

Therapeutic Drug Monitoring of Antiretroviral Drugs with HPLC-MS

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Abstract

Prospective and retrospective studies have provided some evidence of the clinical and virological benefit of incorporating TDM into routine patient care. Because antiretroviral therapy consists always of a combination of different drugs, analysis can be simplified if different drugs are measured at the same time. Therefore, LC-MS or LC-MS/MS is nowadays the analytical method of choice.

In 2003 we have published an LC-MS method [1] for the quantification of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir after solid-phase extraction. In the meantime atazanavir and tipranavir have been introduced into the market. In the process of including them in our analytical procedure we have reduced the sample volume, simplified sample preparation and shortened the chromatographic run time.

Sample preparation consists now in the addition of a solution of the internal standard (protease inhibitor analogue) in a mixture of methanol, 0.1M ZnSO₄ and acetonitrile to 100 µl serum which results in protein precipitation. After centrifugation the supernatant is diluted with buffer before injection into the HPLC system. The different drugs are analyzed by reversed-phase chromatography and detected by negative or positive atmospheric pressure chemical ionization (APCI) mass spectrometry.

Depending on the target concentrations in patients, the calibration curves of the new method are linear in the range of 0.01 – 30.0 mg/l. The limit of quantification is accordingly between 0.01 and 0.3 mg/l. The imprecision is < 10% and the accuracy 92 – 108%. The absence of ion suppression has been demonstrated. The performance data of the new and simplified method are comparable or even better to the published numbers (1) and demonstrate that this method allows the quantification of 10 different protease inhibitors or non-nucleoside reverse transcriptase inhibitors in patients with HIV infection.

1. Introduction

Prospective and retrospective studies have provided some evidence of the clinical and virological benefit of incorporating TDM into routine patient care but still large-scale prospective studies showing that TDM is a useful tool to improve the management of all HIV-infected subjects who start an antiretroviral regime are lacking. In Europe several countries have TDM included into the national HIV treatment guidelines (the United Kingdom, the Netherlands, France and Italy). The bases for using TDM with today's knowledge are data demonstrating considerable inter-individual variability in the concentration of antiretroviral drugs among patients and data demonstrating relationships between drug concentrations and responses, either virological or toxicological.

Because antiretroviral therapy consists always of a combination of different drugs, analysis can be simplified if different drugs are measured at the same

time. Therefore, LC-MS or LC-MS/MS is nowadays the analytical method of choice. Due to the high specificity of the mass spectrometric detection the times of analysis can be shortened and the risk of interferences can be minimized.

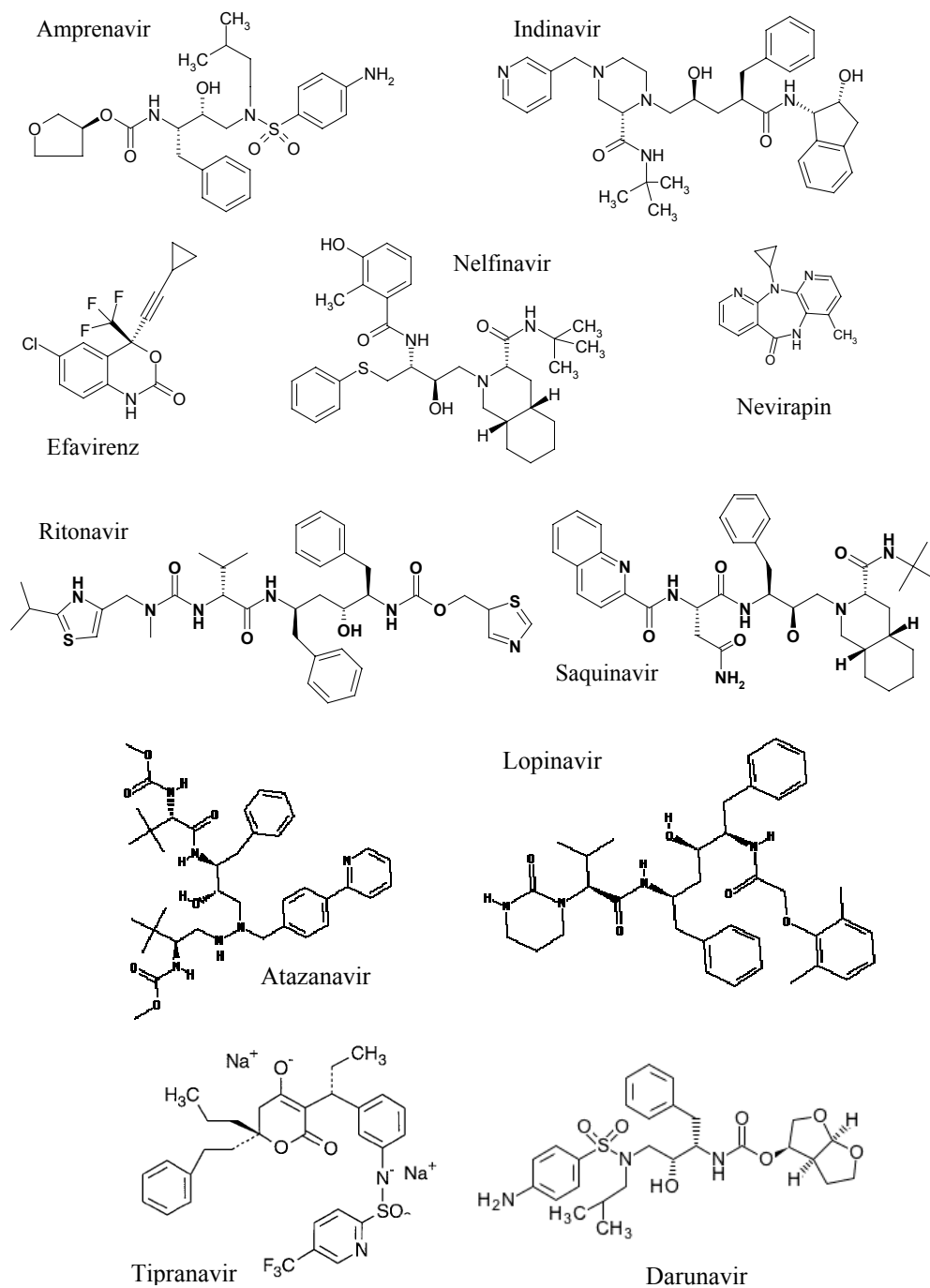


Fig. 1: Chemical structure of the different antiretroviral drugs analysed

Due to the pharmacological properties of the different drug classes TDM in plasma samples is only reasonable for the proteinase inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The nucleoside reverse transcriptase inhibitors need intracellular metabolic activation implicating that the intracellular metabolites need to be quantified for optimisation of the therapy. The chemical structures of the different compounds included in our method are depicted in Figure 1.

In 2003 we published an analytical method for the antiretroviral drugs which have been available at that time using solid-phase extraction [1]. In the meantime new antiretroviral drugs have been introduced into the clinics and have periodically been introduced in our procedure. But tipranavir having a sulphonamide like structure could not be extracted with the established method. Therefore, we optimized the method including all antiretroviral drugs available in Switzerland in one extraction step.

2. Methods

The chromatographic separation was performed with a C18 column (125 x 2 mm) and the mobile phase consisted of acetonitrile, methanol and ammonium carbonate buffer with a pH of 9.3. The gradients were adapted in order to shorten the time of analysis and in addition to the gradient of the mobile phases a flow rate gradient was introduced. The mass spectrometry was performed on a LCQ Deca (Thermo, San José, USA) using atmospheric pressure chemical ionization in the negative as well the positive mode.

In order to evaluate the presence of ion suppression blank plasma samples have been injected with a concomitant continuous infusion of a mixture of all antiretroviral drugs. The absence of negative peaks demonstrates the absence of ion suppression.

3. Results

The chromatograms of a spiked plasma sample is shown in Figure 1. The ion suppression experiments demonstrated the absence of ion suppression. The accuracy and precision of the new method have been compared with the established procedure. Despite the much simpler sample preparation the results were comparable or even better (Figure 2).

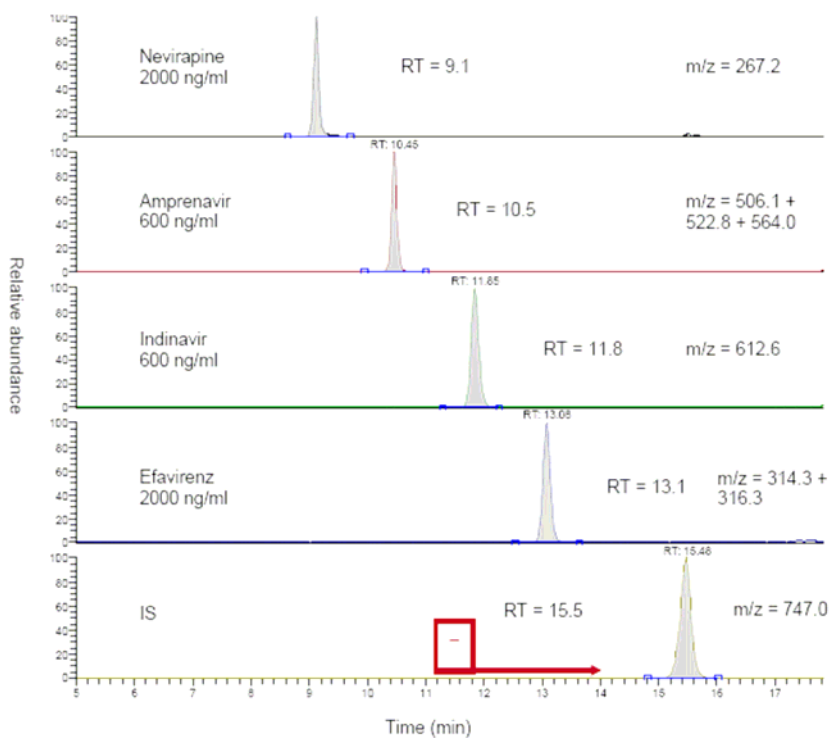
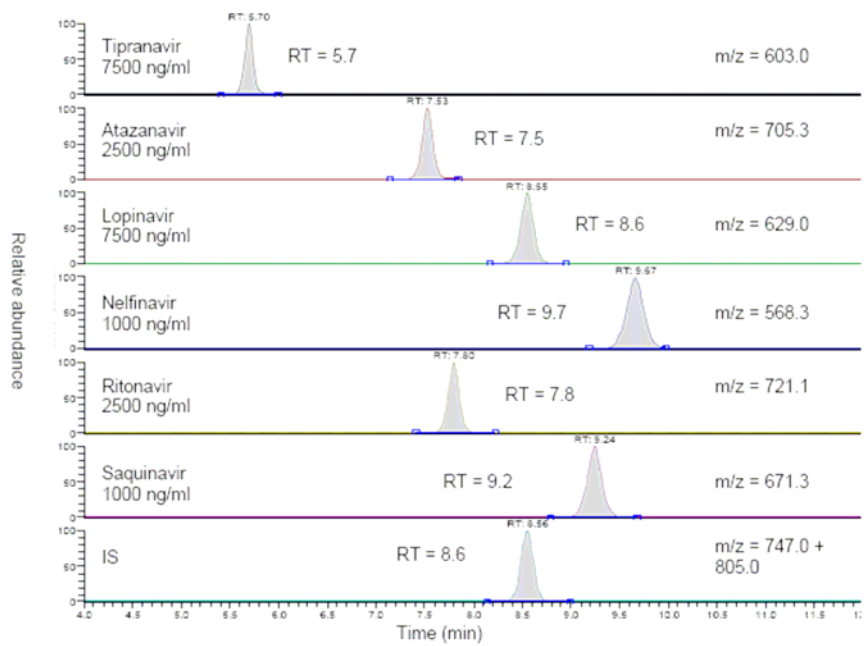


Fig. 1: Chromatograms of a spiked plasma sample containing all compounds analysed (RT = retention time)

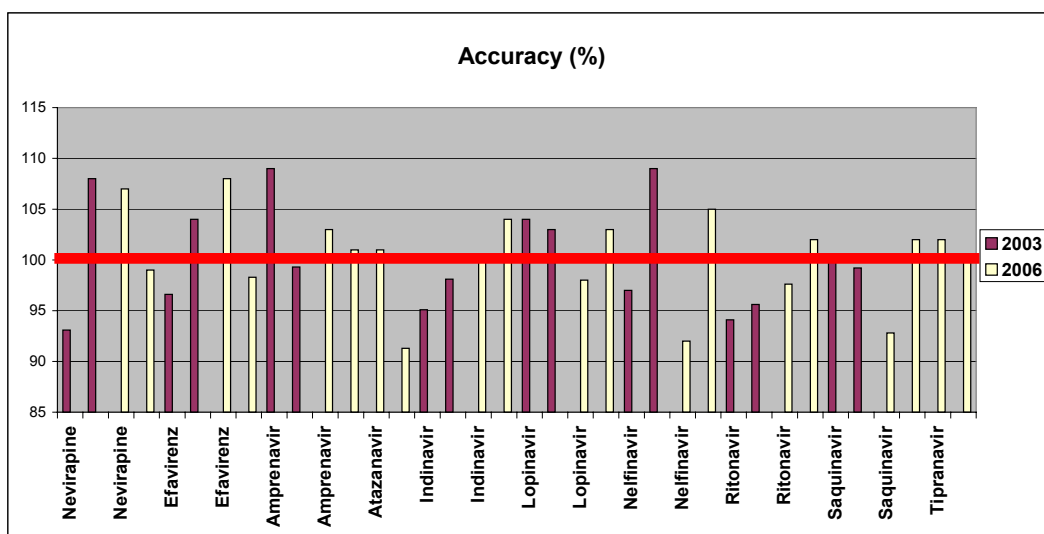
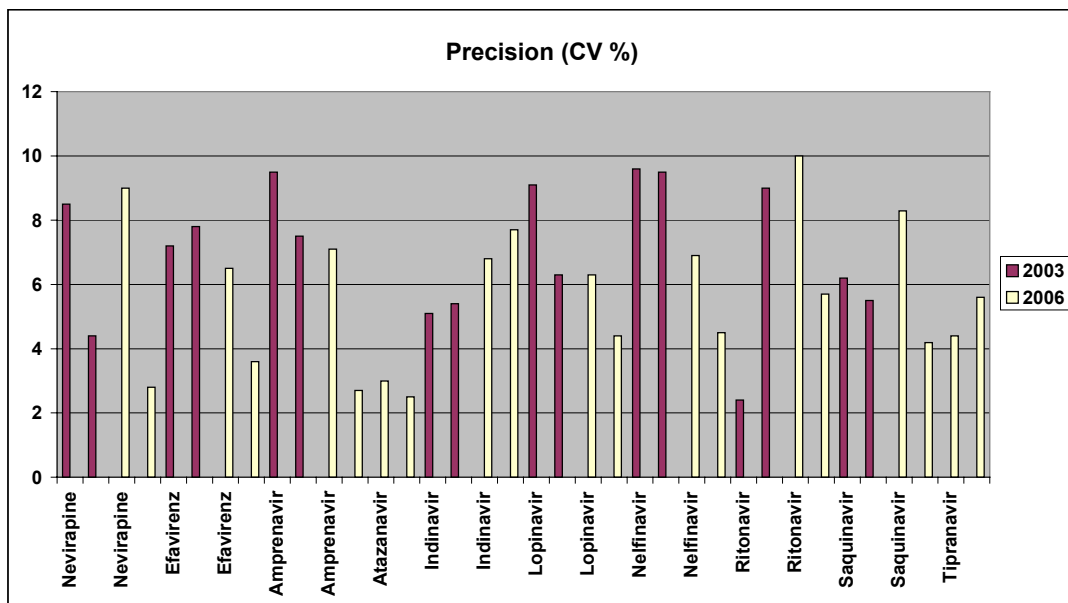


Fig. 2: Precision and accuracy data

4. Conclusions

The new HPLC-MS method for the quantification of all NNRTIs and PIs available in Switzerland is less laborious, needs less instrument time and is cheaper concerning consumables. Nevertheless it is as precise, as accurate and as robust as the old method.

5. Literature

- [1] Rentsch, KM (2003) Sensitive and specific determination of eight antiretroviral agents in plasma by high-performance liquid chromatography-mass spectrometry J Chromatogr B Analyt Technol Biomed Life Sci 788: 339-350.

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