The Association for Mass Spectrometry: Applications to the Clinical Lab

Poster # 21h

# Development and Validation of the First UHPLC-MS/MS Method for the Quantification of the New Anti-Ebola Drug Remdesivir: application to healthy volunteers.

*Authors:* **Valeria Avataneo 1,** Amedeo De Nicolò 1, Miriam Antonucci 1, Elisa De Vivo 1, Jessica Cusato 1, Mohammed Lamorde 2, Giovanni Di Perri 1 and **Antonio D'Avolio 1,3.**

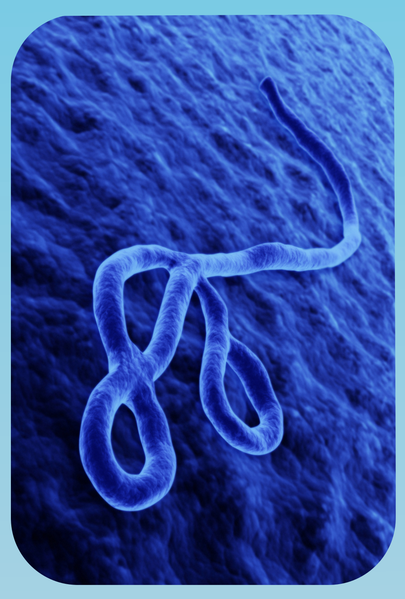
*Affiliations:* 1-Laboratory of Clinical Pharmacology and Pharmacogenetics, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy.
2-Infectious Diseases Institute, Makerere University College of Health Sciences, P.O. Box 22418, Kampala, Uganda.
3-CoQua Lab s.r.l., Turin, Italy.

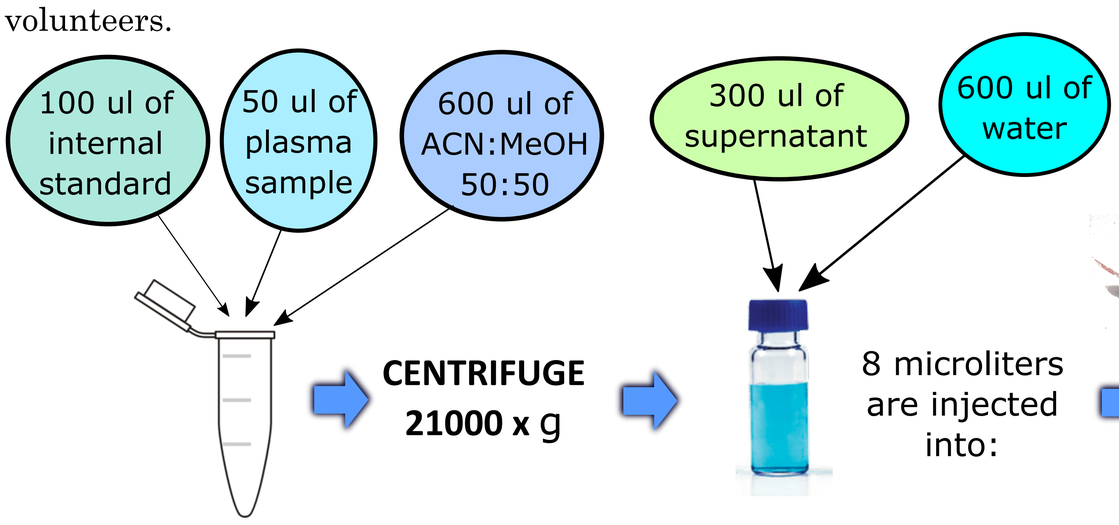
## INTRODUCTION and AIM

Ebola virus disease shows a very high death rate (up to 90%) and the 2014-16 outbreak has been one of the deadliest since 1976, year in which ebola virus was identified. Nevertheless, up to now, any effective pharmacological treatment has been discovered. Some molecules are under study and, among all, remdesivir (RDV) revealed really promising and is now on fase II/III studies. Unfortunately, detailed information about RDV pharmacokinetics are still lacking and no methods for its quantification in patient's plasma have been reported in literature.

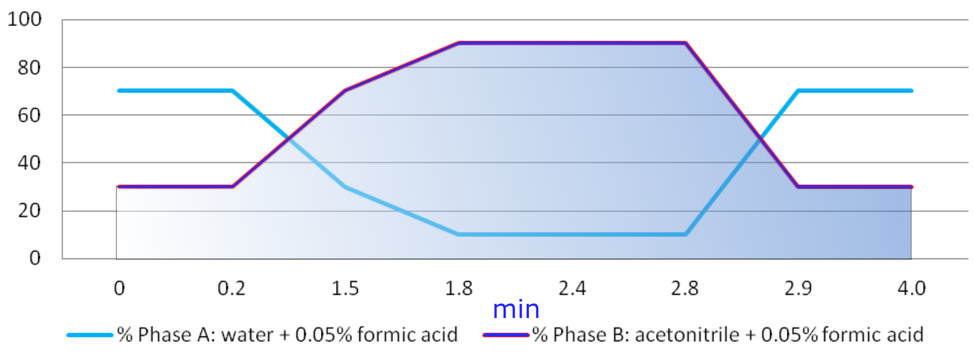
The aim of this work is the development and validation of a method for the Therapeutic Drug Monitoring (TDM) of RDV using liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), in order to describe its pharmacokinetics in healthy volunteers.

## MATERIALS and METHODS






Internal standard: a mixture of 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline (QX) and tenofovir-alafenamide (TAF).
Analytical column: HSS T3 1.8µm 2.1x50 mm

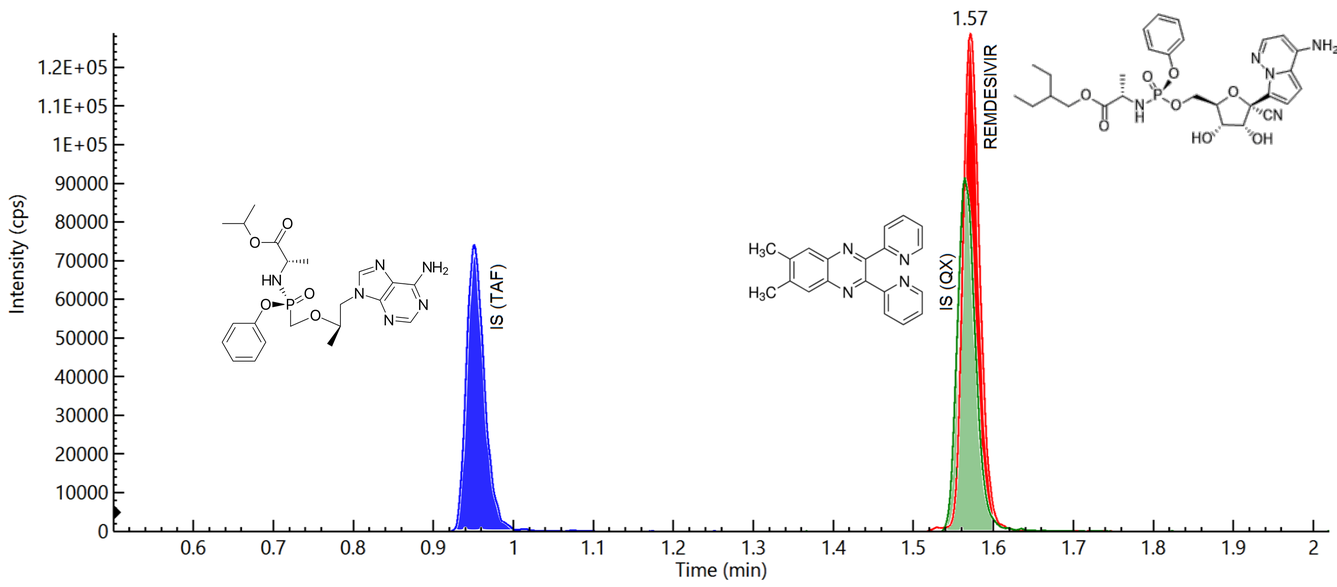



| ESI + | Quantification traces (m/z) |
|---|---|
| RDV | 603.15>200 |
| IS-QX | 313.2>78.05 |
| IS-TAF | 477.20>346.05 |

Liquid Chromatography: Perkin Elmer LX-50
Tandem Mass Spectrometry: Perkin Elmer Triple Quadrupole QSight 220

## RESULTS

All analytes are successfully retained by the column: TAF retention time is 0.95 min (column "dead-time" is 0.32 min) while QX and RDV co-elute at 1.57 min. Preliminary data are very encouraging: for remdesivir, recovery (REC) resulted among 70 and 80%, matrix effect (ME) ranged among -1 and +9%, extraction efficiency (EE) was approximately 73%; accuracy and precision data are also within the limits indicated by the guidelines. RDV calibration curves resulted linear (r2 > 0.996) with 1/X weighting. Stability tests indicated that RDV is very stable for 30 days at -80°C in plasma, for 24 hours in the autosampler (after extraction, at 10°C) and at -20°C in plasma, it is only quite stable at +4°C in plasma and, on the contrary, it is absolutely not stable at room temperature and at 37°C in plasma.




$$IS-nME\% = \left[\left(\frac{Response\ in\ matrix}{Response\ in\ neat}\right) - 1\right] * 100 = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}}\right) - 1\right] * 100$$

| | ACCURACY | IMPRECISION RSD% | | MEAN RECOVERY | MEAN EXTRACTION EFFICIENCY | MEAN MATRIX EFFECT | MEAN IS-NORMALIZED MATRIX EFFECT |
|---|---|---|---|---|---|---|---|
| | | INTRA-DAY | INTER-DAY | (RSD%) | (RSD%) | (RSD%) | (RSD%) |
| HIGH QC LEVEL | 108% | 3% | 4% | 81% | 76% | +7% | +1% |
| | | | | (11) | (8) | (9) | (9) |
| MEDIUM QC LEVEL | 95% | 3% | 4% | 68% | 64% | -1% | +1% |
| | | | | (14) | (11) | (8) | (7) |
| LOW QC LEVEL | 94% | 9% | 15% | 75% | 71% | +9% | +6% |
| | | | | (11) | (14) | (12) | (14) |

| % of degradation | 24h RT | 24h 37°C | 24h 4°C | 24h AUTOSAMPLER (10°C) | 24h -20°C | 2° freeze-and-thaw | 3° freeze-and-thaw | 30 days -80°C |
|---|---|---|---|---|---|---|---|---|
| **H** (800 ng/mL) | 93 | 99 | 17 | 0 | 0 | 0 | 8 | 0 |
| **M** (100 ng/mL) | 95 | 100 | 17 | 4 | 0 | 0 | 12 | 8 |
| **L** (10 ng/mL) | 100 | 100 | 22 | 7 | 2 | 0 | 14 | 16 |

## CONCLUSIONS

This method is currently being validated according to FDA and EMA guidelines, and results the first for RDV quantification. Its main features are the very short analytical run (4 min) and the very small amount of plasma required (50µl). Precipitation and strong dilution of samples (45-folds) contribute to a low instrumental contamination and low matrix effect.
The validated method will be now applied to real samples from healthy patients, enrolled in the CAPA-CT-II study, all giving informed consent, and then published.

SCAN ME

## ACKNOWLEDGMENTS

*This study was supported by the CAPA-CT-II funds by EDCTP.*

## CONTACTS

**Prof. Antonio D'Avolio: antonio.davolio@unito.it**
**Dr. Valeria Avataneo: valeria.avataneo@unito.it**
**Website: www.tdm-torino.org**