Relationship between concentrations of antiretroviral drugs in plasma and saliva of HIV-1 infected individuals

(HIV-1 患者における血中と唾液中の抗 HIV 薬濃度の関係)

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Abstract

Therapeutic drug monitoring (TDM) is valuable to evaluate the efficacy of a regimen or monitor the compliance of patients treated with a drug. However, TDM requires frequent blood sampling, which carries a risk of viral transmission to medical staff and causes pain to patients. In contrast, saliva sampling is safe and easily performed, so the possibility of using saliva for TDM as an alternative body fluid was examined. Firstly, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the determination of abacavir (ABC), tenofovir (TFV), darunavir (DRV), and raltegravir (RAL) in human plasma and saliva was developed and validated. Analytes were detected using LC-MS/MS, and the linearity of the assay was assessed in the range 1–10,000 ng/mL for all four drugs. Within-run and between-run mean accuracy, precision, and the extraction recovery for all drugs were−14.5 to 18.1%, 1.2 to 13.1%, and 86.0 to 111.1%, respectively. The proposed assay is sufficiently sensitive and accurate to quantify these drugs in plasma and saliva. Secondly, the drug concentrations in plasma, plasma ultrafiltrate, and saliva of 30 human immunodeficiency virus (HIV)-1 infected outpatients who had been treated with these drugs for >1 month were measured. Ratios of concentrations in saliva to plasma were 62.3% for ABC, 2.4% for TFV, 6.5% for DRV, and 13.5% for RAL. Significant correlations were evident between drug concentrations in saliva and those in plasma or plasma ultrafiltrate for ABC, DRV, and RAL, therefore the possibility of using saliva for TDM was suggested for these three drugs. Moreover, ABC, DRV, and RAL concentrations in saliva correlated more strongly with those in plasma ultrafiltrate than in plasma, suggesting that non-protein-bound drug may be secreted more easily to saliva. In contrast, saliva cannot be used for this purpose with TFV, which exhibited a very low

saliva concentration and no correlation between concentrations in saliva and plasma. ABC was the most abundant in saliva, and those of ABC were above IC_{50} for half of patients. An ABC-containing regimen may be a candidate for pre-exposure prophylaxis against oral HIV transmission. In conclusion, this study facilitates the use of saliva as an alternative body fluid of blood for TDM of ART of HIV infection by developing the innovative method.

Keywords:

Antiretroviral drugs

Human plasma

Saliva

LC-MS/MS

Therapeutic drug monitoring

1. Introduction

For patients treated with antiretroviral therapy (ART), therapeutic drug monitoring (TDM) is valuable to evaluate the efficacy of a regimen or monitor the compliance of patients treated with a drug [1]. However, TDM requires frequent blood sampling, which carries a risk of viral transmission to medical staff and causes pain to patients. In contrast, saliva sampling is noninvasive, safe, cheap, and easily performed, even at home by the patient if it is a child [2,3].

Patients with human immunodeficiency virus type 1 (HIV-1) infection have been successfully treated with ART. Among numerous antiretroviral drugs that have been approved to date, abacavir (ABC) and tenofovir disoproxil fumarate (TDF) are recommended as backbone drugs, and darunavir (DRV) and raltegravir (RAL) are recommended as key drugs by several guidelines [4,5] and are the most widely used antiretroviral agents. Several methods have been described to determine the concentrations for these four drugs in plasma [6–12] and applied for pharmacokinetics in patients receiving treatment [13–16]. However, the relationship between the plasma and saliva concentrations is poorly understood [16].

In this study, I developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay to determine the concentrations of ABC, tenofovir (TFV), DRV, and RAL in plasma and saliva. Next, the drug concentrations in plasma, plasma ultrafiltrate, and saliva of 30 human immunodeficiency virus (HIV)-1 infected outpatients who had been treated with these drugs for >1 month were measured. Then, I examined the possibility of using saliva for TDM of these agents.

2. Materials and methods

2.1 Development and validation of the quantification method

2.1.1 Chemicals

ABC was purchased from Sigma-Aldrich (Tokyo, Japan), and TFV was purchased from Wako (Osaka, Japan). DRV and RAL were kindly provided by Janssen (Tokyo, Japan) and MSD (Tokyo, Japan). Chemical structures of the drugs are shown in Fig. 1. HPLC-grade acetonitrile (ACN), analytical-grade methanol (MeOH), and formic acid (FA) were obtained from Nakalai Tesque (Kyoto, Japan). Water was deionized and osmosed using a Milli-Q system (Merck Millipore, Billerica, MA). Plasma (citrate) was purchased from Kohjin Bio (Saitama, Japan), and plasma samples were obtained from a healthy individual and four patients using EDTA as an anticoagulant. Saliva samples were obtained without stimulation from two healthy individuals and four patients.

2.1.2 Instruments

The assay was performed using an LCMS-8030 system (Shimadzu, Kyoto, Japan) consisting of an LC pump (LC-20AD), an autosampler (SIL-20ACHT UFTC), a thermostated column compartment (CTO-20A/20AC), UV-VIS detector (SPD-20A), and a triple quadrupole mass spectrometer (LCMS-8030). The analytes were separated on a reverse phase C_{18} column (1.5 × 50 mm, 5 μm) (GL Science, Tokyo, Japan) at a flow rate of 0.2 mL/min. The column temperature was maintained at 40°C. Drugs in the eluent were detected by tandem mass spectrometry with electrospray

ionization in positive-ion mode. Argon was used as the nebulizer and desolvation gas. The gas temperature, neutralizer gas flow, drying gas flow, capillary voltage, and nebulizer pressure were set at 230 kPa, 3.0 L/min, 15.0 L/min, 6000 V, and 250°C, respectively. The collision energy voltage, fragmentation voltage, and capillary voltage that were adjusted to provide the highest sensitivity, and selected multiple reaction monitoring (MRM) transitions are reported in Table 1. LubSolutions software (Shimadzu, Tokyo, Japan) was used for system control and data analysis. The run time for all drugs was 6 min.

2.1.3 Mobile phases

Liquid chromatography was performed isocratically using 5 mM FA in water:ACN (97:3, v/v) as the mobile phase for ABC and TFV and 5 mM FA in water:ACN (65:35, v/v) as the mobile phase for DRV and RAL.

2.1.4 Preparation of standards

Stock solutions (1 mg/mL) were prepared by dissolving base powder in the following solvents: water for ABC and TFV, MeOH for DRV, and MeOH:water (50:50, v/v) for RAL. Stock solutions were stored at −20°C and returned to room temperature before use. The working solution for each drug was prepared by diluting the stock solution in the mobile phase specific for the drug. This method used TFV as the internal standard (IS) for ABC, ABC as the IS for TFV, RAL as the IS for DRV, and DRV as the IS for RAL.

2.1.5 Sample preparation

Plasma or saliva samples containing various concentrations of drug were prepared by mixing 36μL of plasma or saliva and 4 μL of the working solution. Protein was removed by adding one volume of ACN, vortexing for 10 s, and centrifuging at 16,000 g for 3 min at room temperature. Aliquots (40 μL) of the supernatant were taken and dried in a centrifugal evaporator and then dissolved in 20 μL of the mobile phase specific for each drug. A 1-μL aliquot of each sample was injected into the LC-MS/MS system. All samples were kept at 4°C in the autosampler tray during the experiment.

2.1.6 Analytical method validation

Validation of the assay for all four drugs was conducted according to Guidance for Industry, Bioanalytical Method Validation published by the Food and Drug Administration (FDA, 2009) [17].

2.1.6.1 Selectivity

Selectivity with respect to endogenous and exogenous components in the matrix (plasma or saliva) was evaluated using six blank samples from two healthy donors and four patients treated with antiretroviral agents other than the target analytes for this study. The blank samples were spiked with the analyte at 1 ng/mL, which was the common lower limit of quantification (LLOQ) for all of the analytes, and assayed in triplicate. When a drug used as the IS had been used for ART, the IS was not added and the peak area of the drug in the spiked sample was directly compared with that of the working solution at LLOQ.

2.1.6.2 Accuracy

The accuracy of the method was assessed on three separate days through triplicate analyses of samples at five concentrations: LLOQ (1 ng/mL), upper limit of quantification (ULOQ, 10,000 ng/mL), low quality control (QC) (10 ng/mL), middle QC (100 ng/mL), and high QC (1,000 ng/mL). Accuracy was calculated from five determinations per concentration as the percent deviation between the nominal and mean values. The acceptance criterion was within ±15%, except at LLOQ, for which within ±20% was acceptable.

2.1.6.3 Precision

The precision of the method was determined at the same five concentrations used to determine the method's accuracy. Five determinations per concentration were analyzed in triplicate on three separate days. Precision was calculated as the coefficient of variation during a single run (within-run) and between three assays on separate days (between-run) according to Clinical and Laboratory Standards Institute criteria [18]. The acceptance criterion was <15% of the coefficient of variation (CV), except at LLOQ, for which <20% of the CV was acceptable.

2.1.6.4. Recovery

The extraction recovery was determined by comparing the peak areas of each analyte in two extracts: one was prepared from matrix spiked with the standard; the other was prepared by adding the standard to the extract from blank matrix. Recovery was assessed at three concentrations: low QC,

middle QC, and high QC, with three determinations per concentration.

The matrix effect was determined by comparing the peak area of each analyte in the extract prepared by adding the standard to the extract from blank matrix with the peak area of the authentic standard dissolved in the mobile at the same concentrations indicated above.

2.1.6.5 Calibration curve

Calibration curves were generated for each analyte using one blank sample, one zero sample (blank matrix with IS), and standard samples spiked at seven concentrations: 1, 5, 20, 100, 500, 2,000, and 10,000 ng/mL. Analyte to IS peak area ratios were analyzed using linear regression with a weighting factor of $1/(\text{nominal concentration})^2$. The LLOQ was estimated to meet the conditions of the analyte peak area being at least 5 times that of blank, accuracy within ±20%, and precision <20%. A calibration curve was created for each assay, and at least five of the seven non-zero samples were required to meet the criterion that the back-calculated values of the standards were within ±15% of the nominal concentration, except at LLOQ, for which ±20% was acceptable. Standards not meeting this criterion were excluded from the curve calculation.

2.1.6.6 Stability

The stability of the analytes in particular matrices and under various storage conditions was evaluated by analyzing low QC and high QC samples in triplicate. To assess analyte stability afterthree freeze/thaw cycles, aliquots were stored at −20°C for 12 h and thawed by allowing to stand at room temperature. Aliquots of thawed and freshly prepared samples were

then analyzed. Short-term stability of the extracts was assessed by allowing samples to stand at room temperature for 6 h. The stability of extracts stored in the autosampler at 4°C for 6 h was also tested to show that analysis time had no effect on the results. Long-term stability of the analytes in plasma and saliva was assessed after storage at −20°C for 1 month by comparison with freshly prepared QCs. The stability of stock solutions of ABC, TFV, DRV, and RAL allowed to stand at room temperature for 6 h was evaluated by comparison with stock solutions kept at −20°C until the time of analysis.

2.1.7 Data analysis

Results were processed using the Excel 2013 Quick Analysis Tool (Microsoft, Redmond, WA, USA).

2.2 Sample measurement

2.2.1 Patients

I obtained blood and saliva samples from 30 HIV-1-infected outpatients who had been treated with ABC, TDF, DRV, or RAL for >1 month and provided informed consent to participate in this study. Blood and saliva samples were collected within 1 h of each other. No one had abnormal liver or kidney function.

2.2.2 Sample treatment

Blood samples were collected into a 10-mL EDTA tube and centrifuged at 1,500 g for 8 min at 25°C to obtain plasma. Saliva samples (2–3 mL) were

collected without stimulation into a 50-mL tube by the patient. Plasma and saliva samples were stored at −80°C until drug concentration was measured. These samples were thawed at room temperature, then half of the plasma sample was centrifuged at 1500 g for 20 min at 25°C with Centrifree® ultrafiltration devices (Merck Millipore, Darmstadt, Germany)to obtain non-protein-bound drug fraction which is called plasma ultrafiltrate thereafter. Thirty-six microliters of the plasma, plasma ultrafiltrate, or saliva sample was added with 4μL of IS and one volume of acetonitrileto remove protein, then centrifuged at 16,000 g for 3 min at 25°C; 40 μL of supernatant were then dried in a vacuum centrifuge and dissolved in 20 μL of the mobile phase specific for each drug.

2.2.3 Measurement of drug concentration

The drug concentrations in plasma, plasma ultrafiltrate, and saliva of the clinical samples were measured using the method mentioned in 2.1.

2.2.4 Statistics

The drug concentrations in different compartments were processed using the Excel 2013 Quick Analysis Tool and analysed by using the Statcel 3 statistical software package (OMS, Japan).

3. Results

3.1 Validation of the method

3.1.1 Optimization of procedures

I developed an LC-MS/MS assay that enables the measurement of several antiretroviral drugs currently used for treating HIV infection (ABC, TFV, DRV, and RAL). The assay enables determination of these analytes in both plasma and saliva using common extraction procedures and chromatography conditions. Mass spectrometry was performed in positive ionization mode using precursor/product transitions (*m/z*) of 287.3/191.2 for ABC, 288.5/176.2 for TFV, 548.3/392.3 for DRV, and 445.3/109.5 for RAL. Each run required only 6 min, and the retention time was approximately 2.0 min for TFV, 2.5 min for RAL, and 4–4.5 min for ABC and DRV. Typical chromatograms for these four drugs are shown in Figs. 2 and 3. As IS, TFV was chosen for ABC, ABC was chosen for TFV, RAL for DRV, and DRV for RAL.

3.1.2 Linearity

The linearity of the calibration curve for each drug was evaluated by plotting the ratio of the peak area of the analyte to that of the IS against the nominal concentration of the analyte over the concentration range 1–10,000 ng/mL. Calibration curves were analyzed using linear regression with a weighting factor of $1/(\text{nominal concentration})^2$. The slopes, intercepts, and coefficients of determination are presented in Table 2.

3.1.3 Selectivity

The selectivity of the method in plasma and saliva was evaluated using six blank samples obtained from two healthy donors and four patients treated with other antiretroviral drugs. None of them had renal failure or liver disease. The chromatograms of blank plasma and saliva samples showed no interfering peaks at any retention time (data not shown). No interference associated with different blanks was detected in analyses of blank plasma and saliva samples spiked with each drug at LLOQ (Table 3), suggesting that the method is specific for ABC, TFV, DRV, and RAL.

3.1.4 Accuracy and precision

The within- and between-run accuracy and precision were evaluated over three runs, each consisting of the analysis of three plasma and saliva samples at five concentrations (LLOQ, low QC, middle QC, high QC, and ULOQ). The results are presented in Table 4. The within- and between-run precisions (CV) for all drugs were <13.1 and <11.2%, respectively. The within- and between-run accuracies for all drugs were −14.5 to 18.1% and −7.1 to 12.7%, respectively.

3.1.5 Recovery

The extraction recovery for each analyte in plasma and saliva was determined at three different concentrations (low QC, middle QC, and high QC). The results are presented in Tables 5 and 6. The extraction recoveries for all drugs at three concentrations were in the range 86.0–111.1%, and the matrix effect was −52.1–14.0%. The proposed extraction procedure is simple,

requiring only the addition of ACN to remove protein in the plasma and saliva samples, which may achieve a high recovery rate and low LLOQ compared with other methods [6-12].

3.1.6 Stability

The stability of the drugs under various conditions was evaluated using low and high QC (Table 7). No change in the concentration of any analyte was observed after three freeze/thaw cycles (95.4–107.4%) or after storage at room temperature for 6 h (89.8–103.5%). The extracted drugs were stable in the autosampler (4°C) for 6 h (94.7–108.7%). The drugs were also stable for 1 month at −20°C (91.9–109.0%). Stock solutions left at room temperature were stable for at least 6 h (93.3–103.9%).

3.1.7 LLOQ and ULOQ

The LLOQ and ULOQ for all drugs were 1 ng/mL and 10,000 ng/mL, respectively, based on CV (%) of <20% for the LLOQ and <15% for the ULOQ (Table 4). The 1–10,000 ng/mL working concentration range covers the concentrations of the four drugs in the plasma of patients undergoing ART.

3.2 Sample measurement

3.2.1 Patients

Drug concentrations of blood and saliva samples from 30 HIV-1-infected outpatients (28 men, two women) who had been treated with ABC, TDF, DRV, or RAL for >1 month and provided informed consent to

participate in this study were measured. Blood and saliva samples were collected within 1 h of each other. The times since the last drug intake were shown in Table 8. Clinical characteristics and the median CD4 cell counts were described in Table 9.

3.2.2 Drug concentration

Drug concentrations of ABC, TDF, DRV, or RAL in plasma, plasma ultrafiltrate, and saliva samples were determined by LC-MS/MS (Table10). Plasma protein binding ratio of each drug was 58.3±15.2% for ABC (49% in the manufacture's specifications), under 10.9% for TFV (below 7.2%), 74.9±6.0% for DRV (95%), and 44.4±20.1% for RAL (83%). ABC was distributed most abundantly in saliva when compared with blood: the median ABC drug concentration in saliva was 60% higher than that in plasma ultrafiltrate. The ABC drug concentration in saliva was above the inhibitory concentration (IC_{50}, 0.38 ng/mL[19]) in six of 12 patients. In contrast, TFV was less abundant in saliva and its concentration was not above the IC_{50} level in any patients (290 ng/mL [19]).

3.2.3 Correlation of drug concentrations

Significant correlations were evident between drug concentrations in saliva, plasma, and plasma ultrafiltrate for ABC, DRV, and RAL ($P < 0.05$ or 0.001, Table 11), suggesting the possibility of using saliva for TDM for these three drugs. Drug concentrations in saliva correlated more strongly with those in plasma ultrafiltrate than in plasma (Table 11). However, TFV exhibited no correlation between concentrations in saliva and plasma, showing that saliva cannot be used for TDM of TDF.

4. Discussion

4.1 Validation of the method

A rapid, sensitive, and accurate measurement method was developed. Each run required only 6 min, and this run time is shorter than those of previously reported similar assays [8–11]. Moreover, this may enable higher throughput at lower cost.

ABC was used as the IS for TFV, and vice versa, whereas DRV was used as the IS for RAL, and vice versa. This was possible because pairs of the drugs (TFV/RAL and DRV/ABC) exhibited similar retention times with the common mobile phase. Furthermore, current ART guidelines [4,5] recommend against the simultaneous use of both ABC and TDF or both DRV and RAL. An advantage of the IS selection method I used is that synthesis of expensive isotopic isomers is not required.

Furthermore, plasma and saliva samples were processed in only three steps: (1) addition of one volume of ACN to the samples for protein removal; (2) drying of the supernatant; and (3) regeneration in the mobile phase. This procedure is simple and does not require purification of the drugs using reverse-phase or ion-exchange cartridges, thus potentially leading to higher and more reproducible drug recovery rates compared with previous assays [7,8,10–12]. Another advantage of the proposed method is that only 40 μL of plasma or saliva sample is required.

4.2 Sample measurement

The possibility of using saliva for TDM as an alternative body fluid was suggested for ABC, DRV, and RAL. Moreover, drug concentrations of these

three drugs in saliva correlated more strongly with those in plasma ultrafiltrate than in plasma, suggesting that non-protein-bound drug may be secreted more easily to saliva than protein-bound drug.

The ABC concentration has been reported to be high in breast milk (85% of that in plasma [20]) but low in cerebrospinal fluid (CSF) (10% [21]). The ABC concentration in saliva was found to be 62.3% of that in plasma in this study. The distribution rate of the drug in each compartment may depend on the mechanisms of secretion and the structure of transport barriers.

The ABC drug concentration in saliva was above the IC_{50} for half of patients. Although HIV transmission via oral cavity is infrequent [22,23], a regimen containing ABC may play a role in pre-exposure prophylaxis (PrEP) against oral HIV transmission.

The plasma protein binding rates determined in this study differs to some extent from those in manufacture's specifications. This may be due to differences in the method to quantify non-protein bound drug: the Centrifree® ultrafiltration device was used in this study and dialysis membranes were commonly used by the manufacturers (personal communications).

TFV has been reported to penetrate into semen, genital tracts, rectal tissue, and amniotic fluid at high levels [24], but into breast milk and CSF at low levels [25,26]. In this study, TFV exhibited a very low saliva concentration and no correlation between concentrations in saliva and plasma. A low ratio of the TFV concentration in saliva to that in plasma (3%) and no correlation between saliva and plasma concentrations were also reported [27]. A low concentration of TFV in saliva may be due to its poor penetration through plasma membrane because of an ionized property.

In conclusion, because ABC, DRV, and RAL had significant correlations

between drug concentrations in saliva and those in plasma or plasma ultrafiltrate, the possibility of using saliva for TDM as alternative body fluid was suggested. Moreover, ABC drug concentrations in saliva were above IC_{50} for mostly patients, therefore a regimen containing ABC may be a candidate for PrEP against oral HIV transmission.

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preexposure prophylaxis of oral HIV acquisition. Antimicrob Agents Chemother 55(10) (2011) 4905-4907

Fig.1.

The chemical structures of (A) ABC, (B) TFV, (C) DRV, and (D) RAL.

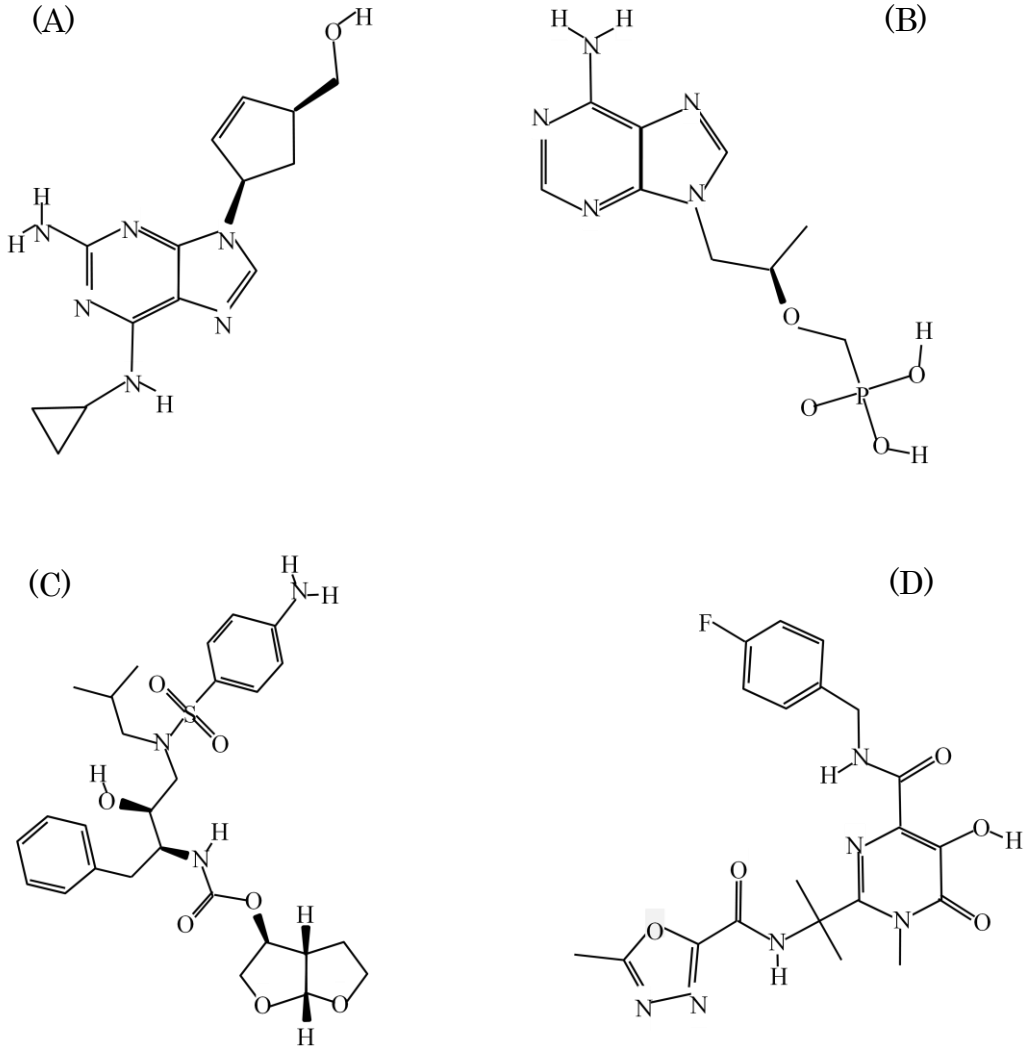

Fig. 2.

LC-MS/MS chromatograms of (A) ABC, (B) TFV, (C) DRV, and (D) RAL extracted from plasma spiked with each drug at LLOQ.

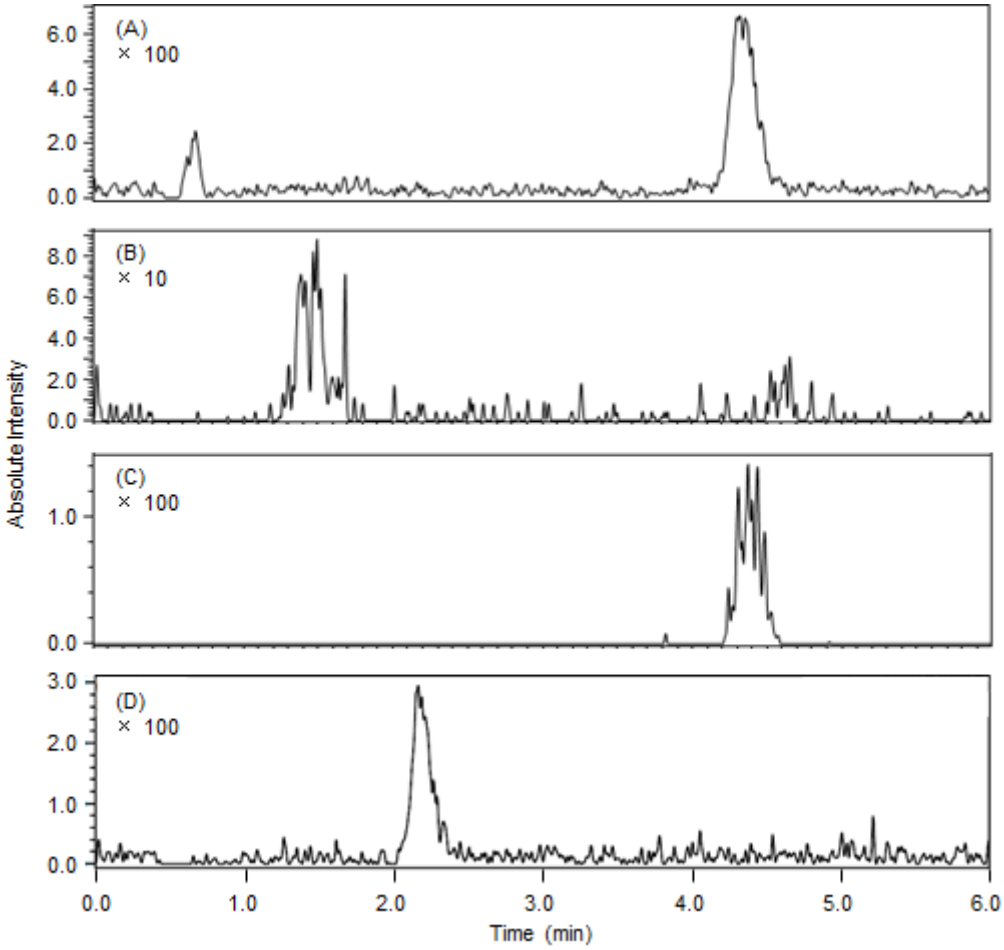

Fig. 3.

LC-MS/MS chromatograms of (A) ABC, (B) TFV, (C) DRV, and (D) RAL extracted from saliva spiked with each drug at LLOQ.

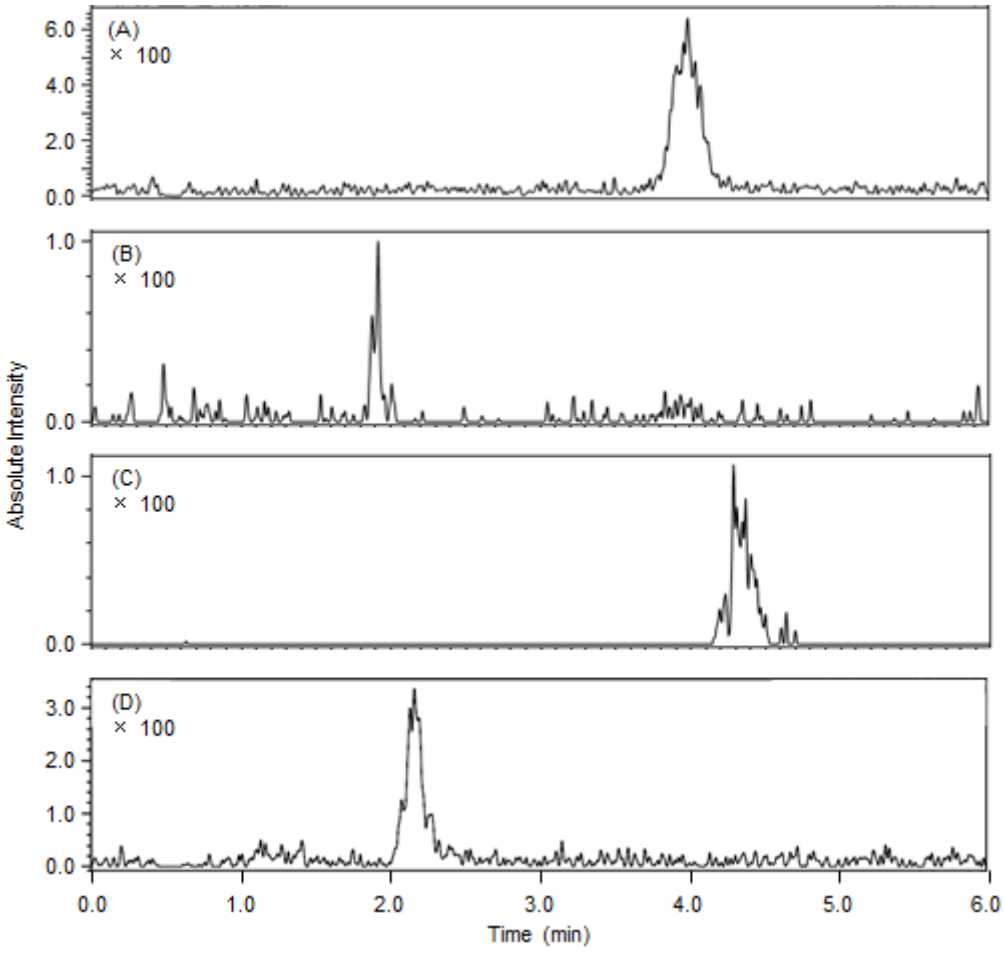

Table 1.

Mass spectrometry parameters.

Drug	MRM	Q1(V)	Collision energy voltage (V)	Q3(V)
ABC	287.3/191.2	−14.0	−20.0	−21.0
TFV	288.2/176.2	−15.0	−20.0	−28.0
DRV	548.3/392.3	−28.0	−15.0	−21.0
RAL	445.3/109.5	−17.0	−35.0	−21.0

Table 2.

Calibration curve coefficients (r^2) and regression line parameters for plasma and saliva over the concentration range 1–10,000 ng/mL (n = 5).

Drug	plasma			saliva		
	Slope (SD)	Intercept (SD)	Average r^2	Slope (SD)	Intercept (SD)	Average r^2
ABC	0.0148 (0.0009)	0.0011 (0.0024)	0.9998	0.0300 (0.0110)	0.0036 (0.0047)	0.9997
TFV	0.000069 (0.000024)	−0.000014 (0.000006)	0.9998	0.000041 (0.000013)	0.000012 (0.000031)	0.9991
DRV	0.00039 (0.00012)	−0.00046 (0.00011)	0.9991	0.00032 (0.00012)	−0.00006 (0.00011)	0.9992
RAL	0.00272 (0.00099)	0.00040 (0.00079)	0.9994	0.00638 (0.00452)	0.00014 (0.00252)	0.9992

Table 3.

Selectivity for ABC, TFV, DRV, and RAL in plasma and saliva at LLOQ (1 ng/mL).

Drug spiked	Patient[a]	Drugs used for ART[b]	plasma		saliva	
			Measured conc. (ng/mL)	CV (%)	Measured conc. (ng/mL)	CV (%)
ABC	A	None	0.96	7.3	0.93	3.8
	B	None	1.00	8.2	0.84	4.9
	C	EFV+TDF/FTC	1.01	8.1	0.97	3.3
	D	DRV+TDF/FTC	1.00	10.2	1.02	0.6
	E	ATV+TDF/FTC	1.02	1.1	1.07	5.6
	F	ATV/RTV+TDF/FTC	0.97	8.9	0.99	1.5
TFV	A	None	1.00	7.0	0.84	6.9
	B	None	1.19	18.6	0.99	19.9
	G	DRV + ABC/3TC	0.97	12.4	0.95	12.7
	H	DRV + ABC/3TC	0.91	12.5	0.93	20.0
	I	ATV/RTV+ABC/3TC	1.12	17.0	0.85	18.4
	J	DRV + ABC/3TC	0.98	5.3	0.96	19.6
DRV	A	None	1.07	6.7	0.97	8.2
	B	None	1.04	5.3	1.01	7.0
	K	ATV + TDF/FTC	1.02	7.3	1.08	5.6
	L	ATV/RTV+TDF/FTC	1.07	2.7	1.03	11.3
	M	ATV/RTV+TDF/FTC	1.00	6.0	0.82	8.8
	N	ATV/RTV+ABC/3TC	0.99	2.0	0.85	11.3
RAL	A	None	0.93	8.7	0.88	4.3
	B	None	0.85	9.2	1.02	3.0

O	ATV + TDF/FTC	0.94	13.9	0.91	11.1
P	ATV/RTV+TDF/FTC	0.90	9.2	0.91	7.7
Q	ATV/RTV+TDF/FTC	1.07	4.9	0.98	7.7
R	ATV/RTV+ABC/3TC	0.90	10.2	0.97	1.9

[a] Patients A and B were healthy donors. Patients C–R were treated with antiretroviral drugs other than a drug used for selectivity test.

[b] Abbreviations for antiretroviral drugs are EFV (efavirenz), FTC (emtricitabine), ATV (atazanavir), RTV (ritonavir), and 3TC (lamivudine).

Table 4.

Accuracy and precision of the proposed method for drugs in plasma and saliva.

Drug	Nominal conc. (ng/mL)	plasma						saliva					
		Within-run				Between-run		Within-run				Between-run	
		accuracy (%RE)			CV (%)	accuracy (%RE)	CV (%)	accuracy (%RE)			CV (%)	accuracy (%RE)	CV (%)
		run1	run2	run3				run1	run2	run3			
ABC	1	11.2	8.2	13.9	2.5	11.1	5.6	−11.0	5.6	15.7	13.0	3.4	6.2
	10	4.6	7.8	14.3	4.6	8.9	4.8	−13.6	11.3	6.7	13.1	1.5	4.7
	100	6.5	12.3	11.1	2.8	10.0	7.8	−5.2	−14.5	1.4	8.5	−6.1	9.7
	1,000	1.0	10.9	8.2	4.8	6.7	4.9	−10.4	8.4	1.9	9.5	−0.1	6.9
	10,000	10.5	−3.6	−0.8	7.4	2.0	3.1	−10.3	7.2	1.4	5.7	−0.6	3.2
TFV	1	6.3	1.6	18.1	7.8	8.7	11.2	−11.0	5.6	15.7	6.1	3.4	9.4
	10	3.7	7.9	11.4	3.6	7.7	3.0	−13.6	11.3	6.7	5.5	1.5	4.1
	100	2.9	10.6	11.2	4.3	8.2	3.9	−5.2	−14.5	1.4	9.8	−6.1	6.6

	1,000	7.6	4.1	12.0	3.7	7.9	5.0	−10.4	8.4	1.9	9.9	−0.1	6.5
	10,000	−0.8	7.4	−1.0	4.7	1.9	5.4	−10.3	7.2	1.4	9.0	−0.6	8.3
DRV	1	−3.0	−7.4	−6.5	2.5	−5.6	9.3	15.9	10.1	−4.7	9.9	7.1	4.1
	10	12.5	11.4	14.1	1.2	12.7	7.9	13.5	−10.5	−4.1	12.5	−0.3	8.1
	100	13.9	2.6	6.3	5.3	7.6	3.1	7.0	−3.8	3.7	5.4	2.3	4.5
	1,000	−4.6	5.4	10.0	7.2	3.6	4.6	−4.7	−0.1	−3.8	2.5	−2.9	3.1
	10,000	−7.5	−4.0	13.3	11.1	0.6	2.9	11.8	1.5	12.8	6.4	10.5	9.6
RAL	1	−10.3	−14.0	3.1	9.7	−7.1	4.3	7.8	−1.8	14.1	7.5	6.7	6.0
	10	2.0	1.1	−6.4	4.7	−1.1	5.3	−14.1	8.2	−2.8	11.5	−2.9	7.6
	100	−2.7	−1.0	−12.1	6.3	−5.3	6.0	−5.2	13.7	5.1	9.0	4.6	5.0
	1,000	9.4	2.4	−10.6	10.1	0.4	5.2	−4.2	−0.4	3.3	3.8	−0.4	2.3
	10,000	8.3	−8.9	−6.0	9.4	−2.2	9.3	−13.6	9.3	5.9	12.3	0.5	6.1

Table 5.

The extraction recovery in plasma and saliva.

Drug	Recovery ± SD (%)					
	plasma			saliva		
	QC level			QC level		
	low QC	middle QC	high QC	low QC	middle QC	high QC
ABC	97.1±5.9	100.8±3.8	90.8±4.4	98.4±2.9	99.5±0.7	98.6±1.0
TFV	97.9±3.0	100.4±5.8	96.3±1.1	94.1±3.1	97.2±3.6	104.1±5.4
DRV	104.6±3.1	91.1±4.7	98.5±3.8	101.9±5.4	86.0±1.3	94.9±1.8
RAL	107.9±5.9	111.1±3.1	111.1±5.8	98.8±5.2	104.6±6.6	107.7±1.2

Table.6

The matrix effect in plasma and saliva.

Drug	Matrix effect ± SD (%)					
	plasma			saliva		
	QC level			QC level		
	low QC	middle QC	high QC	low QC	middle QC	high QC
ABC	9.1±6.7	−2.3±3.7	−10.5±4.4	0.1±2.9	−1.3±7.0	−1.2±10.1
TFV	6.3±3.2	4.8±6.1	−0.9±1.2	−52.1±1.6	−51.5±1.8	−48.5±2.7
DRV	14.0±3.4	−11.4±4.5	−5.2±3.7	6.2±5.6	−15.1±1.3	−7.9±1.7
RAL	4.8±5.8	6.1±2.9	−3.2±5.1	3.4±5.5	−4.4±6.0	−3.5±1.0

Table 7.

Stability of ABC, TFV, DRV, RAL (%).

Condition	Drug	plasma		saliva		Stock
		low QC	high QC	low QC	high QC	
Freeze/thaw	ABC	99.9	95.4	101.4	107.4	
	TFV	102.6	98.7	103.8	100.0	
	DRV	96.5	99.1	101.8	101.2	
	RAL	98.8	99.8	98.8	96.8	
Short-term	ABC	99.1	98.6	97.6	102.2	103.9
(at room	TFV	97.8	98.6	95.9	89.8	101.0
temperature	DRV	100.8	99.1	97.7	99.0	93.3
for 6 h)	RAL	97.3	99.7	103.5	101.5	97.7
Short-term	ABC	97.6	99.9	108.7	97.8	
(in	TFV	105.5	95.8	108.1	94.7	
autosampler	DRV	96.7	96.3	102.9	99.6	
for 6 h)	RAL	98.8	96.7	95.5	95.4	
Long-term	ABC	97.6	102.5	95.0	109.0	
(−20°C for	TFV	98.3	104.7	94.5	98.9	
1 month)	DRV	106.9	108.8	95.2	91.9	
	RAL	105.8	108.8	102.2	104.9	

Table 8.

The times since the last drug intake.

Drug	*n*	plasma (mean ± SD)(h)	saliva (mean ± SD)(h)
ABC	16	10.7±6.3	10.8±6.3
TDF	13	12.2±6.9	12.1±6.6
DRV	8	13.2±6.8	13.2±6.8
RAL	9	4.8±2.2	4.9±2.2

Table 9.

Patient characteristics.

Drug	*n*	Median age (range) (yr)	Median no. of CD4 cells/mm^3 (range)	No.(%) with HIV viral loads of < 20 copies/ml	Median month treated with ART (range)
ABC	16	46 (28–74)	581 (82–784)	81.3	17 (1–53)
TDF	13	39 (30–64)	457 (102–615)	84.6	22 (5–57)
DRV	8	39 (28–55)	438 (209–655)	87.5	20 (1–36)
RAL	9	45 (30–74)	546 (82–784)	88.9	9 (1–22)

Table 10.

Concentrations of DRV, RAL, ABC, and TFV in plasma, plasma ultrafiltrate, and saliva.

Drug	*n*	plasma concn (μg/mL)		plasma ultrafiltrate concn (μg/mL)		saliva concn (μg/mL)	
		Median	Range	Median	Range	Median	Range
ABC	16	0.12	0.010–11	0.058	0.0059–2.9	0.066	0.0080–6.9
TFV	13	0.12	0.052–0.48	0.14	0.046–0.46	0.0020	0.00036–0.0097
DRV	8	4.6	2.4–12	1.0	0.73–3.1	0.23	0.12–1.7
RAL	9	1.0	0.40–3.5	0.56	0.21–1.9	0.13	0.051–0.53

Table 11.

Ratios and correlation coefficients (*r*) between drug concentrations in plasma, plasma ultrafiltrate, and saliva.

Drug	plasma ultrafiltrate to plasma		saliva to plasma		saliva to plasma ultrafiltrate	
	Ratio (%) (Mean ± SD)	*r*	Ratio (%) (Mean ± SD)	*r*	Ratio (%) (Mean ± SD)	*r*
ABC	41.7±15.2	0.98[b]	62.3±19.2	0.94[b]	164.5±60.7	0.98[b]
TFV	113.6±24.5	0.98[b]	2.4±1.8	0.32 ($P = 0.29$)	2.2±1.7	0.33 ($P = 0.27$)
DRV	25.1±6.0	0.94[a]	6.5±3.4	0.88[a]	27.3±15.5	0.92[a]
RAL	55.6±20.0	0.92[b]	13.5±5.7	0.92[b]	26.0±11.7	0.95[b]

[a] $P < 0.05$

[b] $P < 0.001$