The determination of human active renin and prorenin by LC-MS: the impact of injection solvent composition and material on precision and signal intensity

Ilja Burdman, Bjoern B. Burckhardt

Heinrich Heine University, Institute of Clinical Pharmacy and Pharmacotherapy, Dusseldorf, Germany

Background

Representing the extended renin-angiotensin-aldosterone system, active renin accompanied by its precursor prorenin plays a crucial role in physiological as well as pathological health states. In clinical analysis, active renin is used for diagnosis of hyperaldosteronism which is characterized by low picogram per milliliter concentrations and might be affected by inaccurate measurement due to cross-reactivity of immunoassays with catalytical active prorenin. LC-MS is a reliable and selective alternative. Currently, no reliable LC-MS method is available for clinical analysis of active renin and prorenin. Subsequently, a hybrid assay was developed incorporating proteolytical digestion. However, in very low concentrations, the analysis suffers from poor repeatability affecting the precision of the assay. Since unspecific peptide adsorption has been identified as a major concern for this imprecision, a systematic investigation of the impact of the injection solvent composition and applied material on the reliable quantification of active renin and prorenin was performed.

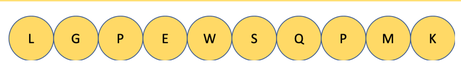


Fig. 1 Pro-part signature peptide of pro-segment

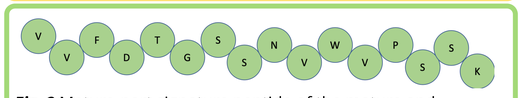


Fig. 2 Mature-part signature peptide of the mature and active renin

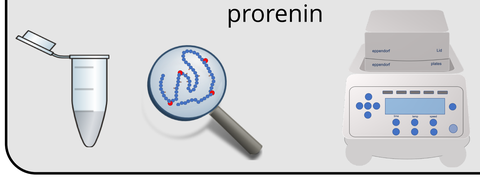
Objective

Improvement of precision and signal intensity for pro-part and mature-part signature peptides by avoiding unspecific adsorption and solubility issues.

Methods

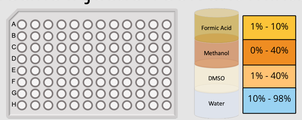
(1)

Proteolytical digestion of active renin and prorenin



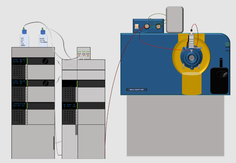
(2)

Evaporation of the digestion buffer and reconstitution of the dried samples in the specific injection solvent mixture



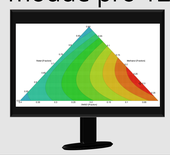
(3)

LC-HRMS aquisition



(4)

D-optimal evaluation by Umetrics modde pro 12



Results

Goodness of fit

Pro-part signature peptide	Eppendorf® Protein LowBind	Waters® regular	Waters® Quan Recovery	Brand®	Greiner®
R^2	0.76	0.51	0.77	0.94	0.93
Q^2	0.68	0.35	0.74	0.90	0.88
Repeatability	0.78	0.72	0.72	0.95	0.96

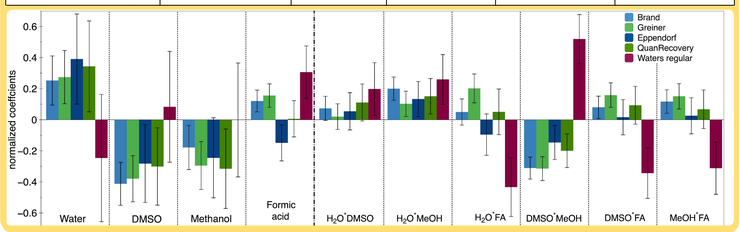


Fig. 3 Coefficient effect on adsorption and solubility of the pro-part signature peptide influenced by water (H_2O); dimethylsulfoxide (DMSO); methanol (MeOH); formic acid (FA).

Mature-part signature peptide	Eppendorf® Protein LowBind	Waters® regular	Waters® Quan Recovery	Brand®	Greiner®
R^2	0.86	0.87	0.88	0.91	0,95
Q^2	0.80	0.84	0.91	0.87	0.94
Repeatability	0.89	0.95	0.92	0.97	0.97

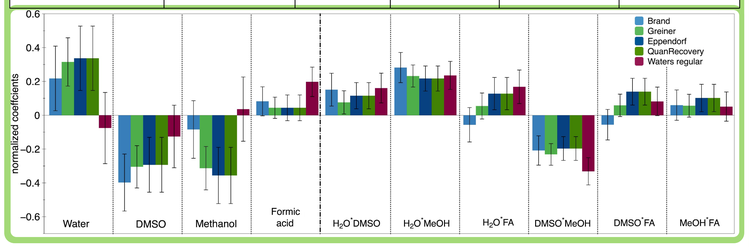


Fig. 4 Coefficient effect on adsorption and solubility of the mature-part signature peptide influenced by water (H_2O); dimethylsulfoxide (DMSO); methanol (MeOH); formic acid (FA). *Confidence interval 95% (Student's t-test)*

DoE optimized mixtures

Pro-part signature peptide

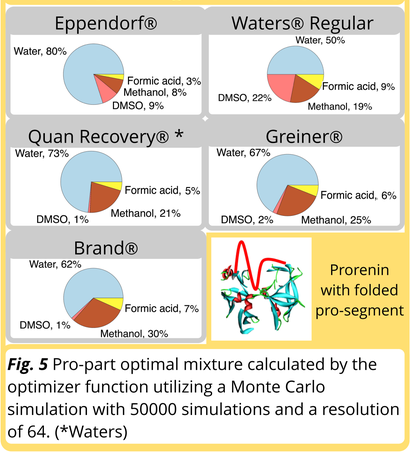


Fig. 5 Pro-part optimal mixture calculated by the optimizer function utilizing a Monte Carlo simulation with 50000 simulations and a resolution of 64. (*Waters)

Mature-part signature peptide

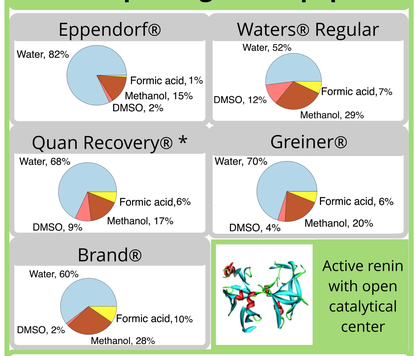


Fig. 6 Mature-part optimal mixture calculated by the optimizer function utilizing a Monte Carlo simulation with 50000 simulations and a resolution of 64. (*Waters)

LC-HRMS of optimized mixtures

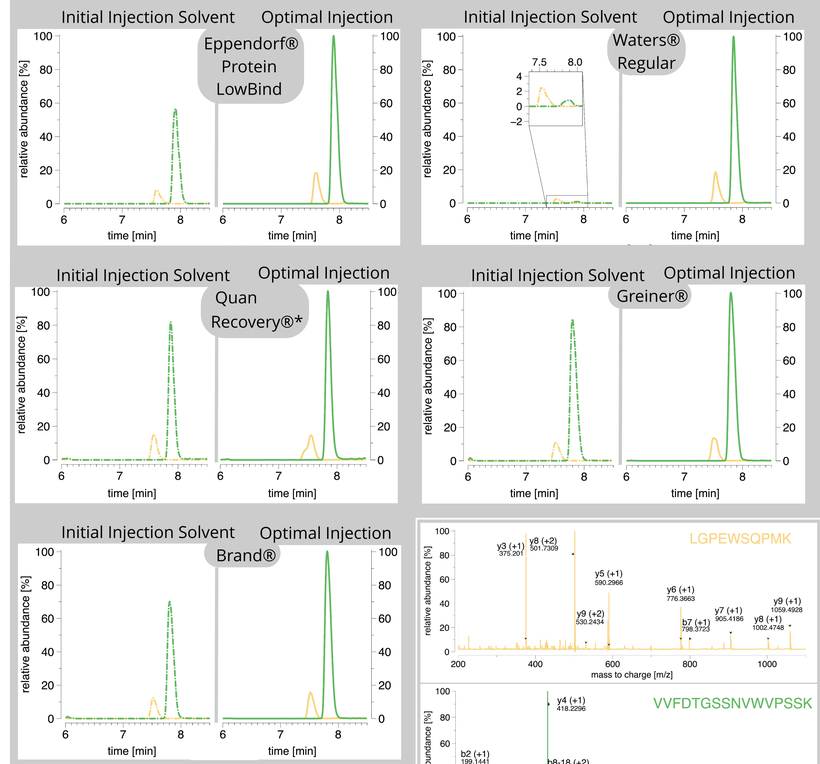


Fig. 7 Comparison between initial injection solvent mixture (left side, dotted chromatogram) and Design of Experiments optimized injection solvent mixture (right side) on Eppendorf protein low binding, Waters regular and QuanRecovery, Greiner and Brand 96 well plates. The yellow line: pro-part signature peptide;
the green line: mature-part signature peptide. (*Waters)

Fig. 8 High resolution product ion scan of pro-part and mature-part signature peptides with typical y- and b-fragments. Pro-part signature peptide is unique for prorenin and mature-part signature peptide is part of both proteins.

Conclusion

The most suitable material and optimal injection solvent composition made endogenous human active renin and prorenin detectable on the LC-MS system by a substantial gain in sensitivity. Besides the higher intensity, a robust precision accomplished the pre-validation for a hybrid immunocapture LC-MS assay. After a full validation, active renin and prorenin will be accessible for clinical application.

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Contact

ilja.burdman@hhu.de