ORIGINAL

Development and application of a simple LC-MS method for the determination of plasma rilpivirine (TMC-278) concentrations

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Abstract : Rilpivirine (TMC-278) is a second-generation non-nucleoside reverse transcriptase inhibitor that is high potent against both wild-type and drug-resistant HIV-1 strains. Therefore, rilpivirine is expected to treat therapy-experienced patients who failed to use current drugs due to the emergence of drug-resistant HIV mutants. The quantification of rilpivirine in human plasma is important to support clinical studies and determine pharmacokinetic parameters of rilpivirine in HIV-1 infected patients. Consequently, simple and easy system to determine plasma rilpivirine concentrations has been required. In this study, we developed a conventional LC-MS method to quantify plasma rilpivirine. Subsequently the method was validated by estimating the precision and accuracy for inter- and intraday analysis in the concentration range of 18-715 ng/ml. The calibration curve was linear in this range. Average accuracy ranged from 100.0 to 100.6%. Relative standard deviations of both inter- and intraday assays were less than 3.3%. Recovery of rilpivirine was more than 82.0%. These results demonstrate that our LC-MS method provides a conventional, accurate and precise way to determine rilpivirine in human plasma. This method can be used in routine clinical application for HIV-1 infected patients, and permits management of drug interactions and toxicity for rilpivirine. J. Med. Invest. 60 : 35-40, February, 2013

INTRODUCTION

The clinical treatment of patients with human immunodeficiency virus (HIV)-1 infection has been advanced by the success of highly active antiretroviral therapy. The latest treatment guidelines recommend regimen including efavirenz, a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI), as one of the standard first-line regimen (1). However, efavirenz use is limited by low genetic barrier to resistance and central nervous system toxity (2, 3). Therefore, new antiretroviral drugs, which have long-term efficacy and good tolerability, are required to continue effective therapy for the treatment of HIV-1.

Rilpivirine (TMC-278) is a second-generation NNRTI that is high potent against both wild-type and drug-resistant HIV-1 strains (4). Consequently, rilpivirine is expected to treat therapy-experienced patients who failed to use current drugs due to the emergence of drug-resistant HIV mutants. In addition, rilpivirine shows a favourable safety profile (5-7). The recommended dose of rilpivirine is 25 mg (one tablet) once daily in combination with other antiretroviral agents. No dose adjustment is required in patients with moderate hepatic or renal impairment. However, rilpivirine is primarily metabolized by cytochrome P450 (CYP)3A. Therefore, co-administration of rilpivirine and CYP3A inducer may result in decreased plasma concentrations of rilpivirine, loss of virologic response, and possible resistance to rilpivirine. To avoid these risks, therapeutic drug monitoring of rilpivirine is essential.

Else *et al.* (8) recently determined plasma rilpivirine concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, more simple and easy system to determine plasma rilpivirine concentrations has been required. Now we have a routine system, by which antiretroviral drug plasma concentrations are easily determined by HPLC (9). According to our preliminary HPLC application, the sensitivity of LC-MS method must at least be essential for quantification of plasma rilpivirine. In this study, we intended to develop a conventional method for determining plasma rilpivirine concentrations by LC-MS.

MATERIALS AND METHODS

Chemicals and Reagents

Rilpivirine was supplied by Janssen Pharmaceutica (Turnhoutseweg, Beerse, Belgium) and the internal standard (IS), 6,7-Dimethyl-2,3-di (2-pyridyl)-quinoxaline, was purchased from Sigma-Aldrich (St Louis, MO, USA). Methanol, *n*-hexane, ethyl acetate, and acetonitrile (Kanto Chemical, Tokyo, Japan) were HPLC grade. Ammonium acetate, EDTA and acetic acid were purchased from Katayama Chemical (Osaka, Japan). Water was deionized and osmosed using a Milli-Q® system (Millipore Corp., Bedford, MA, USA). All other chemicals and solvents were of analytical grade.

Equipment

A Waters Alliance 2695 HPLC and a Micromass ZQ-2000 MS (Waters Assoc., Milford, MA, USA),

controlled with MassLynx version 4.0 software, were used for detection. The analytical column was a SunFire C_{18} column (3.5 μm, 2.1×50 mm, Waters), protected by a SunFire C_{18} Guard Column.

Chromatographic and mass spectrometric conditions

The mobile phase was a mixture of 0.1 mM EDTA in 0.1% acetic acid (A), 100% acetonitrile (B) and 100% methanol (C). An isocratic mobile phase consisting of A-B-C (65 : 15 : 20) was used during the first 2 min of the run, followed by a linear gradient elution consisting of A-B-C (30 : 50 : 20) for the next 8 min. The final conditions were maintained for the final 5 min. The system was then reequilibrated for an additional 25 min using the initial conditions. The flow rate of the mobile phase was 0.2 ml/min, the column temperature was 40°C, and the amount of injected sample was 5 μl.

The mass spectrometer was operated in positive ion electrospray mode. The capillary sprayer voltage was 3.5 kV and the sample cone voltage was 30 V for both rilpivirine and the internal standard. The source temperature was 120°C and the desolvation temperature was 350°C. The desolvation and cone gas flow-rates were set to 600 and 50 L/h, respectively. The acquisition mass range is *m/z* 200-800 at 0.5 s per scan with a 0.1 s interscan delay. All mass spectra are acquired in centroid mode.

Quantitative analysis, carried out in selected-ion recording (SIR) mode, detected rilpivirine at *m/z* 367, and the IS, at *m/z* 313, all in the form of ions. The quantitation calculations were performed using analytical software, MassLynx version 4.0 (Waters).

Standard Solutions

Stock solutions of rilpivirine and the IS were prepared by dissolving accurately weighed amounts of each reference compound in water/ethanol (50 : 50, v/v) to yield concentrations of 143.0 μg/ml of rilpivirine and 588.0 μg/ml of the IS. These stock solutions were stored at -80°C and thawed on the day of analysis. The stock solution was diluted in drug-free plasma to yield rilpivirine concentrations of 18, 72, 143, 358 and 715 ng/ml.

Sample Preparation

Two milliliters of ethyl acetatate/*n*-hexane (50 : 50, v/v) containing the IS (177.5 ng/ml) and 1.0 ml of 0.2 M ammonium acetate were added to a 500 μl plasma sample prepared from peripheral blood anticoagulated with heparin. The mixture was vortexed for 5 min and then centrifuged at 3,500 g

for 5 min. The upper layer was separated and evaporated dry. The dried material was then dissolved in 50 μl of a mobile phase solution. Lastly, 5 μl of the upper solution was injected into the LC-MS system. The institutional review board of National Hospital Organization Nagoya Medical Center approved this study and each subject provided written informed consent.

Validation

Inter- and intraday precision values using this method were estimated by assaying control plasma containing five different concentrations of rilpivirine five times on the same day and on three separate days to obtain the relative standard deviation (RSD). The measured value was calculated as the peak area ratio of rilpivirine to the internal standard. The extraction recovery was determined by comparing the peak areas obtained from the extracted samples in plasma with those of direct injected standards, at the same concentrations. The mean recoveries were determined in triplicate. Accuracy was determined as the percentage of the nominal concentration.

RESULTS

LC-MS Chromatograms

Figures 1A and B show selected-ion recording

chromatograms obtained from a spiked plasma sample containing 143.0 ng/ml of rilpivirine and 177.5 ng/ml of the IS. Under the described chromatographic conditions, retention times were 5.3 min for rilpivirine and 10.0 min for IS. Figures 1C and D show chromatograms obtained from a blank plasma sample. Assays performed on drug-free human plasma succeeded to show no interfering peaks during the interested intervals of the retention times. Figure 1D is the expanded figure of the baseline part of Fig. 1B. These peaks did not affect the quantification of the IS. Figures 1E and F show chromatograms of a plasma sample from an HIV-1-infected patient treated with rilpivirine. There were no interfering peaks affecting quantification of rilpivirine in this chromatogram. Anticoagulants of heparin and EDTA did not hinder the selected-ion recording chromatograms for rilpivirine and the IS.

Validation : Linearity, Precision, Accuracy and Recovery

Calibration curves of rilpivirine appeared linear in the concentration range of 18 to 715 ng/ml with a correlation of 0.995.

Precision, accuracy, and recovery of our LC-MS method are shown in Table 1. The selected concentration of rilpivirine covers the expected plasma concentrations found in the patients. The RSDs calculated for rilpivirine in the inter- and intraday
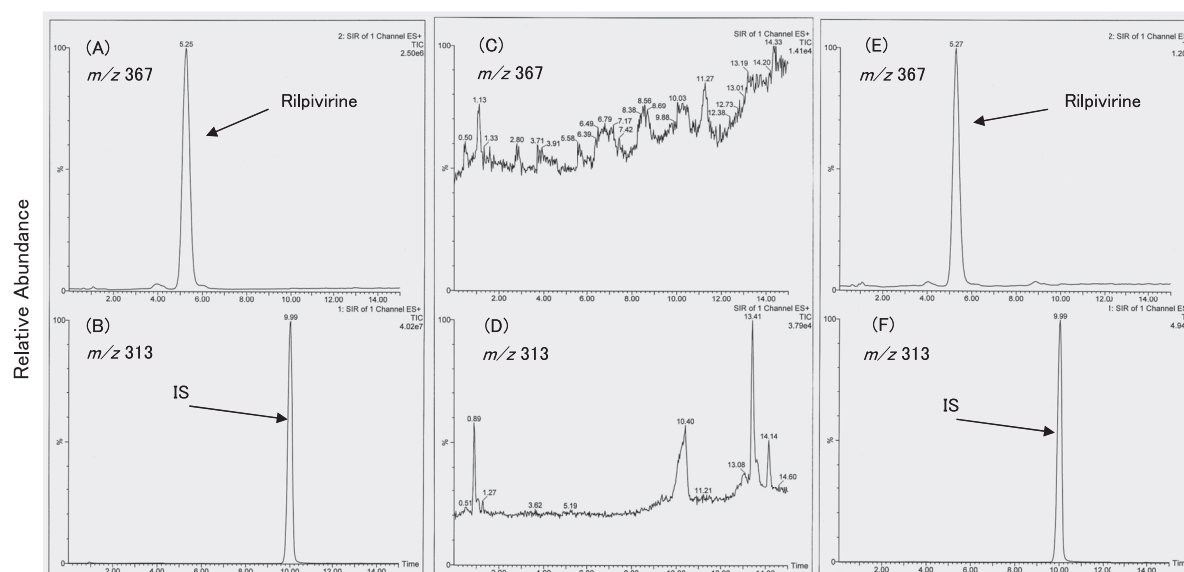

Figure 1.   Selected-ion recording chromatograms for rilpivirine and the internal standard.
(A) and (B) were obtained from a spiked plasma containing 143 ng/ml of rilpivirine and 178 ng/ml of the internal standard (IS). (C) and (D) were obtained from a blank plasma sample. (E) and (F) were obtained from a plasma sample from an HIV-1-infected patient on rilpivirine at 16 h after orally administration. (A), (C) and (E) were monitored with *m/z* 367. (B), (D) and (F) were monitored with *m/z* 313. (C) and (D) are the expanded figures of the baselines in (A) and (B), respectively.

Table 1.   Intraday and interday precision and accuracy for rilpivirine

Expected (ng/ml)	Intraday	(n=5)	Interday	(n=15)	Accuracy (%)	Recovery (%)
	Measured (ng/ml)	RSD (%)	Measured (ng/ml)	RSD (%)		
18	18.1± 0.2	1.0	18.0± 0.4	2.4	100.3± 1.0	85.1± 1.3
72	72.3± 1.4	1.9	72.0± 2.3	3.3	100.4± 1.9	82.0± 3.7
143	143.7± 3.0	2.1	143.9± 3.1	2.2	100.5± 2.1	87.6± 0.6
358	360.2± 2.9	0.8	357.8± 5.2	1.5	100.6± 0.8	88.3± 7.2
715	715.0± 8.6	1.2	716.3± 6.1	0.8	100.0± 1.2	84.0± 8.6

RSD, relative standard deviation
Means± SD

assays ranged from 0.8 to 3.3%, which are similar to values reported by LC-MS/MS method (8). Accuracies ranged from 100.0 to 100.6%. Recoveries from plasma ranged from 82.0 to 88.3%. These results indicate that this method achieves a high degree of reproducibility and accuracy.

Clinical application

Figure 2 shows the distribution of plasma rilpivirine concentrations in 6 Japanese HIV-1 infected patients. Rilpivirine plasma concentrations were measured at trough level (14-22 h after orally administration). Mean rilpivirine plasma concentration was 49± 22 ng/ml (n=6, range : 23-90 ng/ml). Rilpivirine has been just approved at May 2012 in Japan. This result is the first rilpivirine concentration data for Japanese HIV-1 infected patients. These rilpivirine concentrations were similar to values reported by foreign healthy volunteers (12).
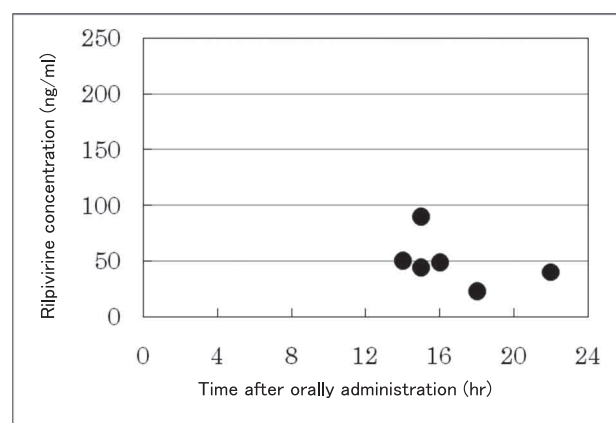

Figure 2.   Distribution of rilpivirine plasma concentrations in 6 Japanese HIV-1 infected patients.

DISCUSSION

In NNRTI-based regimens, efavirenz is recommended as an initial combination regimen for antiretroviral-naïve patients, because no regime has proven superior to efavirenz-based regimens with respect to virologic responses. However, efavirenz-based regimens are associated with rash and central nervous system adverse effects (1-3). Clinical trials of rilpivirine (TMC-278) have showed the same efficacy compared with efavirenz, with a slightly increased incidence of virological failures, but a more favourable safety and tolerability profile (10, 11). Therefore, rilpivirine can be an alternative NNRTI-based regimen for antiretroviral therapy-naive patients infected with HIV-1.

Rilpivirine is a substrate of CYP3A4 and its pharmacokinetics is likely to be modulated by inhibitors and inducers of these enzymes. To manage these drug interactions and ensure optimal drug efficacy, monitoring plasma rilpivirine concentrations is essential. For this purpose, we developed a method for determining plasma rilpivirine concentrations using LC-MS. The principal advantages of our method are rapid liquid-liquid drug extraction from plasma and use of an available IS, a commercial compound. Validation showed our method was successful in measuring plasma rilpivirine with high precision and satisfactory RSD values. The rilpivirine calibration curve was linear at the concentration range of 18 to 715 ng/ml, and the average accuracy ranged from 100.0 to 100.6%. Both inter- and intraday RSDs for rilpivirine were less than 3.3%, which is similar to previously reported values by LC-MS/MS (8). Recovery of rilpivirine was more than 82.0%. These results indicate our newly developed method achieves the same level of reproducibility and accuracy as the LC-MS/MS method. As plasma concentrations of rilpivirine are expected in the 67 to 204 ng/ml range when rilpivirine is administered at single dose of 25 mg for healthy volunteers (12), our method successfully covers this region with good precision and accuracy. In clinical practice, mean rilpivirine plasma concentration

at trough was 49 ng/ml. This level compared favourably with trough concentrations of about 50-80 ng/ml seen in ECHO and THRIVE trials (10, 11, 13).

In conclusion, our LC-MS method provides a conventional, accurate and precise way to determine rilpivirine in human plasma. This method can be used in routine clinical application for HIV-1 infected patients, and permits management of drug interactions and toxicity for rilpivirine.

ACKNOWLEDGEMENTS

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