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

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【Background】

Rilpivirine is a second-generation non-nucleoside reverse transcriptase inhibitor that is high potent against both wild-type and drug-resistant HIV-1 strains. Consequently, rilpivirine is expected to treat therapy-experienced patients who failed to use current drugs due to the emergence of drug-resistant HIV mutants. 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 and loss of virologic response and possible resistance to rilpivirine. To avoid these risks, therapeutic drug monitoring of rilpivirine is essential. In this study, we intended to develop a conventional method for determining plasma rilpivirine concentrations and compare plasma rilpivirine concentrations of Japanese HIV-1 infected patients with that of foreign healthy volunteers.

【Material & Methods】

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). All other chemicals and solvents were of analytical grade.

We used a Waters Alliance 2695 HPLC and a Micromass ZQ-2000 MS, controlled with MassLynx version 4.0 software. Our method involves rapid liquid-liquid drug extraction from plasma and use of gradient elution on a reversed-phase C18 column. We recruited 34 Japanese HIV-1 infected patients who were treated with rilpivirine containing regimen at the National Hospital Organization Nagoya Medical Center, Japan. All patients had been administered with 75mg rilpivirine once daily in combination with other antiretrovirals.

【Results】

Figures 1A and B show selected-ion recording chromatograms obtained from a spiked plasma sample containing 143 ng/ml of rilpivirine and 177.5 ng/ml of the 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. There were no interfering peaks affecting quantification of rilpivirine in this chromatogram. 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 relative standard deviation (RSDs) calculated for rilpivirine in the inter- and intraday assays ranged from 0.8 to 3.3%, which are similar to values reported by LC-MS/MS method previously. Accuracies ranged from 100.0 to 100.6%. Recoveries from plasma ranged from 82.0 to 88.3%.

Figure 2 shows the distribution of plasma rilpivirine concentrations in 24 Japanese HIV-1 infected patients. In this study, mean rilpivirine plasma concentration for Japanese patients at trough was 58 ng/ml (n=18, range: 20-90 ng/ml). Mean rilpivirine concentration at peak was 126 ng/ml (n=6, range: 85-170 ng/ml). These levels were slightly higher than rilpivirine concentrations seen in foreign healthy volunteers' trials.

Table 1. Intraday and interday precision and accuracy for rilpivirine

	Intraday (n=5)		Interday (n=15)			
Expected (ng/ml)	Measured (ng/ml)	RSD (%)	Measured (ng/ml)	RSD (%)	Accuracy (%)	Recovery (%)
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

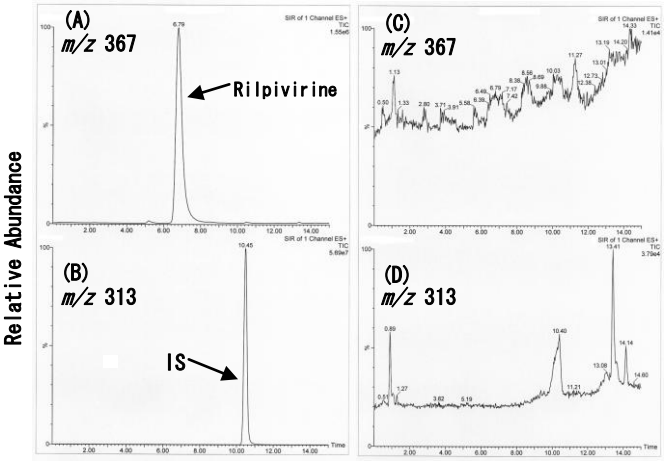

Figure 1. Selected-ion recording chromatograms for rilpivirine and the internal standard (IS). (A)and (B) were obtained from a spiked plasma containing 143 ng/ml of rilpivirine and 178 ng/ml of IS. (C) and (D) were obtained from a blank plasma sample. (A) and (C) were monitored with *m/z* 367. (B) and (D) were monitored with *m/z* 313. (C) and (D) are the expanded figures of the baselines in (A) and (B), respectively.

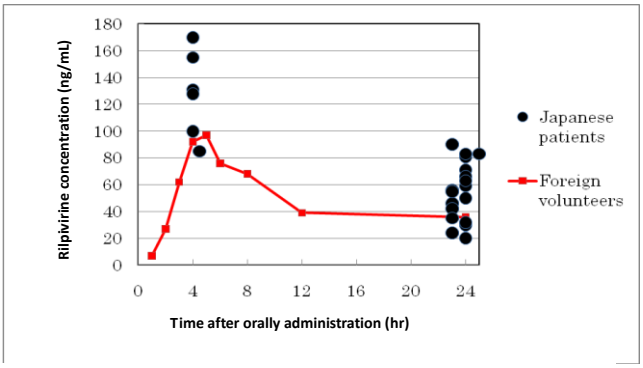

Figure 2. Distribution of plasma rilpivirine concentrations in 24 Japanese HIV-1 infected patients.

【Conclusions】

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 %. These results indicate our newly developed method achieves the same level of reproducibility and accuracy as the LC-MS/MS method. As the mean trough plasma concentration of rilpivirine was 79±35 ng/ml when rilpivirine was administered at rilpivirine 25 mg once daily for foreign HIV-1 infected patients, our method successfully covers this region with good precision and accuracy. In this study, Japanese mean rilpivirine plasma concentration at trough was 58 ng/ml. This level compared favourably with trough concentrations of about 50-80 ng/ml seen in ECHO and THRIVE trials. However, AUC of rilpivirine for Japanese HIV-1 infected patients slightly increased in comparison with foreign data. We think that it was caused by poor build of Japanese HIV-1 infected patients. 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.