CHAPTER V
CONCLUSIONS

An accurate and precise simultaneous LC-MS method was developed and validated for the qualitative and quantitative assessment of eight potential small organic acids in plasma and urine. This method will provide an important clinical tool for studying the pathogenesis of acidosis in patients with severe malaria and other conditions associated with acidosis. The method was shown to be sensitive, reproducible and suitable for small volumes of plasma or urine (100 µL). The method utilized SPE in a 96-well format, which permits high-throughput processing and automation of routine analysis of clinical samples. The presented LC-MS method in this study provides an accurate tool for the identification and quantification of unknown acids in patients with severe malaria.

Clinical applicability and relevance was demonstrated and discussed by analysing samples from three sample groups: severe malaria, uncomplicated malaria and healthy control. Four of the eight acids (LA, aHBA, bHBA and pHPLA) were found as potential acids for acidosis in severe malaria. Seven of the eight acids (LA, aHBA, bHBA , pHPLA, MMA, EMA and aKGA) were found in the urine of patients but they could not be considered as potential acids for acidosis in severe malaria. Furthermore, only aKGA could not be found in healthy urine individuals.

Pattern recognition was performed for an evaluation of acid concentration profiles in plasma as a main contributor of acidosis and in urine as additional information. According to the results from unsupervised pattern recognition (PCA), plasma concentration profiles (LA, aHBA, bHBA and pHPLA) could be considered as potential contributors of acidosis in severe malaria and could be used to classify malaria patients (including severe and uncomplicated groups) and healthy individuals completely. However, patients with severe malaria and uncomplicated malaria could not be classified completely by using plasma acid concentration profiles. Furthermore, urine acid concentration profiles (LA, aHBA, bHBA, pHPLA, MMA, EMA and aKGA) could not be used to classify malaria patients and healthy individuals completely.

This pilot study presented a metabolomic study approach for the assessment of organic acids potentially contributing to acidosis in severe malaria in human plasma and urine. The use of reliable and robust pattern recognition method

combine with quantitative LC-MS data (acid concentration profiles) was performed. This method could be an alternative novel clinical tool for the assessment of prognostic and pathophysiological significance in this devastating disease. Furthermore, this approach can be readily extended for screening clinical samples (plasma and urine) to explore other potentially relevant acids in larger studies or in other conditions associated with acidosis.