Pharmaceutica Analytica Acta - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/2153-2435.1000102

An LC–MS/MS Method for Simultaneous Quantification of Seven Anti-HIV Medicines in Plasma of HIV-infected Patients

Li-jun Zhang[1,3]*, Ya-min Yao[1]*, Jian-jun Sun[1], Jun Chen[1], Xiao-fang Jia[1], Fang Shen[1] and Hong-zhou Lu[1,2]*

[1]*Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China*
[2]*Huashan Hospital, 12 Wu Lu Mu Qi Medium Road, Shanghai 200040, China*
[3]*Institute of Clinical Pharmacology, Pharmacogenetics Research Institute, Changsha, Hunan 410078, China*

Abstract

A quick and high-through LC-MS/MS method has been developed and applied to simultaneously quantify lamivudine (3TC), stavudine(d4T), zidovudine(AZT), efavirenz(EFV), nevirapine(NVP), and Lopinavir/Ritonavir (LPV/RTV) plasma concentrations. A combination of protein precipitation and liquid–liquid extraction was used to extract all compounds. The method showed a good linearity in a concentration range of 20–3200μg/L for 3TC, d4T, AZT, 40-6400μg/L for EFV and NVP, 62.5-10000μg/L for LPV and 12.5-2000μg/L for RTV. Mean intra- and inter-day precision were within ±20% at the LLOQ and ±15% at the other QC level. The accuracy were between 85% and 115% for all seven analytes. The whole run is 13 minutes. This method has been successfully used for analyzing 133 samples from 84 HIV-positive patients being treated with the combination therapy in China. To our knowledge, it is the first time to use LC-MS/MS method to simultaneously quantify 3TC, d4T, AZT, EFV, NVP, LPV and RTV in China for investigating the relationship between blood drug concentrations and efficacy and/or toxicity.

Keywords: LC–MS/MS; Plasma concentration; Anti-HIV medicine; HIV-positive patient

Introduction

In recent years, highly active antiretroviral therapy (HAART) has become the standard care for the management of patients with human immunodeficiency virus (HIV) infection [1]. With the wide use of these antiretroviral medicines, the survive ratio of HIV-infected patients has greatly improved. However, some new questions come out, such as drug resistance, drug toxicity and drug-drug interactions. Several studies [2-4] have demonstrated a relationship between plasma drug concentrations and efficacy and/or toxicity. Therefore, therapeutic drug monitoring (TDM) of antiretroviral drugs is important in clinical care[5,6] to determine the best dosage regimen adapted to each individual to reduce the risk of virologic failure from low plasma drug concentrations and to limit the toxicity linked to high plasma drug concentrations.

In China, the first-line antiretroviral combination includes two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitors (NNRTI) or Protease Inhibitor (PI), such as AZT+3TC+EFV/NVP/(LPV/RTV) and d4T+3TC+NVP /EFV/(LPV/RTV), which are widely used in clinical practice. Since only few combination options are available in China and the dosage of these medicines is primarily based on the clinical data from Caucasians. Is it the most appropriate dosage for Chinese patients with HAART?

In order to answer the above questions, first of all, what we need to do is to build a quick and high-through method to detect these medicines. Now several bioanalytical methods, mostly employing either high performance liquid chromatography (HPLC) with UV detection [7] or HPLC tandem mass spectrometry (LC-MS/MS) [8-11] have been reported for quantifying AZT, 3TC, d4T, EFV, NVP, etc. Some of them [8,11] used SPE columns, however, SPE column is expensive and not very suit for Chinese clinical use.

So in this work, we developed and validated a simple and sensitive LC-MS method for simultaneous detection of AZT, 3TC, d4T, EFV, NVP and LPV/RTV in human plasma. This method was successfully used to study the relationship between the plasma drug concentration and therapeutic efficacy in China.

Material and Methods

Chemicals

Working standard of lamivudine (3TC), stavudine (d4T), zidovudine (AZT), efavirenz (EFV), nevirapine (NVP), lopinavir (LPV), ritonavir (RTV) and telmisartan (internal standard, IS) were supplied by U.S Pharmacopeia. HPLC grade methanol and acetonitrile were purchased from Merck Ltd., Germany. Analytical reagent (AR) grade ammonium acetate and glacial acetic acid were purchased from Sinopharm chemical Ltd., China. Blank human blood was collected in the tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) from healthy and drug free volunteers. After centrifugation at 4000 rpm at room temperature, plasma was collected and stored at -20°C.

LC-MS/MS conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary LC-20AD prominence pump, autosampler (SIL-HTC), solvent degasser (DGU-20A3 prominence) and a temperature controlled compartment for column (CTO10AVP) were used for all the analytes. The chromatographic system consisted of Eclipse XDB-C18 (150 mm × 4.6 mm, 5μm) analytical column bought from Agilent Company (USA). The flow rate of the mobile phase was kept at 0.5 mL/min. The autosampler temperature was set at 4°C and the injection volume was 10μL. The mobile phase consisted of A (0.1% formic acid in

***Corresponding authors:** Li-jun Zhang, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China, E-mail: zhanglijun1221@163.com

Hongzhou Lu, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China, E-mail: luhongzhou@fudan.edu.cn

Received August 23, 2010; **Accepted** September 23, 2010 **Published** September 25, 2010

Citation: Zhang LJ, Yao YM, Sun JJ, Chen J, Jia XF, et al. (2010) An LC–MS/MS Method for Simultaneous Quantification of Seven Anti-HIV Medicines in Plasma of HIV-infected Patients. Pharm Anal Acta 1:102. doi:10.4172/2153-2435.1000102

water): B (0.1% formic acid in methanol) (20:80, v/v). The column oven temperature was maintained at 30°C. The pressure of the system was 4.5 MPa and the total LC run time was 13 minutes.

Detection of analytes and IS were performed on a triple quadrupole mass spectrometer, API-3200 (Applied Biosystems, USA) equipped with turbo ion spray ionization source and operating in the positive ion mode. Analyst software version 1.4.2 was used to control all parameters of LC and MS. Quantification was performed using multiple reaction monitoring (MRM) mode based on the precursor m/z and its fragment m/z (MRM transition) for each analyte. Source-dependent parameters optimized were: gas 1 (nebulizer gas), 40 psi; gas 2 (heater gas), 50psi; ion spray voltage (ISV), 4500V; temperature (TEM), 550°C. Compound-dependent parameters like declustering potential (DP), entrance potential (EP), collision energy (CE), cell exit potential (CXP) and dwell time were optimized. Nitrogen was used as collision activated dissociation (CAD) gas and was set at 6 psi. Quadrupoles 1 and 3 were maintained at low and unit resolution, respectively.

Stock solutions

Stock solutions of AZT, d4T, 3TC, NVP, LPV and RTV were prepared as 1.0 g/L concentrations in 50% methanol/water and EFV prepared as 1.0 g/L in 70% methanol/water. A 0.1g/L stock solution of internal standard (telmisartan, IS) in 50% methanol/water was prepared. All stock solutions were stored in -20°C.

Standard and quality control preparation

Each standard stock solution was diluted in 50% methanol to give a working solution containing all drugs at 200, 400, 800, 1600, 3200, 6400, 12,800, 25,600 and 32,000μg/L for d4T, 3TC and AZT, 400, 800, 1600, 3200, 6400, 12,800, 25,600, 51,200 and 64,000μg/L for EFV and NVP, 625, 800, 6250, 8000, 12,500, 25,000, 50,000, 80,000 and 100,000μg/L for LPV, 125, 150, 1250, 1500, 2500, 5000, 10,000, 15,000 and 20,000μg/L for RTV. The internal standard was diluted in 50% methanol to give a working solution of 100 ng/mL. 20μL working standard and 20μL IS was further diluted in 200μL drug free human plasma to prepare 6 plasma calibrators at 10-fold dilution. Quality controls samples were made in blank human plasma at 40, 320 and 2560μg/L for d4T, 3TC and AZT, 80, 640 and 5120μg/L for EFV and NVP, 80, 800 and 8000μg/L for LPV and 15, 150 and 1500μg/L for RTV.

Sample preparation

Owing to the varying physicochemical properties of the compounds, a protein precipitation followed by a liquid–liquid extraction method was used to prepare plasma samples for the quantification of antiretroviral agents. Samples in 0.2 mL plasma were transferred into a 2.0 mL centrifuge tubes and 0.2 mL of organic solvent (50mM NH_4Ac:ACN=1:6) were added for protein precipitation and votexed 3 min for sufficient mixing. Then 1.0mL ethyl acetate were added to above samples to extract anlytes to organic layer and keep the endogenous materials from plasma which were mostly water soluble in aqueous layer to reduce matrix effect. The resulting samples were vortexed for 5 minutes and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was transferred to a new tube and evaporated under nitrogen at 37°C. A second ethyl acetate extraction was performed after centrifugation and the organic layer was added to the first dried extract and evaporated as described and the residue was redissolved in 200μL mobile phase. A 10μL aliquot was injected into the HPLC-MS system.

Method validation

Specificity: Analytic interferences from endogenous substances were investigated by analyzing drug-free human plasma samples from six individuals.

Matrix effects: Ion suppression/enhancement due to matrix was checked according to the previous publications [8,12]. Five different batches of blank plasma were extracted as described in section 2.5 and then spiked with the analytes at high quality control (HQC), middle quality control (MQC) and low quality control (LQC). The corresponding peak areas of the analytes in spiked plasma post-extraction (A) were then compared to those of the aqueous standards in 50% methanol (B) at equivalent concentrations. The ratio (A/B×100) is defined as the matrix effect (ME). A ME value of 100% indicates that the responses for analytes in the mobile phase and in the plasma extracts were the same and that no absolute ME was observed. A value of >100% indicates ionization enhancement and a value of<100% indicates ionization suppression.

Limit of quantization (LOQ) and limit of detection (LOD): LOD was set to the ratio of signal/noise (S/N) bigger than 3.0 in chromatogram. LOQ was set to S/N more than 10.

Calibration curves: Calibration curves were analyzed by weighted linear regression ($1/X^2$) of the area ratio of each antiretroviral drug to internal standard versus nominal drug concentrations. Each calibration standard were prepared and analyzed in five independent batches. To assess linearity, deviations of the mean calculated concentrations should be within ±15% while lower limit of quantification (LLOQ) within ±20% from nominal concentrations for the non-zero calibration standards. Unknown concentrations were calculated from the linear regression equation of the peak area ratio against the concentration of each antiretroviral agent.

Precision, accuracy and recovery: The precision of the method was determined by the replicate analyses of QC samples. For intra-day precision, three QCs (HQC, MQC and LQC) were involved. Five independent samples were prepared for each QC and analyzed in the same standard curve. For inter-day CV, 15 independent samples were prepared and analyzed in triplicate days. The accuracy of the method was expressed by ((mean observed concentration)/(spiked concentration))×100%. The absolute recovery was determined by comparing the peak area of analyte extracted from plasma to that of standards injected directly.

Analysis of clinical samples: The developed method was used to analyze 133 samples from 84 patients (69 males and 15 females) aged 18 years or older who participated in our TDM study that was approved by the Ethical Committee of Shanghai Public Health Clinical Center and with the patients' consent. According to patients' baseline, six types of antiretroviral combination were used, including AZT/3TC/NVP (34 samples, 25.6%); AZT/3TC/EFV (50, 37.6%); d4T/3TC/NVP (18, 13.5%); d4T/3TC/EFV (17, 12.8%); AZT/3TC/LPV/RTV (12, 9.0%) and 3TC/EFV/LPV/RTV (2, 1.5%). The dosage and times of ARV are as follows: AZT, 300mg, twice a day; 3TC, 300mg, once daily; d4T, 30mg, twice a day; NVP, 200mg, twice a day; EFV, 600mg, once daily and LPV (400mg) / RTV (100mg), twice a day. Blood samples were collected during the follow up in our clinic or hospital. Blood samples were collected into 5 ml EDTA tubes. The drug concentrations were assayed using the LC-MS/MS procedure. Clinical samples were diluted or concentrated so that the drugs concentration obtained were within the linear range of the assay if necessary.

Results and Discussions

LC-MS of AZT, d4T, 3TC, EFV, NVP, LPV, RTV and IS and specificity of this method

For optimum detection and simultaneous quantification of AZT, d4T, 3TC, EFV NVP, LPV and RTV with IS in human plasma, it was necessary to adjust not only the chromatographic conditions and mass parameters but also to develop an efficient extraction method that gives consistent and reproducible recovery of analyte from plasma. Precursor ions and product ions were optimized by infusing 1000μg/L solutions into mass spectrometer in suitable mass range respectively, both in positive and negative polarity modes using Electrospray Ionization technique. Best intensity for $[M+H]^+$ ions was found in positive mode for all eight analytes including IS as they have an ability to accept protons. The compounds and their molecullar weight, precursor ions, product ions, retention time, were listed in Table 1.

The LC-MS/MS method has high selectivity for only a precursor→product ion of the analyte of interest monitored by MRM. The selectivity towards endogenous plasma components was assessed in six different batches of human plasma samples by analyzing blanks and spiked samples at LQC levels. Endogenous peaks at the retention time of the analytes were not observed in any of the plasma batches (Figure 1A), all analytes in the plasma spiked at the LQC level (Figure 1B) and clinical samples (Figure 1C) can be detected in their retention time with single peak. This indicated that there was no significant direct interference in the MRM channel for the analytes at the expected retention time. This method is quick and easy for all analytes can be analyzed with the total run time of 13 minutes under positive mode.

Matrix effects (ME)

The ME data at QC concentrations of analytes in five different lots of human plasma are presented in Table 2. There was some ME, as indicated by values of slightly <100% or >100% for plasma samples spiked post-extraction. The ME observed was similar to the QC concentration ranges, without showing any analyte concentration-dependence for different lots of human plasma. This indicated a small ionization enhancement or suppression for analytes (86.2–116.2%) under the present chromatographic and extraction conditions when the ESI interface was employed. However, such ionization suppression did not affect the slopes and linearity of the established calibration curves for the matrix matched standards over the analytical period.

LOQ and LOD

Results for LOQ and LOD were shown in Table 3. The LOQs are much lower than the reports especially for d4T and 3TC [8, 11]. In these two studies, LOQ was 20 to 50μg/L. During the clinical application, we detected a lot of samples with plasma concentration lower than 20μg/L for d4T and 3TC. So it is necessary to improve the LOQ of previous method [8]. In this research, we improved the LOQs of these antiretroviral agents. This method might be much suitable for clinical research at least in China.

Antiretroviral agents	Molecullar weight	precursor ion $[M+H]^+$	product ion $[M+H]^+$	retention time (minutes)
AZT	267	268.0	127.0	3.22
3TC	229	230.2	112.2	2.67
d4T	224	225.0	126.8	3.00
NVP	266	267.0	226.0	3.55
EFV	315	316.0	243.9	6.62
LPV	628	629.3	155.4	10.66
RTV	720	721.6	268.3	7.68
IS	513	514.8	275.9	3.23

Table 1: The ion information of 7 analytes and IS.

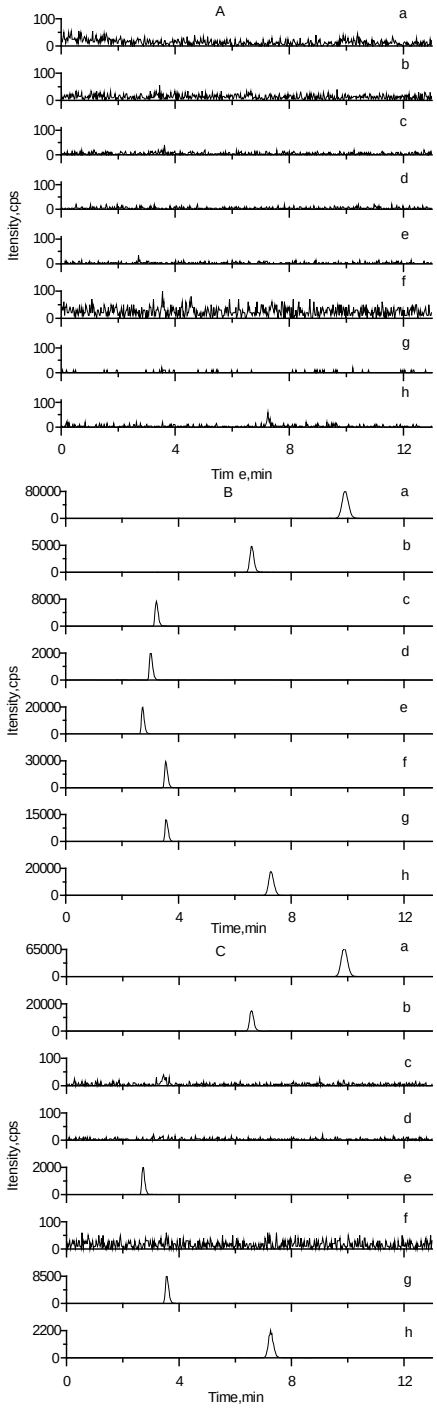

Figure 1: Specificity of method. A) MRM of blank plasma, B) MRM of spiked plasma, C) MRM of samples from patients after NVP+AZT+LPV+RTV treatment, a,b,c,d,e,f,g and h represent the spectrum of IS, NVP, 3TC, d4T, AZT, EFV, LPV and RTV respectively.

Recovery and stability

Five replicates at LQC, MQC and HQC level were prepared for

Analytes	NVP	AZT	D4T	3TC	EFV	LPV	RTV
	ME(%)						
LQC	96.7	86.2	91.8	91.3	97.3	116.2	92.4
MQC	97.0	92.3	91.8	95.5	93.1	113.3	94.8
HQC	101.3	95.9	95.3	99.0	101.2	104.3	111.3

Table 2: Matrix effect data for seven analytes at LQC, MQC, HQC in five different lots of human plasma (n=5).

Compounds	LOD (μg·L^{-1})	LOQ (μg·L^{-1})
AZT	0.25	1
3TC	0.5	2
d4T	1.0	3
EFV	1.0	2
NVP	0.2	0.5
LPV	0.015	0.05
RTV	0.008	0.015

Table 3: Limits of detection (LOD) and Limits of quantitation (LOQ) of AZT, 3TC, d4T, EFV, NVP, LPV and RTV in human plasma(n=3).

Analytes	Concentration (μg·L^{-1})	Recovery (%)	Precision (%)
AZT	40	82.4	6.6
	320	88.5	6.7
	2560	97.2	6.8
3TC	40	59.3	8.7
	320	53.3	9.3
	2560	57.1	8.3
d4T	40	67.6	12.3
	320	70.3	12.9
	2560	73.3	9.5
NVP	80	105.4	10.4
	640	99.8	8.9
	5120	101.2	8.7
EFV	80	89.7	6.6
	640	87.1	7.9
	5120	93.3	8.7
LPV	80	79.7	10.9
	800	76.1	7.5
	8000	82.2	7.3
RTV	15	87.5	9.0
	150	75.7	7.0
	1500	81.6	6.1

Table 4: Absolute Recovery of the determination of AZT, 3TC, d4T, EFV, NVP, LPV and RTV in human plasma (n=5).

recovery determination. The data for absolute recovery are given in Table 4. The absolute recovery for 3TC and d4T were about 57% and 70%, respectively, which were lower than that from the previous report [8]. It may be due to the complicated but cheap sample extraction procession. The recovery of other five analytes was high to 80% recovery, which indicated that our method was very suitable for analyzing these five analytes. For d4T and 3TC, although the lower recovery was detected, our research was not affected. As shown in Table 3, lower LOQ was detected in this work than the report [8] and 133 clinical samples were successfully analyzed.

Stock solution of all seven analysts and IS were stable at room temperature for 24 h and at 2–8°C for 30 days. 3TC, d4T, AZT, NVP, EFV, LPV and RTV in control human plasma at room temperature were stable at least for 56 h and for minimum of three freeze and thaw cycles. Spiked plasma samples stored at -20 C were stable for minimum 30 days. There was no significant degradation observed since the deviations in concentration was within 15% of their nominal values (Data not shown).

Linearity

The correlation coefficients (*r*) of the calibration curves were >0.99 for all seven analytes as determined by linear analysis with a $1/x^2$ regression over a concentration range of 20–3200μg/L for AZT, 3TC and d4T, 40-6400μg/L for EFV and NVP, 62.5-10000μg/L for LPV and 12.5-2000μg/L for RTV respectively. The results showed that the linearity range of this method is wide and suit for clinical research in China.

Accuracy and precision

Intra-day and inter-day accuracy and precision was evaluated at three different concentrations (LQC, MQC and HQC) for each analyte. For inter-batch, three runs and for intra-batch, a single run was assayed. Each run consisted of five replicates.

The results of the precision and accuracy experiments were given in Table 5. Intra-day accuracy varied from 92.1% to 113.8% and inter-day accuracy from 95.3% to 107.4%, respectively. While intra-day precision varied from 2.1% to 11.0% and inter-day from 6.1% to 10.9%. These values are well within the acceptance criteria recommended by the China FDA guideline (2005, China Pharmacopoeia).

Analytes	Concentration (μg·L^{-1})	Intra-day (n=5)			Inter-day (n=15)		
		Mean	Accuracy (%)	Precision (%)	Mean	Accuracy (%)	Precision (%)
AZT	40	38.1	95.2	11.0	38.5	96.3	9.4
	320	310.4	96.9	7.5	313.9	98.1	9.0
	2560	2568.0	100.3	2.1	2610.7	102.0	6.8
3TC	40	40.2	100.6	8.7	38.1	95.3	8.5
	320	297.6	93.0	4.6	308.5	96.4	8.5
	2560	2516.0	98.2	10.4	2566.0	100.2	9.1
d4T	40	37.6	94.0	10.1	38.2	95.4	8.7
	320	302.0	94.4	4.8	312.3	97.6	9.4
	2560	2586.0	100.9	2.1	2590.0	101.2	6.6
NVP	80	79.5	99.4	5.9	80.1	100.2	7.6
	640	662.8	103.5	9.0	658.3	102.9	7.0
	5120	5044.0	98.5	6.1	4981.3	97.3	8.1
EFV	80	73.7	92.1	7.1	77.4	96.7	9.2
	640	651.8	102.0	7.7	648.7	101.4	8.4
	5120	5178.0	101.1	5.1	5190.0	101.4	6.9
LPV	80	91.1	113.8	6.6	85.4	106.7	10.9
	800	885.0	110.8	3.6	844.7	105.7	7.5
	8000	7900	98.7	3.8	7767.3	97.1	7.3
RTV	15	14.9	99.1	9.6	15.0	99.7	9.0
	150	152.6	101.9	9.0	161.1	107.4	7.0
	1500	1494.0	99.8	4.9	1592.7	106.3	6.1

Table 5: Precision and accuracy of AZT, 3TC, d4T, EFV, NVP, LPV and RTV in plasma (n=5).

Antiretroviral agents	Samples(n)	Mean±SD (μg/L)	Range(μg/L)
AZT	96	226.1±412.0	5.11-2600
3TC	133	1020±1412.1	7.16-6020
D4T	35	199.6±262.7	6.13-1030
NVP	52	5192.5±2264.6	1880-12220
EFV	69	2429.06±1090.44	875-5850
LPV	14	6529±573.2	1320-20400
RTV	14	454.5±61.66	62-2460

Table 6: The antiretroviral drug concentrations in Chinese patients.

Application of the method in clinical samples

The validated method was successfully applied to detect the antiretroviral drug concentrations in Chinese patients with HIV infection. The observed plasma concentrations (μg/L) of AZT, 3TC, d4T, NVP, EFV, LPV and RTV are listed in Table 6. The observed anti-HIV medicines plasma concentration and those postdosing time are presented in Figure 2. As shown in Figure 2, for 3TC, d4T and AZT, a large inter-patient variability of the drug concentrations (μg/L) was noted and our results were consistent with the previous report [13]. For NVP, there were 36 patients enrolled in this study, of which, 10 with drug concentrations higher than 6000 μg/L and 4 with drug concentration lower than 3400μg/L. According to a previous paper [14], the therapeutic range for NVP was 3400–6000μg/L[15]. So near 40% samples have the concentration beyond therapeutic range. For EFV, there were 45 patients enrolled in this trial and plasma samples were collected from each subject, 1-4 samples per patient with a total of 69. There were 6 patients with the EFV concentrations beyond the therapeutic window (1000-4000μg/L), one below 1000μg/L and 5 above 4000μg/L. In this work, 14 samples containing LPV and RTV were analyzed. Our results showed a large inter-patient variability,with drug range (μg/L) from 1320-20400 for LPV and 62-2460 for RTV. In the future, it will be necessary to use more samples and obtain more significant statistic data which can truly represent Chinese group.

Discussion

LC-MS method

To screen and quantify the currently available antiretroviral agents in China, a novel analytical method with high-throughout and low-cost is necessary. There are several HPLC and LC-MS/MS methods for measuring antiretroviral agents[8-11,16,17]. However, some of them [8-11] used SPE columns which is expensive, and some determined only two or three drugs in a combination therapy [8,16,17] or certain classes of antiretrovirals [18,19], which had lower throughout. So far a few methods have been previously reported for simultaneously determining NRTI, NNRTI and PI agents[10,20,21], however, in all

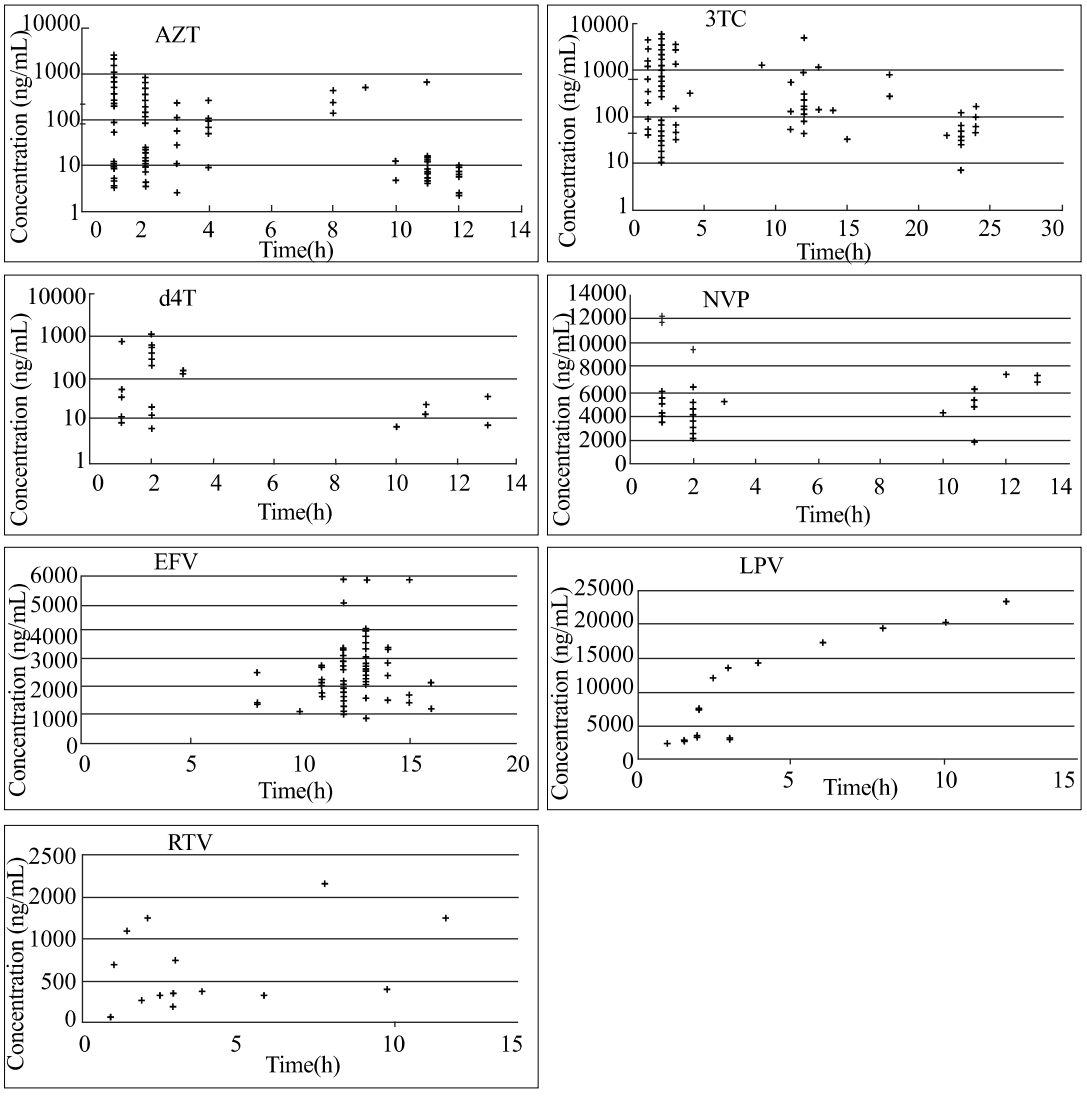

Figure 2: The plasma concentration of AZT, 3TC, d4T, NVP , EFV, LPV and RTV.

these methods, both negative and positive MS/MS modes were used. This polarity switch might affect the replication and resolution of analysis method.

Application of the method in clinical samples

As described in introduction section, only few antivirus medicines are available in China and the dosage of these medicines is primarily based on the clinical data from Caucasians. So it is very necessary to carry out clinical research such as TDM, PK-PD, drug-drug interaction in China. In this study, 133 samples were analyzed and the concentration ranges of 7 anti-HIV drugs were shown in Table 6. These results show that the developed LC-MS method is an ideal method to measure drug concentrations in Chinese. However, due to the small sample size, the statistic data is not very significant and might not represent the whole Chinese population. In the future, it will be necessary to extend sample size and further study the pharmacokinetics of these anti-HIV drugs in the Chinese population and know the differences between other races and Chinese.

Conclusion

In conclusion, we developed and verified an LC-MS method for simultaneously quantifying the AZT, 3TC, d4T, EFV, NVP, LPV and RTV in plasma from Chinese patients infected by HIV. The sample preparation is cheap and easy for only protein precipitation followed by a liquid–liquid extraction. The LC-MS running time is short to 13 minutes. The main ARV medicines taken by Chinese were analyzed simultaneously. This method has lower LOQ [8] and wider quantification range [10] and is very suitable for PK-PD analysis, patient's adhesion detection and drug-drug interaction in China according the results from 133 clinical samples. To our knowledge, this was the first time to simultaneously quantify AZT, 3TC, d4T, EFV, NVP, LPV and RTV in Chinese patients with HIV infection and offer a right method for PK-PD analysis, drug-drug interaction and so on.

Acknowledgment

This work was supported by a Basic Research grant from the Ministry of Health, the People's Republic of China (WA-2007-04) and "National Key Technology R&D Program of China (Grant No. 2008ZX10001-008)". We thank Professor Qing Ma from University at Buffalo for reviewing this manuscript. We also acknowledge the patients and the staff at Shanghai Public Health Clinical Center.

References

1. De Clercq E (2002) New developments in anti-HIV chemotherapy. Biochim Biophys Acta 1587: 258-275.
2. Gerber JG, Acosta EP (2003) Therapeutic drug monitoring in the treatment of HIV-infection. J Clin Virol 27: 117-128.
3. Kiser JJ, Anderson PL, Gerber JG (2005) Therapeutic drug monitoring: pharmacologic considerations for antiretroviral drugs. Curr HIV/AIDS Rep 2: 61-67.
4. Durand-Gasselin L, Pruvost A, Dehée A, Vaudre G, Tabone MD, et al. (2008) High levels of zidovudine (AZT) and its intracellular phosphate metabolites in AZT- and AZT-lamivudine-treated newborns of human immunodeficiency virus-infected mothers. Antimicrob Agents Chemother 52: 2555-2563.
5. Holland DT, DiFrancesco R, Connor JD, Morse GD (2006) Quality assurance program for pharmacokinetic assay of antiretrovirals: ACTG proficiency testing for pediatric and adult pharmacology support laboratories, 2003 to 2004: a requirement for therapeutic drug monitoring. Ther Drug Monit 28: 367-374.
6. Back D, Gatti G, Fletcher C, Garaffo R, Haubrich R, et al. (2002) Therapeutic drug monitoring in HIV infection: current status and future directions. Aids 16: S5-37.
7. Poirier JM, Robidou P, Jaillon P (2005) Simple and simultaneous determination of the hiv-protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir plus M8 nelfinavir metabolite and the nonnucleoside reverse transcriptase inhibitors efavirenz and nevirapine in human plasma by reversed-phase liquid chromatography. Ther Drug Monit 27: 186-192.
8. Mistri HN, Jangid AG, Pudage A, Gomes N, Sanyal M, et al. (2007) High throughput LC-MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 853: 320-332.
9. Rouzes A, Berthoin K, Xuereb F, Djabarouti S, Pellegrin I, et al. (2004) Simultaneous determination of the antiretroviral agents: amprenavir, lopinavir, ritonavir, saquinavir and efavirenz in human peripheral blood mononuclear cells by high-performance liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 813: 209-216.
10. Jung BH, Rezk NL, Bridges AS, Corbett AH, Kashuba AD (2007) Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. Biomed Chromatogr 21: 1095-1104.
11. Fan B, Bartlett MG, Stewart JT (2002) Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch. Biomed Chromatogr 16: 383-389.
12. Matuszewski BK, Constanzer ML, Chavez-Eng CM (1998) Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal Chem 70: 882-889.
13. Molto J, Blanco A, Miranda C, Miranda J, Puig J, et al. (2006) Variability in non-nucleoside reverse transcriptase and protease inhibitor concentrations among HIV-infected adults in routine clinical practice. Br J Clin Pharmacol 62: 560-566.
14. Smith PF, DiCenzo R, Morse GD (2001) Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin Pharmacokinet 40: 893-905.
15. Duong M, Buisson M, Peytavin G, Kohli E, Piroth L, et al. (2005) Low trough plasma concentrations of nevirapine associated with virologic rebounds in HIV-infected patients who switched from protease inhibitors. Ann Pharmacother 39: 603-609.
16. Delahunty T, Bushman L, Robbins B, Fletcher CV (2009) The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. J Chromatogr B Analyt Technol Biomed Life Sci 877: 1907-1914.
17. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, et al. (2008) Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. Rapid Commun Mass Spectrom 22: 3401-3409.
18. Rezk NL, Crutchley RD, Yeh RF, Kashuba AD (2006) Full validation of an analytical method for the HIV-protease inhibitor atazanavir in combination with 8 other antiretroviral agents and its applicability to therapeutic drug monitoring. Ther Drug Monit 28: 517-525.
19. Rezk NL, Tidwell RR, Kashuba AD (2004) High-performance liquid chromatography assay for the quantification of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 805: 241-247.
20. Volosov A, Alexander C, Ting L, Soldin SJ (2002) Simple rapid method for quantification of antiretrovirals by liquid chromatography-tandem mass-spectrometry. Clin Biochem 35: 99-103.
21. Ghoshal AK, Soldin SJ (2003) Improved method for concurrent quantification of antiretrovirals by liquid chromatography-tandem mass spectrometry. Ther Drug Monit 25: 541-543.