Quantitative analysis of antiretroviral drugs by MALDI-TOF mass spectrometry

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INTRODUCTION

We aim to develop a fast, sensitive and reliable assay for quantitative analysis of antiretroviral drugs. MALDI-TOF mass spectrometry was chosen for our assay, because it is able to analyze compounds at the femtomole level in high throughput mode and is relative tolerant towards contaminants. However, reliable quantitative analysis by MALDI-TOF mass spectrometry remains a point of discussion.[1] In our experience, MALDI-TOF mass spectrometry can be used for reliable quantitative analysis of HIV-1 protease inhibitors by use of an internal standard and preparation of homogeneous matrix / sample crystals.

We used the cationizing matrix meso-Tetrakis-(pentafluorophenyl)porphyrin (F20TPP, MW 974.6 Da) because of its limited interference in the low mass range.[2] Homogeneous matrix / sample crystals were obtained by preparing a thin matrix layer on an AnchorChip™ target plate and subsequent sample deposition on the matrix crystals. F20TPP in combination with various alkali halides (lithium-, sodium-, potassium-, rubidium-, and cesium iodide) were tested for the sensitivity to cationize the HIV-1 protease inhibitors nelfinavir (MW 567.8 Da), indinavir (MW 613.8 Da), lopinavir (MW 628.8 Da), saquinavir (MW 670.9 Da) and ritonavir (MW 721.0 Da). A calibration curve of lopinavir was obtained using indinavir as internal standard. The intrarun precision and accuracy were compared to the criteria of the FDA on Bioanalytical Method Validation.[3]

METHODS

Matrices: 10 mg/mL F20TPP with 19.2 mM LiI, NaI, KI, RbI or CsI (in 100% acetone for LiI and NaI. For KI, RbI and CsI in 80% acetone and 20% MeOH). 10 mg/mL F20TPP with 3.84 mM LiI, NaI, KI, RbI and CsI in 83% acetone and 17% MeOH. Thin matrix layers were prepared on a 800 µm AnchorChip™ target plate (Bruker Daltonics, Germany) by stroking a pipette tip filled with 10 µL matrix solution on one row (24 anchors).

Samples: Pure lopinavir (LPV) and ritonavir (RTV), indinavir (IDV), nelfinavir (NFV), and saquinavir (SQV) were kindly provided by Abbott Laboratories, Merck Sharp & Dohme, Pfizer Inc. and F. Hoffmann-La Roche, respectively. Drugs were dissolved in 100% EtOH or 50% EtOH. Samples (0.5 µL) were deposited on the thin layer matrix crystals. The matrix crystals did not dissolve by the EtOH or water in the samples.

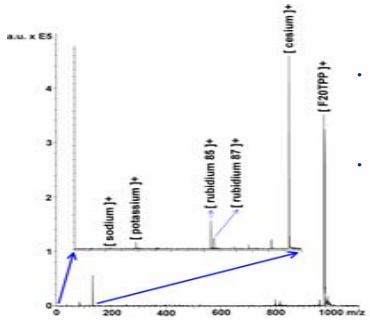
Mass spectrometry: Samples were automatically measured on a MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) in the positive reflectron mode (1000 shots). Laser power and movement were kept constant for all samples. Raw mass spectra were processed with FlexAnalysis™ v2.2 (Bruker Daltonics, Germany) and MS Integrity™ v1.0 (Cerno Bioscience, USA).

FDA definitions: Precision is defined as the closeness of individual measures of an analyte when the procedure is applied to multiple replicates of the same sample. Precision is expressed as the % of coefficient of variation (% CV). Accuracy is defined as the closeness of the mean measured concentration of an analyte to the true concentration of the analyte. Accuracy is expressed as % deviation (bias) of the measured concentration to the true concentration.

FDA criteria for calibration curve: The Lower Limit of Quantification (LLOQ) is the lowest standard on the calibration curve that meets the following conditions: 1] Analyte response is at least 5 times the blank response. 2] Precision is ≤ 20%. 3] Accuracy expressed as bias is ≤ 20%. Other standards than the LLOQ on the calibration curve should meet the following criteria: 1] Precision is ≤ 15%. 2] Accuracy expressed as bias is ≤ 15%.

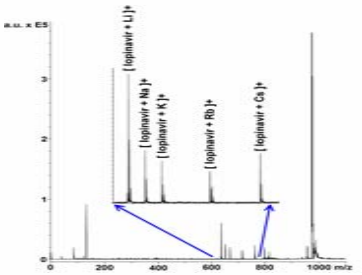
RESULTS

Matrix



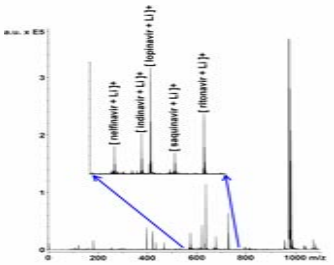
- Matrix: 10 mg/mL F20TPP with 3.84 mM LiI, NaI, KI, RbI and CsI.
- Free lithium+ is not detected.

Detection of lopinavir



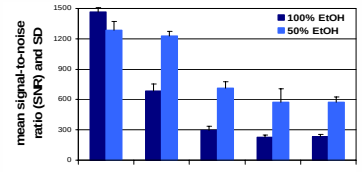
- Sample: 500 femtomole (fmol) lopinavir in 50% EtOH.
- Matrix: 10 mg/mL F20TPP with 3.84 mM LiI, NaI, KI, RbI and CsI.

Detection of nelfinavir, indinavir, lopinavir, saquinavir and ritonavir



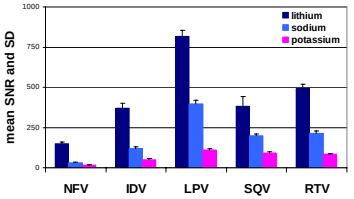
- Sample: 100 femtomole NFV, IDV, LPV, SQV and RTV in 50% EtOH.
- Matrix: 10 mg/mL F20TPP with 19.2mM LiI.

Cationization of lopinavir



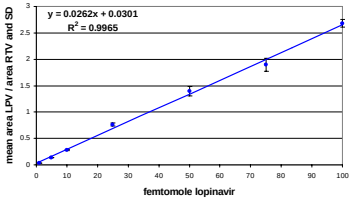
- Sample: 500 femtomole lopinavir in 100% EtOH or 50% EtOH.
- Matrix: 10 mg/mL F20TPP with 19.2 mM LiI or NaI or KI or RbI or CsI.
- Replicates: Six per matrix.

Cationization of NFV, IDV, LPV, SQV and RTV



- Sample: 100 fmol NFV, IDV, LPV, SQV and RTV in 50% EtOH.
- Matrix: 10 mg/mL F20TPP with 19.2 mM LiI or NaI or KI.
- Replicates: Six per matrix.

Lopinavir calibration curve



	FDA precision (% CV)		FDA accuracy (% bias)	
fmol LPV	FlexAnalysis™	MS Integrity™	FlexAnalysis™	MS Integrity™
100	5.7	5.6	- 1.5	1.9
75	10.3	13.2	5.0	7.6
50	13.0	12.9	- 3.0	- 1.6
25	9.4	7.7	- 11.1	- 11.0
10	6.3	7.8	8.4	0.6
5	9.8	7.2	15.9	6.4
1	32.7	16.5	37.7	- 3.5

- Sample: different concentrations of lopinavir spiked with 100 femtomole indinavir in 50% EtOH.
- Matrix: 10 mg/mL F20TPP with 19.2 mM LiI.
- Replicates: Five per lopinavir concentration.
- In general, precision and accuracy were better when raw data were processed with MS Integrity™ rather than FlexAnalysis™.
- According to the FDA criteria the LLOQ was 1 femtomole when MS Integrity™ was used and 5 femtomole when FlexAnalysis™ was used.
- The mean SNR of 1 femtomole LPV was 14.4 with a CV of 16.6%.

CONCLUSIONS

- F20TPP is a suitable matrix for analysis of HIV-1 protease inhibitors with MALDI-TOF mass spectrometry.
- Best sensitivity is obtained when F20TPP is used in combination with lithium iodide.
- The addition of water to samples has a profound effect on the SNR of lopinavir cationized by various alkali metals.
- MALDI-TOF mass spectrometry can be used for accurate, precise and sensitive quantitative analysis of lopinavir.
- The type of software used to process raw mass spectra has a profound effect on the precision and accuracy of the lopinavir calibration curve.
- The value of MALDI-TOF mass spectrometry for quantitative drug analysis in clinical studies is being investigated.

REFERENCES

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2. Ayorinde FO, Hambright P, Porter TN, Keith QL, Jr. Use of meso- tetrakis(pentafluorophenyl)porphyrin as a matrix for low molecular weight alkylphenol ethoxylates in laser desorption/ ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 1999;13(24):2474-9.
3. US Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: Bioanalytical Method Validation. 2001. Available: www.fda.gov/cder/guidance/4252fnl.htm.

ACKNOWLEDGEMENTS

- Aids Fonds, The Netherlands (project number 2004051) and VIRGO for financial support.
- Cerno Bioscience for data processing with MS Integrity™.
- Abbott Laboratories for supply of lopinavir and ritonavir.
- Merck Sharp & Dohme for supply of indinavir.
- F. Hoffmann–La Roche for supply of saquinavir.
- Pfizer Inc. for supply of nelfinavir.