NBUP and ARVs.

2.2. Chemicals and reagents

BUP, its metabolite NBUP, and the deuterated internal standards, d4-BUP and d3-NBUP were purchased from Cerriliant (Round Rock, TX). Ammonium hydroxide was purchased from Mallinckrodt-Baker (Phillipsburg, NJ). Ammonium acetate, acetic acid, hydrochloric acid, water and methanol were obtained from Fisher Scientific (Fair Lawn, NJ). All solvents, including water, used in sample preparation and chromatographic separations were of HPLC grade quality. Blank EDTA plasma was purchased from Interstate Blood Bank, Inc. (Memphis, TN).

2.3. Preparation of standards and samples

Stock solutions of BUP and NBUP were supplied as 100 μg/mL solution in methanol. Dilutions were prepared from the stocks separately for calibrators working solutions and quality control (QC) spiking solutions. Calibrator stocks were prepared in methanol to yield 100, 250–500 ng/mL methanol calibrator working solutions for both analytes. These calibrator working solutions were diluted 10-fold to yield methanol calibrators at 10, 25 and 50 ng/mL. The 25 and 50 ng/mL calibrators were both diluted 10-fold once more to obtain the last two calibrator working solutions at 2.5 and 5 ng/mL concentrations. All stocks and working solutions were stored in amber glass vials at −70 °C. To prepare calibrators in plasma, 50 μL of working calibrator solution was spiked into 0.5 mL of plasma and mixed. Calibrators are prepared daily.

To maintain quality of the assay, three levels of controls (low, medium, high) were prepared by using the purchased stock solutions and the diluted QC spiking solutions. These QC spiking solutions were prepared at 400 and 4000 ng/mL in methanol. The low, medium and high controls were prepared measuring volumes of the solutions into volumetric flasks and adding human plasma to yield the final QC pools. Final concentrations of the QCs were 40, 4 and 0.4 ng/mL of each analyte. Aliquots of 1200 μL of the QCs in polypropylene tubes were stored at −70 °C.

Plasma samples were prepared using a solid phase extraction (SPE) method. After 50 μL of internal standard were added to 500 μL of each of the plasma samples, 750 μL of 0.1N HCl

were added. After mixing, 1 mL of each acidified sample was applied to a preconditioned Waters Oasis MCX cartridge (1 mL capacity). Each cartridge was then washed twice with 1 mL 0.1N HCl, followed by a final rinse of 5% methanol in 0.1N HCl. After drying the cartridges, the samples were eluted with two 1 mL rinses of 1% ammonium hydroxide in methanol. The final samples were evaporated to dryness using a Zymark Turbo Vap LV (Hopkinton, MA) at 50 °C.

The samples were reconstituted with 50 μL of mobile phase mix of 75% A and 25% B, transferred to polyethylene microfuge tubes and centrifuged at 1018 × *g* for 5 min to remove any particulates. The supernatant was transferred to polyethylene sample inserts and 20 μL utilized for sample injection into the chromatographic system.

2.4. LC-MS/MS

ARVs were monitored by high performance liquid chromatographic methods that were previously published [25,26]. To measure BUP and its active metabolite, NBUP, a liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method was developed and validated using collective information from several published methods [27–30]. Validation studies were performed according to the Bioanalytical Method Validation provided by the FDA [31, 2001]. The Pharmacology Laboratory is certified by the New York State Department of Health's Clinical Laboratory Evaluation Program for therapeutic drug monitoring of ARVs and maintains CLIA compliant operations [32].

The HPLC-MS/MS system consisted of an Agilent series 1100 autosampler, pumps and degasser used for the chromatographic separation and a Sciex API 3000 as the mass spectrometer (MS). The entire system was controlled by Analyst software Version 1.4 (Applied Biosystems, Foster CA). A column diverter was placed prior to the interface that diverted flow before and after the chromatographic retention times of interest.

The analytes were eluted from a Waters 3.5 μm Symmetry™ shield C18, 2.1 mm × 30 mm column protected with a Waters Symmetry™ C18 5 μm guard column. Gradient elution was used throughout the separation. Two mobile phases were used: 5% methanol in 5 mM ammonium acetate buffer, pH 3.5 (A); and 95% methanol in 5 mM ammonium acetate buffer, pH 3.5 (B). The flow rate for the final choice of separation utilized was 0.25 mL/min for over a 6 min gradient with 2.5 min required for re-equilibration. Initial conditions were 70% A, changing to 35% A by 2 min, 30% by 3.5 min, 25% by 5.5 and holding until 5.9 min. The column temperature was held room temperature.

The TurboIon spray source was used to interface the HPLC and the MS. The voltage was held at 4000 V and the temperature at 500 °C. Nitrogen was used as the nebulizer gas and the collision gas. The mass transition of BUP and its deuterated form was monitored as 468.4/396.2 *m/z* and 472.4/400.3 *m/z*, respectively. The detection of NBUP and its deuterated form was monitored in single ion monitoring as 414.7 amu and 417.5 amu, respectively, as neither compound was fragmented in the collision cell. Mixed-Reaction-Monitoring (MRM) mode with the low-resolution option was employed.

2.5. Validation of the quantitative analysis

To determine the accuracy and precision at the lower limit of quantitation, replicates of the lowest calibrator were prepared separately as unknowns for each validation day. Day 1 the lower limit attempted was 0.1 ng/mL. Day 2 both 0.1 and 0.25 ng/mL calibrators were performed in triplicate. Days 3, 4 and 5, the 0.25 ng/mL was replicated 6 times. All data for the 0.25 ng/mL calibrator on Days 2–5 were used to validate this lower limit of quantitation. The limit of detection for BUP and NBUP was measured each day during the validation using three-times the signal-to-noise measurement for each replicate at the lowest calibrator concentration.

To verify accuracy of the method, 24 samples assayed by another laboratory for BUP and NBUP content were assayed by this new method. The other laboratory also used LC-MS/MS for determining analyte concentrations. Results were compared using a paired *t*-test analysis of the results and Pearson's correlation.

For validation purposes, six replicates of each QC were assayed each day to measure intra-assay variation over 6 days; inter-assay variation was determined statistically across all 6 days. Precision was expressed as relative standard deviation between controls of the same value. Inter-assay relative standard deviation was calculated by determining the relative standard deviation over all days for each compound at each control level.

Initial development of the method included testing of 10 matrices' lots for recovery testing to determine what gradient separation measured consistent and adequate recovery. Each lot was tested by spiking with analytes and internal standards prior to extraction (pre-spike), and with no analyte spike (single determinations). Area responses of the analytes were compared to the mean area response of a non-matrix sample assayed in triplicate by calculating the % response in the presence of the matrix.

After the initial choice of the gradient method, ion suppression, recovery and accuracy were determined using six blank plasma lots. Each lot was tested by spiking with analytes and internal standards prior to extraction (pre-spike), spiking with analytes and internal standards after extraction (post-spike) and with no analyte spike (single determination). Each pre-spike and post-spike were prepared in triplicate and only tested by the final choice of gradient separation. In addition, the pure response for analytes was determined by adding the spike to the final elution buffer prior to drying and reconstituting. All determinations were performed in triplicate except were noted. The percent recovery was determined for each matrix by dividing the mean response for the pre-spiked samples by the mean response for the post-spike. The amount of ion suppression for each matrix was calculated by dividing the mean response for the post-spike by the mean response of analytes spiked in elution buffer. Accuracy for each matrix was determined by calculating the concentration from a calibration curve extracted with the pre-spike matrix samples. To verify the calibration curve accuracy, two QCs at each of three concentrations were performed. All samples were injected for LC-MS/MS analysis for three different gradient separations to determine which gradient gave the best results.

To determine the accuracy of dilutions, the 40 ng/mL QC was diluted 2-, 5- and 10-fold with blank plasma. The measured concentration was compared to the targeted concentration.

All six matrices tested for ion suppression and recovery experiment were tested as blank preparations to determine if false positive detection of NBUP or BUP would occur.

Several patient samples were also analyzed for this purpose. The patients drug therapies included: venlafaxine, trazadone, imiquimod, ketoconazole, atazanavir, tenofovir, ritonavir, zidovudine, efavirenz, citalopram HBr, fluconazole, zolpidem tartrate, testosterone, sulfamethoxazole, trimethoprim, lisinopril and lamivudine. Some patient samples included methadone, however methadone and buprenorphine were not co-administered.

To allow for use of EDTA as well as heparin in sample collection, plasma collected with EDTA as the anticoagulant was tested for accuracy and specificity. Six lots of EDTA plasma were tested by spiking with known amounts of analytes and assayed in triplicate. Additionally, a blank sample from each lot was tested.

To verify the stability of the sample handling conditions, time held at room temperature and freeze–thaw cycles, the QC samples at high and low concentrations were subjected to each treatment and assayed in triplicate. For freeze–thaw cycles, three cycles were done at room temperature and −70 °C. For room temperature, the samples were held 17 h on bench top. The treated QC results were compared to untreated QC sample results using a *t*-test.

2.6. Calculations and statistics

Daily calibration curves were linearly regressed using concentration as the *x* variable and ratio of the analyte response to its deuterated internal standard response as *y* variable; a 1/(concentration)^2 weighting was applied. Unknown and QC concentrations were extrapolated within the calibration range of the curves. All statistical results were derived using SYSTAT (V11.0) software package (SYSTAT Software, INC). Precision (CV%) was calculated as the percentage of relative standard deviation. Accuracy was calculated as the percentage of measured concentration divided by the target concentration.

3. Results and discussion

3.1. Optimization of LC-MS/MS parameters

Fig. 1 displays the chromatogram overlay for the two compounds, BUP and NBUP. NBUP was detected without fragmentation in the source whereas BUP was fragmented and an ion pair was detected. Both analyte structures are displayed next to their corresponding peak and the fragmentation of BUP is pointed out. Eight calibrated standard concentrations ranged from 0.25 to 50 ng/mL for BUP and NBUP. Variation of calibration standards fittings was below 10%.

The lower limit of quantitation was validated at 0.25 for both BUP and NBUP. The variation accomplished for the lower limit of quantitation ranged from 6 to 15% across 4 days of assay validation for both analytes. Mean accuracy across replicates for each day was 11% or better. Individual determinations were within 20% accuracy for 93% of the determinations. The limits of detection were calculated as 0.04 ng/mL for BUP and 0.1 ng/mL for NBUP.

Table 1a and b display the intra-assay and inter-assay variation of each control level of BUP and NBUP, respectively. Inter- and intra-assay variation was generally consistent, ≤11%, across the concentration ranges.

Initial development of the method was characterized by preparation of 10 spiked matrices and analysis by three gradient methods to determine which would yield the least ion suppression. Fig. 2a shows the initial separation of a blank matrix and a nonmatrix, spiked sample using initial conditions of 85% mobile phase A with a transition to 25% by 2 min (gradient 1). Using these conditions an endogenous unknown peak corresponding to the NBUP ion coelutes with the BUP and its IS. We

Table 1

	BUP level			NBUP level		
	HQC	LQC	MQC	HQC	LQC	MQC
(a) Intra-assay performance						
Target conc. (ng/mL)	40	4	0.4	40	4	0.4
N per day	6	6	6	6	6	6
Days	5	4	5	5	4	5
Accuracy (low)	95.1	89.7	101	103	99.1	97.8
Accuracy (high)	101	103	106	108	111	103
CV% (low)	2.14	6.02	2.93	1.47	3.98	2.17
CV% (high)	5.73	11.2	8.76	5.40	9.41	7.64
(b) Inter-assay performance						
N	30	24	30	30	24	30
Target conc. (ng/mL)	40	0.4	4.0	40	0.4	4.0
Mean	39.4	0.389	4.12	42.1	0.412	4.04
S.D.	1.69	0.0394	0.208	1.71	0.0363	0.181
CV%	4.29	10.1	5.06	4.07	8.8	4.48
%Accuracy	98.5	97.1	103	105	103	101

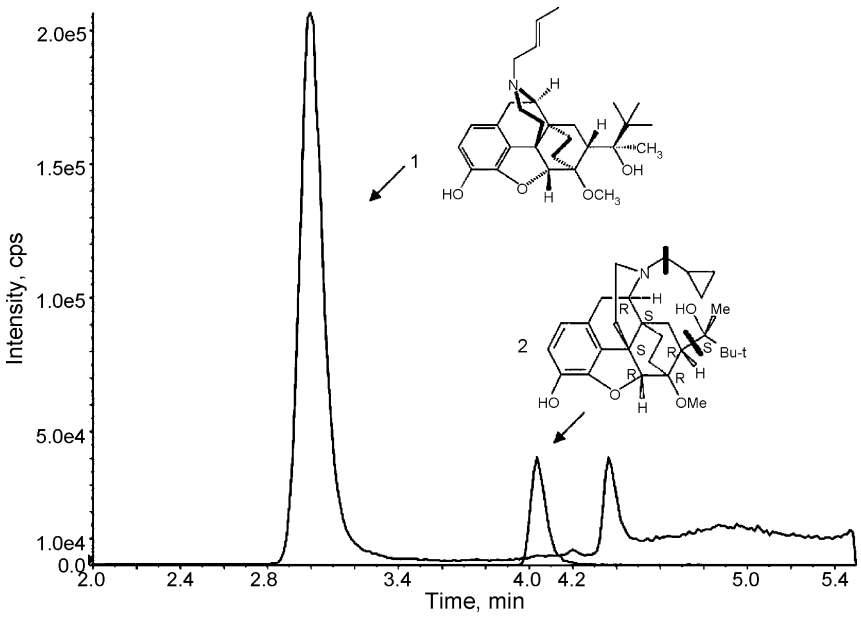

Fig. 1. Chromatogram of norbuprenorphine (NBUP), retention ~3.0 min, monitored at 414.7 and buprenorphine (BUP), retention ~4 min, monitored at 468.4/396.2. Their chromatograms are overlaid. Fragmentation of buprenorphine structure is designated by thick bars.

found that every sample had this peak but the response varied between matrices. Two other gradients were developed to move the endogenous peak away from the BUP elution time. One had a 75% initial condition for mobile phase A with changes at 2, 3.5 and 5.5 min to 35%, 30% and 25% mobile phase A, respectively (gradient 2). The other gradient used a 70% initial condition for mobile phase A with gradient changes identical to gradient 2 (gradient 3). The area ratio responses of the matrices' results using each gradient separation were compared to sample prepared without matrix (response in matrix/response nonmatrix) as a percentage. Table 2 displays the results. The furthest separation between the analytes produced the best results. Fig. 2b shows the separation using the final gradient, gradient 3.

After the gradient separation method was chosen, six sources of blank matrix were spiked with known amounts of the analytes (final concentration 5.8 ng/mL for each analyte), assayed in triplicate, and the accuracy and variation of the results in each matrix were used to determine if various sources of plasma might alter the results. The variation across triplicates ranged from 1 to 4%. Each matrix was also tested without the addition of analytes or internal standards to determine specificity of the method (see specificity sections). Table 3 displays the accuracy, recovery and matrix suppression results for each blank matrix tested.

Table 2
Initial recovery tests with 10 source matrices

	BUP			NBUP		
Retention time (min)	4.38	4.44	4.24	4.08	3.89	3.43
Time between analytes elution (min)	0.30	0.55	0.81	–	–	–
Gradient method[a]	1	2	3	1	2	3
Matrix ID						
1	50%	103%	95%	45%	87%	104%
2	80%	84%	84%	72%	90%	106%
3	22%	67%	69%	23%	83%	103%
4	38%	82%	73%	36%	83%	103%
5	51%	73%	87%	48%	85%	110%
6	63%	90%	74%	62%	101%	108%
7	64%	76%	88%	57%	90%	126%
8	88%	73%	77%	73%	79%	118%
9	79%	74%	83%	64%	84%	116%
10	59%	75%	76%	49%	87%	114%
Mean	59%	80%	81%	53%	87%	111%
S.D.	20%	10%	8%	16%	6%	8%
%cv	34%	13%	10%	30%	7%	7%

[a] 1: Initial conditions 85% mobile phase A changing to 25% by 2 min with an isocratic hold for 2.5 min; 2: Initial conditions 75% mobile phase A with changes at 2, 3.5 and 5.5 min to 35%, 30% and 25% mobile phase A, respectively, and 3: Initial conditions 70% mobile phase A, gradient changes same as #2.

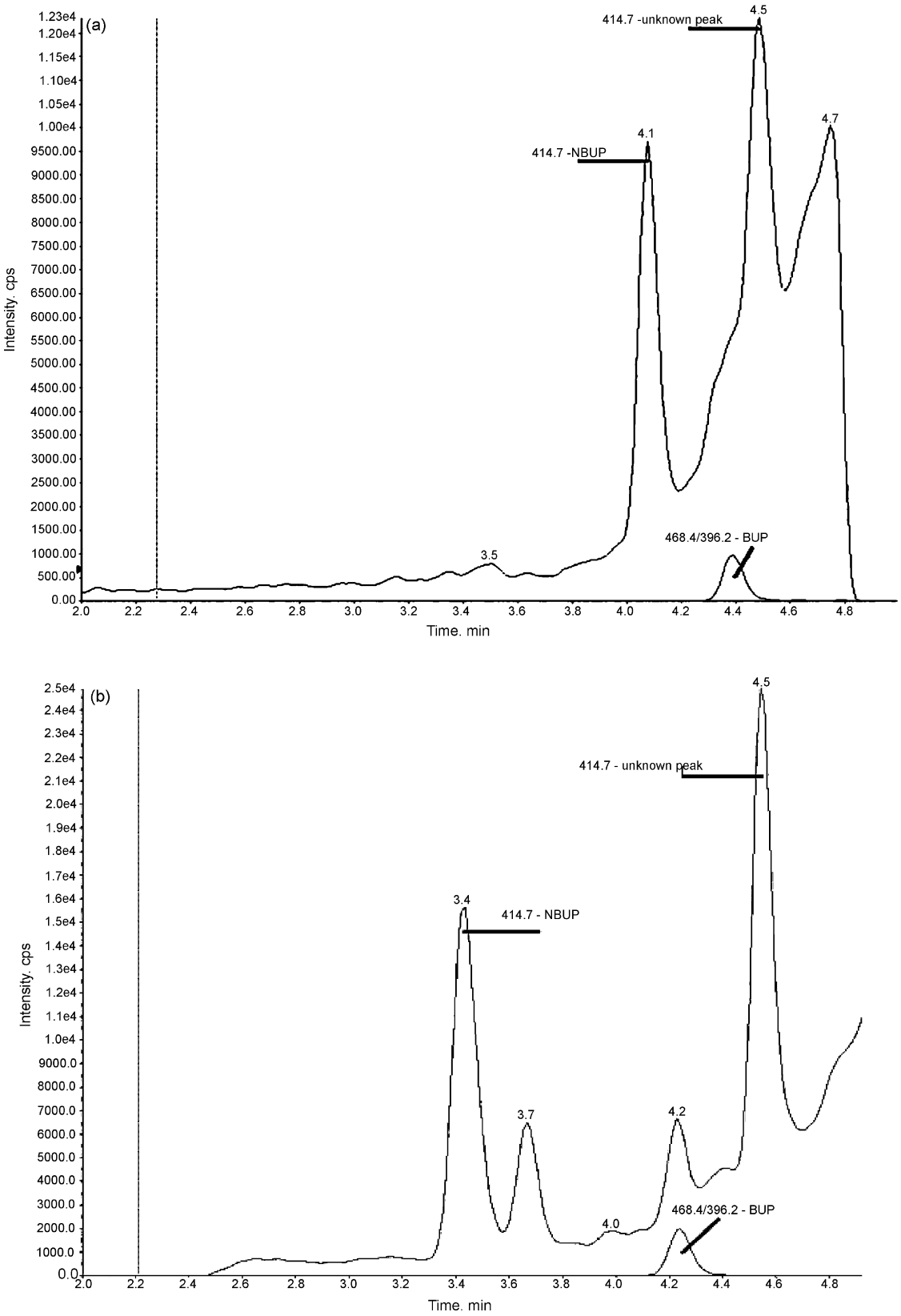

Fig. 2. (a) Total ion chromatogram of norbuprenophine (NBUP) and buprenorphine (BUP) monitoring for 414.7 (NBUP) and 468.4/396.2 (BUP). Chromatograms are overlaid. Initial gradient conditions: 85% mobile A changing to 25% by 2 min and holding to 4.5 min. (b) Total ion chromatogram of norbuprenophine (NBUP) and buprenorphine (BUP) monitoring for 414.7 (NBUP) and 468.4/396.2 (BUP). Chromatograms are overlaid. Gradient was adjusted to resolve BUP from the additional 414.7 peak: 70% mobile phase A changing to 35% at 2 min, 30% at 3.5 min, 25% at 5.5 min and holding until 5.9 min. Final time for injection and re-equilibration is 8.5 min.

Table 3
Recovery, ion suppression and accuracy

Matrix #	BUP			NBUP		
	%Recovery	%Suppression[a]	%Accuracy	%Recovery	%Suppression[a]	%Accuracy
1	80	−18	3	89	+17	3%
2	87	−29	−5	90	+2	5%
3	94	−36	−1	102	+2	2%
4	82	−24	1	87	+7	−5%
5	89	−26	−1	94	+8	−4%
6	98	−27	3	94	+15	−3%
Overall precision	8%			6%		

[a] %Deviation from maintaining 100% signal of analyte.

Recovery was 80–102% across both analytes; NBUP recovery was slightly higher than BUP (93% overall versus 88%). Measurement of precision across matrices was good, 6–8%. Ion suppression of the BUP analyte (−18 to −36%) was apparent whereas for NBUP, enhancement was measured in some of the matrices (+2 to +17%) However, the internal standard corrected for recovery and ion suppression differences adequately and accuracy was within 5% or better.

3.2. Validation results

Dilutions conducted at two-, five- and ten-fold yielded accuracy within ± 8% or better. The initial validation was performed using plasma anti-coagulated with sodium heparin. When patient samples were attempted, there was some difficulty in clogging of the cartridge. Plasma samples anti-coagulated with EDTA were found to be less troublesome. Therefore, the accuracy was tested in six separate EDTA lots. Accuracy was within ±6% for both analytes.

Moody et al. [28] published data supporting the stability of BUP and NBUP in human plasma samples stored at room temperature for 24–72 h and after three freeze–thaw cycles at −20 °C. Musshoff et al. also published data supporting the freeze–thaw stability of BUP [29]. Polettini and Huestis [30] published data supporting the stability of BUP and NBUP in human plasma stored at −20 °C for 6 months. During the validation of this assay, freeze–thaw testing and room temperature stability studies were completed. All p values for testing sample handling were insignificant ($p > 0.05$). This indicates that no significant loss of analytes is to be expected after up to three freeze–thaw cycles or when samples are held at room temperature for 17 h.

Table 4
Atazanavir plasma concentrations before and after BUP therapy

	Visit 1	Visit 2			
	Trough	Trough/DOT	1 h	2 h	3 h
ATV (ng/mL before BUP)	123	92	87	420	1245
ATV (ng/mL during BUP)	76	109	119	122	138

None of the blanks for the six matrices tested above indicated false positive measurements indicating a good specificity of this assay. Also, patient samples did not yield any false positive measurements of any of the analytes.

Twenty four samples tested ranged from 0.5–10 ng/mL for BUP and 0.5–5 ng/mL for NBUP during a cross-laboratory comparison. The statistical tests for the results showed that the NBUP results from this method measured no different than the other lab's values and that correlation was 0.994. The statistical tests for the results showed that the BUP results from this method measured 15% higher than the other lab's values but that correlation was 0.983. The high degree of correlation suggests the methods strongly agree. The higher concentration bias measured by this method indicates an issue in standardization, either by preparations or source. However, internal preparation of new calibrators showed no difference.

Table 5
Efavirenz, BUP and NBUP plasma concentrations before and during BUP therapy

	Visit	Sample collection time (hours post-dose)	EFV (ng/mL)	BUP (ng/mL)	NBUP (ng/mL)
Before BUP	Trough visit 1	11	1145	–	–
	Trough/DOT visit 2	11	2278	–	–
		12	1933	–	–
		13	1987	–	–
		14	1865	–	–
During BUP	Trough visit 1	11	2267	3.89	0.745
	Trough/DOT visit 2	11	2681	5.36	1.36
		12	2348	7.52	1.92
		13	2194	7.79	1.18
		14	2064	6.73	1.17

3.3. Example of BUP analysis in an HIV-infected patient

This patient was enrolled into the PCM research program when BUP was added to their current ARV regimen. The patient completed a trough sample collection and a directly observed therapy (DOT) pharmacokinetic visit. The BUP dose was 4 mg sublingual every 6 h. The atazanavir plasma concentrations for this patient are presented in Table 4. The efavirenz, BUP and NBUP concentrations are presented in Table 5. The ARV regimen included: atazanavir/ritonavir (300/100 qd), tenofovir (300 qd), zidovudine (300 bid) and efavirenz (600 qhs). Atazanavir concentrations were lower after BUP was initiated (<150 ng/mL). Efavirenz concentrations were slightly lower after BUP treatments were initiated, but well within the target concentration range for efavirenz (1000–4000 ng/mL).

4. Conclusions

A sensitive method was developed for the clinical measurement of BUP and its active metabolite, NBUP in HIV-infected patients. The assay quantitates BUP plasma concentrations within the range of anticipated values from current BUP dosing guidelines. The method uses a single plasma sample collection for assay of ARVs and BUP/NBUP. The clinical availability of a BUP assay to determine plasma concentrations allows for a PCM program to support the investigation of optimal approaches of adding BUP treatment to ARV regimens or adding ARVs in patients already on BUP. A prior report identified a potential interaction between atazanavir, ritonavir and BUP [33]. This method we have described allows for individualization of both BUP and ARV therapy. Currently this PCM method is being used to investigate BUP pharmacokinetics, as well as drug interactions in studies with patients who are stabilized on ARVs.

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