Simultaneous determination of lopinavir, saquinavir and ritonavir in human plasma using liquid chromatography–ion trap mass spectrometry

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INTRODUCTION

Lopinavir, **saquinavir**, and **ritonavir** represent inhibitors of human immunodeficiency (HIV) protease, used as components of the highly active antiretroviral therapy (HAART). Recently, it has been suggested that protease inhibitors developed for HIV treatment could be also effective in SARS-CoV-2 infection. Not all HIV-infected patients experience an optimal response to the antiretroviral treatment, and some of those non-responders do not show phenotype- or genotype-related resistance, implicating that systemic exposure to the drug is insufficient to suppress virial replication. This may result from specific pharmacokinetics in the individual subject and the extent to which the patient follows the prescribed therapy. Such information is very important in clinical practice. An optimal way to address it is to evaluate blood concentration of the drug and to conduct therapeutic drug monitoring.

We present here an LC/MS procedure that uses cheapest and most accessible ion trap mass detector that allows simultaneous analysis of three most commonly used protease inhibitor antiviral drugs: lopinavir, saquinavir, and ritonavir. This procedure could be adopted for high throughput method in routine therapeutic drug monitoring (TDM).

RESULTS - Analysis of the control human and patient plasma extracts

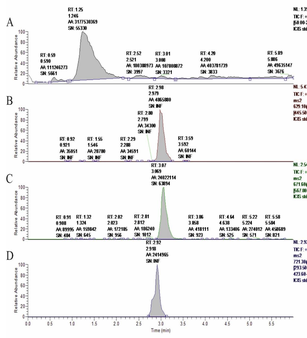

FIG. 1. LC/MS chromatograms for standards of protease inhibitors in MS2 (SRM) mode: A. Total Ion Current. B. lopinavir, C. saquinavir, D. ritonavir.
Samples of human plasma were protein precipitated with 0.3 M zinc sulphate in water/methanol solution (30:70, v/v) and analyzed with reversed-phase chromatography connected to ESI ion source of ion trap mass detector LCQ Advantage Thermo Finnigan operating in MS and MS/MS modes. The response of chromatographic peak areas for chromatograms extracted for selected ions was linear within concentration range between 0.01 and 10 µg/mL. Recoveries of drug standards added to plasma were >90% (TABLE 1).

CONCLUSIONS

- The developed LC/MS procedure emplying basic and most accessible ion-trap LC/MS system could be used for simultaneous selective, rapid and precise determination of three antiviral protease inhibitors and can be adopted for high throughput analyses in routine therapeutic drug monitoring

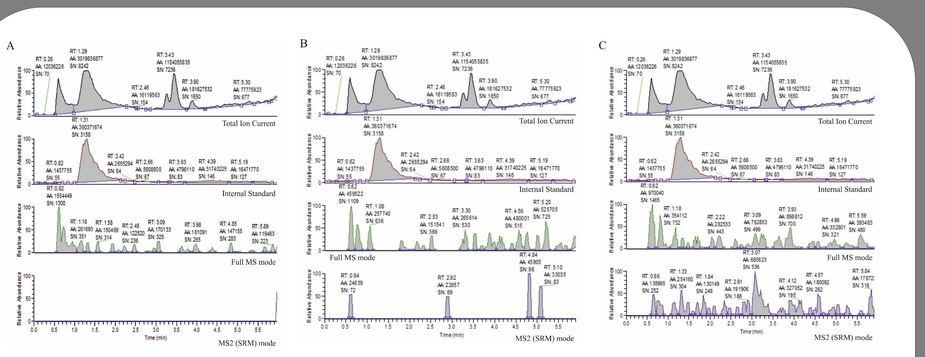

FIG. 2. LC/MS chromatograms for plasma extract of a control subject. Ion chromatograms for: A. lopinavir, B. saquinavir, and C. ritonavir. Each chromatogram consists of: Total Ion Current, Internal standard, Full MS mode, and MS2 (SRM) mode.

RESULTS - Preparation of standards and calibration curve

FIG.2 presents chromatograms from the analysis of the control human plasma extract from an individual non-treated with the analyzed therapeutics. **FIG.3** with representative chromatograms/reports for treated patients plasma extracts analyses demonstrates that the analyzed drugs, if present, form clear peaks.

Our method, although applied for monitoring of just three compounds, can be suitable for monitoring of other drugs in the same sample. Low sensitivity preliminary data could be obtained from full MS trace of already recorded chromatograms while more accurate determination would require extending selective ion monitoring/fragmentation mode and re-run of the separation.

Several previously described analytical methods HPLC for evaluation of the plasma levels of antiretroviral drugs were set only for individual compound, or for more than one but with different extraction procedures and chromatographic conditions for each compound. That type of analytical procedure is expensive and time consuming. Some other methods used liquid chromatography but with different detection modes. Our procedure seems to be much less expensive due to implementation of Ion Trap detector type. While most of the procedures available use solid phase extraction for preparing material, here, methanol/zinc sulphate simple precipitation was used for the drug determination. Finally, the total run time of 6 min is significantly shorter than in many others assays. Then the established method is suitable for routine analyses of large number of samples in a very short time.

300 ng	**lopinavir**	**ritonavir**	**saquinavir**
Av	288.2	280.1	295.7
SD	16.79	24.35	15.32
CV	0.058	0.086	0.051
Rec	96%	93%	98%
30 ng	**lopinavir**	**ritonavir**	**saquinavir**
Av	28.14	27.24	29.02
SD	0.78	1.46	2.1
CV	0.027	0.05	0.071
Rec	93%	90%	96%
3ng	**lopinavir**	**ritonavir**	**saquinavir**
Av	1.78	2.22	2.15
SD	0.45	0.59	0.32
CV	0.25	0.26	0.14
Rec	59%	74%	71%

Table 1: Reproducibility and recovery data from patients plasma extracts analysis at three different concentrations of each drug (3, 0.3 and 0.03 µg/mL). Av: average, SD: standard deviation, CV: coefficient of variation, Rec: percentage of recovery.

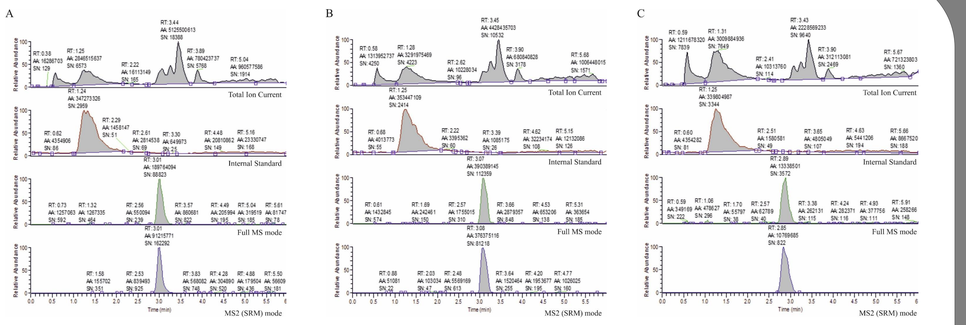

FIG. 3. LC/MS chromatograms for plasma extract from a patient treated with: A. lopinavir, B. saquinavir, and C. ritonavir. Each chromatogram consists of: Total Ion Current, Internal standard, Full MS mode, and MS2 (SRM) mode.