

# PIPERAQUINE AND METABOLITES

## Bioanalysis and Pharmacokinetics

Akademisk avhandling

Som för avläggande av farmacie doktorsexamen vid Sahlgrenska akademien,  
Göteborgs universitet kommer att offentligens försvaras i hörsal Åke Göransson,  
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Avhandlingen baseras på följande delarbeten:

- I. **Mohd Yusmaidie Aziz**, Kurt-Jürgen Hoffmann and Michael Ashton. *LC-MS/MS quantitation of antimalarial drug piperazine and metabolites in human plasma*. Accepted for publication in Journal of Chromatography B, 2017
- II. **Mohd Yusmaidie Aziz**, Kurt-Jürgen Hoffmann and Michael Ashton. *Inhibition of CYP3A by antimalarial piperazine and its metabolites in human liver microsomes with IVIV extrapolation*. Submitted
- III. **Mohd Yusmaidie Aziz**, Kurt-Jürgen Hoffmann and Michael Ashton. *Plasma protein binding of piperazine and its metabolites: Binding to human plasma, serum albumin and  $\alpha$ 1-acid glycoprotein*. In manuscript
- IV. **Mohd Yusmaidie Aziz**, Trinh Ngoc Hai, Emma Johansson, Le Minh Dao, Pham Thi Thinh and Michael Ashton. *Dose- and time-independent pharmacokinetics of piperazine and its metabolites in healthy male Vietnamese subjects after four escalating oral doses separated by one month*. In manuscript

**SAHLGRENSKA AKADEMIN**  
**INSTITUTIONEN FÖR NEUROVETENSKAP OCH FYSIOLOGI**



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## Bioanalysis and Pharmacokinetics

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### ABSTRACT

Antimalarial piperazine (PQ) is currently used as a partner drug with dihydroartemisinin (DHA), exhibiting high cure rates (>95%) for *P. falciparum*. Despite its raising usage worldwide with DHA, PQ is synthetically developed outside of big pharma pipelines. Thus, there is potentially some scientific gap in the information regarding disposition of the drug not being systematically established. This thesis comprised studies on bioanalysis- (Paper I), CYP3A4/5 inhibitory potential- (Paper II), protein binding- (Paper III) and pharmacokinetics (PK) of piperazine and its metabolites (Paper IV) with intention of filling these scientific gaps. PQ in earlier studies metabolized to two main urinary metabolites, M1 which is a carboxylic acid cleavage product and M2, the mono N-oxide of PQ. PQ and M2 were found as potent CYP3A inhibitors whereby M2 showed greater inhibition *in vitro*. Simulation of PQ inhibitory effect, predicted the drug-drug interaction (DDI) between PQ and co-administered midazolam in healthy subjects during antimalarial PQ treatment. Bioanalytical method was developed using a highly sensitive analytical instrument, LC-MS/MS to determine PQ and its metabolites in human plasma. The simultaneous quantitation method of PQ and metabolites was developed and validated for the first time based on the FDA guidelines. The method was applied for PK studies of PQ and metabolites after oral administration of single and escalating dose regimen of Artekin® (DHA-PQ) in Vietnamese healthy subjects. PQ exhibited dose- and time independent kinetics. M2 was found to be circulating metabolites in plasma while M1 was hardly detected. Plasma protein binding of PQ and its metabolites were studied *in vitro* whereby PQ was extensively bound to plasma proteins with higher affinity towards AGP protein than to the albumin while metabolites, exhibited a much lower degree of binding. Unbound fractions of PQ and metabolites were successfully determined in human plasma by ultrafiltration. Generally, the utmost contribution of this thesis is the application of bioanalysis method to quantitate the antimalarial PQ and its metabolites for pharmacokinetics including CYPs- and protein binding studies. As other antimalarials, PQ nowadays should be carefully evaluated for its treatment benefit and risk potential considering the challenge of increasing antimalarial resistance. Furthermore, DHA-PQ is suggested for mass-drug-administration (MDA) to eliminate malaria in Sub-Saharan Africa.

**Keywords:** Piperazine, LC-MS/MS, pharmacokinetics, CYP3A inhibition, protein binding