Measurement of caffeine and its three primary metabolites in human plasma by HPLC-ESI-MS/MS and clinical application

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Introduction

- Caffeine is a mild stimulant with significant potential for abuse.
- Investigations of caffeine pharmacokinetics and pharmacodynamics in humans should include quantification of caffeine's three major metabolites (paraxanthine, theobromine, and theophylline) because they retain central nervous system stimulant activity.
- The study established a method for the simultaneous quantification of caffeine and its three major metabolites in human plasma by HPLC-ESI-MS/MS.
- The validated assay provides a simple and sensitive method with high throughput capacity and low sample volume requirement. The method was applied to the study of caffeine pharmacokinetics in human subjects.

Results

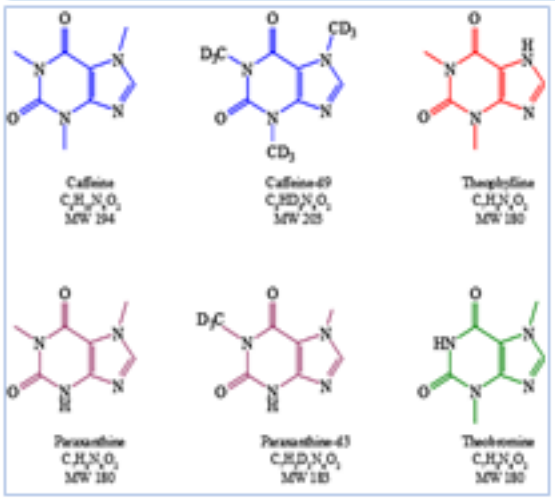

Figure 1. Chemical structures of caffeine (CAF), paraxanthine (PAR), theophylline (THY), theobromine (THM), deuterated caffeine (CAF-d9), and deuterated paraxanthine (PAR-d3).

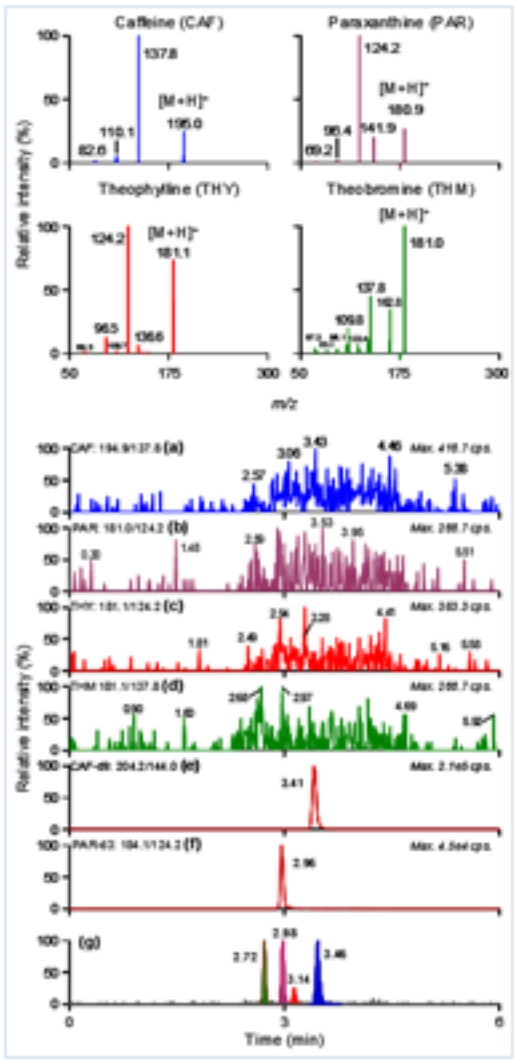

Figure 2

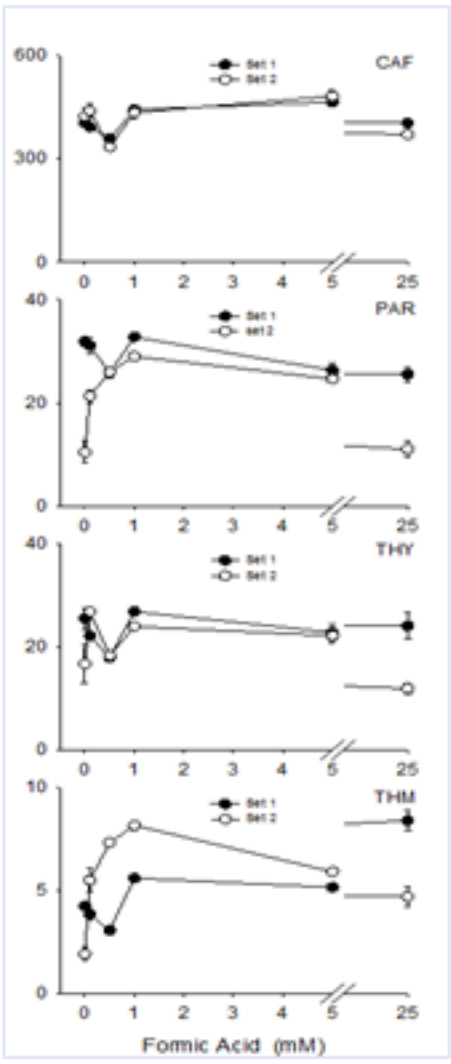

Figure 3

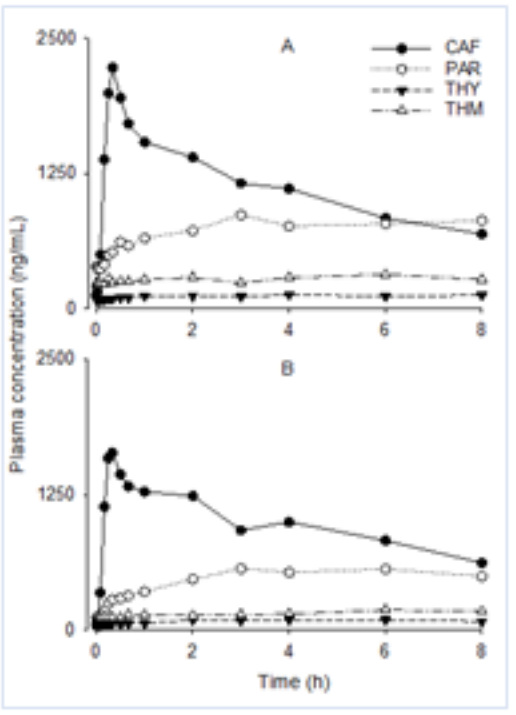

Figure 4. Plasma concentration-time curves of CAF and its three metabolites after administration of CAF to a human subject on separate study days.

- Product ion mass spectra of the protonated molecules of CAF, PAR, THY and THM (top panel); chromatograms of blank human plasma, blank human plasma spiked with deuterated standard solutions, and a study subject plasma sample for the caffeine and its three metabolites (bottom panel) (Figure 2).
- The effect of formic acid concentration in mobile phase on the signal intensity and absolute matrix effects of CAF, PAR, THY, and THM (Figure 3).

Conclusions

- A method with low sample volume consumption, high sensitivity, and simplicity for the simultaneous quantification of caffeine, paraxanthine, theophylline, and theobromine in human plasma by HPLC-ESI-MS/MS was developed and validated.
- The method is the first, to our knowledge, to evaluate different concentrations of formic acid in mobile phase to optimize the ionization and matrix effects.
- This straightforward and easy-to-use strategy can be transferred to other HPLC-ESI-MS/MS-based assays.
- The newly developed method was successfully applied to the analysis of caffeine and its metabolites in human plasma samples.

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