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## ACCEPTED MANUSCRIPT

Development and validation of two LC-MS/MS methods to assay urinary tylerdipine hydrochloride and its metabolites in healthy Chinese subjects

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#### Abstract

For quantitative assaying tylerdipine hydrochloride, and its two primary metabolites (M2 and M4) in human urine, two sensitive and accurate LC-MS/MS methods were firstly developed and validated, where multiple reaction monitoring (MRM) was applied under positive electrospray ionization mode for tylerdipine and negative electrospray ionization mode for M2/M4, respectively. Urinary proteins were precipitated using acetonitrile, and deuterated isotopes of tylerdipine and M4 ([D5]-tylerdipine and [D6]-M4) were used as internal standards. Triton X-100, a good surfactant, was used to prevent the adsorption. An Agilent Poroshell 120 column was employed for chromatographic separation of the analytes with the mobile phases of 2mM ammonium formate solution (containing 0.1% formic acid) and acetonitrile

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