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Development of a liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for characterizing caffeine, methylliberine, and theacrine pharmacokinetics in humans

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ABSTRACT

Coffea liberica possesses stimulant properties without accumulating the methylxanthine caffeine. The basis for this peculiar observation is that methylurates (e.g., theacrine and methylliberine) have replaced caffeine. The stimulant properties of methylurates, alone and in combination with caffeine, have recently been investigated. However, human pharmacokinetics and LC-MS/MS methods for simultaneous measurement of methylxanthines and methylurates are lacking. To address this deficiency, we conducted a pharmacokinetic study in which subjects (n = 12) were orally administered caffeine (150 mg), methylliberine (Dynamine™, 100 mg), and theacrine (TeaCrine®, 50 mg) followed by blood sampling over 24 h. Liquid-liquid extraction of plasma samples containing purine alkaloids and internal standard (^{13}C-Caffeine) were analyzed using a C18 reversed-phase column and gradient elution (acetonitrile and water, both containing 0.1% formic acid). A Waters Xevo TQ-S tandem mass spectrometer (positive mode) was used to detect caffeine, methylliberine, theacrine, and IS transitions of *m/z* 195.11 → 138.01, 225.12 → 168.02, 225.12 → 167.95, and 198.1 → 140.07, respectively. The method was validated for precision, accuracy, selectivity, and linearity and was successfully applied to characterize the oral pharmacokinetics of caffeine, methylliberine, and theacrine in human plasma. Successful development and application of LC-MS/MS-based methods such as ours for the simultaneous measurement of methylxanthines and methylurates are essential for the characterization of potential pharmacokinetic and pharmacodynamic interactions.

1. Introduction

Methylurates (e.g. theacrine, methylliberine, etc.) are purine alkaloids synthesized by a limited number of plant species including coffee (*Coffea liberica*) and tea (*Camellia assamica* var. *kucha*) and represent secondary plant metabolites derived from purine nucleotides [1]. Theacrine and liberine were first isolated from young leaves of various *Coffea* species including *C. liberica* [2]. Subsequently, methylliberine was first identified in very low concentrations (< 0.1%) during vegetative development of *C. liberica* leaves [3]. Mature leaves of *C. liberica*, *C. dewevrei*, and *C. abeokutae* contain the methylurates theacrine, methylliberine, and liberine [2], whereas the leaves of *C. assamica* var. *kucha* contain only theacrine [4]. Metabolism studies analyzing *C. assamica* var. *kucha* leaf discs indicated that theacrine was synthesized from caffeine by successive oxidation and methylation steps with 1,3,7-trimethyluric acid acting as the intermediate [4], whereas caffeine was converted to liberine via theacrine and methylliberine in leaves of *C. dewevrei*, *C. liberica* and *C. abeokutae* [5].

Preclinical and clinical pharmacology studies demonstrated that theacrine, similar to caffeine, activated adenosine and dopamine receptors and signaling pathways responsible for motivation and wakefulness, which results in a subjective increase in energy, mood, focus, concentration, motivation to train and willingness to exercise [6–8]. Interestingly, combining theacrine and caffeine yields enhanced energy, mood and focus compared to theacrine or caffeine alone [7]; presumably due to caffeine's ability to enhance the oral bioavailability of theacrine [9]. Theacrine is dissimilar to caffeine in that it has a longer half-life, no effect on blood pressure, lower likelihood of sleep

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disruption, and a low potential for pharmacologic tolerance [10–12]. Methylliberine reportedly has similar neuro-energetic effects to theacrine and caffeine [13] demonstrating enhanced energy, mood, and focus without anxiety with a more rapid onset of action [14]. Moreover, methylliberine, either alone or in combination with theacrine, did not elicit clinically significant changes in cardiovascular function and blood biomarkers [13].

Several reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection based bioanalytical methods have been published for the quantitation of theacrine and caffeine [15,16]. Most of these reported methods suffer from long HPLC run times and high detection limits [15,16]. In recent years, liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based bioanalytical methods have also been used to quantitate theacrine and caffeine due to the high selectivity and sensitivity of this technique. Several published reports have successfully separated theacrine and caffeine using HPLC with diode array detection coupled with electrospray ionization tandem mass spectrometry (HPLC-DAD/ESI-MS/MS) as well as HPLC and UFLC-Q-TOF-MS/MS [17,18]. However, these methods are also plagued by long chromatographic run times. Further, methylliberine and theacrine are isomeric methylurates with the same MS/MS transition (*m/z* 225.12 → 168.02) that cannot be distinguished based on their masses alone. Thus, chromatographic separation is required to avoid interference.

Anecdotal evidence of enhanced EMF activity when caffeine, methylliberine, and theacrine are combined has led to the marketing of dietary supplements that “stack” or combine methlliberine with caffeine and/or theacrine. We previously demonstrated that caffeine enhanced the oral bioavailability and thus plasma exposure of theacrine [9]. Consequently, there is a need to develop novel bioanalytical methods to support future pharmacokinetic and substantiation studies of methylxanthine/methyurate combination products. To address this unmet need, we aimed to develop a rapid, simple, and sensitive LC–MS/MS method for the simultaneous determination of methylliberine, caffeine, and theacrine in human plasma and to characterize their pharmacokinetics after oral administration to humans.

2. Methods and materials

2.1. Reagents and chemicals

Caffeine and caffeine-$^{13}C_3$ solution (1 mg/mL, Internal Standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Theacrine and methylliberine were acquired from Compound Solution, Inc. (Carlsbad, CA, USA). The identities of standard compounds were certified on the basis of the spectral data (^{1}H- and ^{13}C NMR and HR-ESIMS). The purity was confirmed all above 99% by chromatographic methods. Methanol, acetonitrile and formic acid were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified using a Millipore Synergy UV Water Purification System (Millipore SAS, Molsheim, France).

2.2. Preparation of calibration standards and quality controls

An individual stock solution of standard compounds was prepared at a concentration of 1.0 mg/mL in methanol. An aliquot (100 µL) of a standard solution containing 300 ng/mL of caffeine-$^{13}C_3$ (Internal Standard, IS) was added to a of drug-free human plasma (50 µL) and mixed by vortexing for 1 min. The mixture was then centrifuged (12,000*g* for 10 min at 4 °C) and an aliquot (50 µL) of the supernatant was transferred to another tube and diluted using Millipore water (100 µL) and then mixed thoroughly. Serial dilution of the standard compounds were prepared as final concentrations of 0.67, 1.33, 3.33, 6.67, 13.3, 33.3, 53.3, 66.7, 133, 333, 533, 667, and 1333 ng/mL for each analyte. Quality control (QC) samples were added to the stock solution of standards to drug free human plasma at final concentrations of 0, 6.67, 66.7, and 667 ng/mL. The final concentration of IS was 66.7 ng/mL for routine use in all prepared solutions. On analysis days, calibration curves for caffeine, theacrine, and methylliberine in plasma were derived from their peak area ratios relative to that of caffeine-$^{13}C_3$ regression with 1/x for the three analytes as a weighting factor. The respective QC samples were assayed along with each batch of plasma samples to evaluate the intra- and inter-day precision and accuracy of the method. All prepared solutions were kept at 4 °C until analysis.

2.3. Sample preparation

Methanol (100 µL) containing IS (300 ng/mL) was added to human plasma (50 µL) in a 1.5 mL microcentrifuge tube and mixed by vortexing for 1 min. The mixture was centrifuged at 12,000*g* for 10 min at 4 °C and an aliquot (50 µL) of supernatant was transferred to another tube and diluted by Millipore water (100 µL). The sample was mixed thoroughly and transferred to an LC vial for LC–MS/MS analysis. All prepared samples were kept at 4 °C until LC-MS/MS analysis.

2.4. LC-MS/MS parameters

All analyses were performed on a Waters Acquity UPLC™ I-class system (Waters Corp., Milford, MA, USA) that included a binary solvent manager, sample manager, heated column compartment, and a Xevo TQ-S triple quadrupole mass spectrometry detector. The instrument was controlled by Waters MassLynx 4.1 software. For analysis of caffeine, theacrine, and methylliberine, a Waters UPLC BEH C_{18} column (50 mm × 2.1 mm I.D., 1.8 µm) was used. The column and sample temperature were maintained at 40 °C and 10 °C, respectively. The eluent consisted of water containing 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Analysis was performed using the following gradient elution at a flow rate of 0.5 mL/min: 0–2 min, 5% B to 30% B; 2–2.5 min, 30% to 100% B. The analysis was followed by a one and half minute washing procedure with 100% B and re-equilibration period of 3.5 min with initial condition. A wash solvent (1:1:1:1 methanol/acetonitrile/isopropanol/water, v/v) and purge solvent (1:1 methanol/water, v/v) were used for the autosampler and needle wash. The injection volume was 2 µL.

The tandem mass experiments were carried out on a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) that was connected to the UHPLC system via an electrospray ionization (ESI) interface. The ESI MS/MS parameters such as capillary voltage, cone voltage, collision energy, key fragment ions, etc. were optimized using the IntelliStart autotune feature that was built in Waters MassLynx software. The MS/MS parameters were finally determined as follows: capillary voltage, 1.1 kV; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 1000 L/h, and cone gas flow, 150 L/h. Nitrogen was used as the desolvation and cone gas. Argon (99.99% purity) was introduced as the collision gas into the collision cell at a flow rate of 0.15 mL/min. The effluent was introduced into the TQ-S mass spectrometer equipped with ESI in positive ion mode (ESI +) for the quantification of the analytes. Detection was obtained by Multiple Reaction Monitoring (MRM) mode including two MRMs for confirmation of the analytes. The quantification of each analyte was acquired with transitions of the protonated ion at *m/z* 195.11 → 138.01 (dwell time 32 ms, cone voltage 22 V, and collision energy 16 eV) for caffeine, 198.1 → 140.07 (dwell time 32 ms, cone voltage 22 V, and collision energy 16 eV) for caffeine-$^{13}C_3$ (IS), 225.12 → 168.02 (dwell time 32 ms, cone voltage 42 V, and collision energy 18 eV) for theacrine, and 225.12 → 167.95 (dwell time 32 ms, cone voltage 44 V, and collision energy 20 eV) for methylliberine, respectively. Data acquisition was carried out using the MassLynx 4.1 software (Waters Corp., Milford, MA, USA).

2.5. LC-MS/MS method validation

Method validation was conducted in accordance with suggested criteria outlined in the US Food and Drug Administration (FDA) Guidance for Industry – Bioanalytical Method Validation [19]. Method parameters including specificity, linearity, sensitivity, precision, accuracy, and stability of caffeine, theacrine, and methylliberine were validated in human plasma. Specificity was evaluated by comparing chromatograms of nine different batches of drug-free plasma to ensure that no interfering peaks were present at the retention time of caffeine, caffeine-$^{13}C_3$ (IS), theacrine, and methylliberine.

Calibration curves in human plasma were obtained by plotting the peak ratios of caffeine, theacrine, and methylliberine, respectively, to the IS against the nominal concentrations of the calibration standards at 0.67, 1.33, 3.33, 6.67, 13.3, 33.3, 53.3, 66.7, 133, 333, 533, 667, and 1333 ng/mL for the three analytes. The calibration curves were fitted using a linear least-squares regression with a weighting factor of 1/x for all analytes. The limits of detection (LOD) and lower limit of quantification (LLOQ) for all analytes in plasma samples were defined as the lowest concentration giving a signal-to-noise ratio of 3 and 10, respectively. For LLOQ, the acceptable accuracies of 80–120% and sufficient precisions within 20% were adopted and verified using seven replicate analyses.

Intra-day precision and accuracy were determined by analyzing three different QC samples (6.67, 66.7, and 667 ng/mL) on the same day. Inter-day precision and accuracy were also evaluated by analyzing three different QC samples on three different days (three replicates/day). Precision was expressed as the relative standard deviation (RSD, %) and the accuracy was expressed as [(mean observed concentration)/(nominal concentration) × 100%]. The stability of caffeine, theacrine, and methylliberine in plasma were assessed by analyzing three replicate samples (6.67, 66.7, and 667 ng/mL) for the three analytes under short-term storage (8 h at room temperature) and post-treatment storage (72 h at 4 °C).

2.6. Pharmacokinetic study design

The study was designed for a single oral co-administration of methylliberine (100 mg), caffeine (150 mg), and theacrine (50 mg) in twelve healthy nonsmokers followed by serial blood draws over a 24 h period. Methylliberine (Dynamine™) and theacrine (TeaCrine™) were provided by Compound Solutions (Carlsbad, CA). Caffeine, administered as caffeine anhydrous, was obtained from Nutravative Ingredients (Allen, TX).

2.7. Pharmacokinetic data analysis

Noncompartmental pharmacokinetic analyses of the methylliberine, caffeine, and theacrine concentrations were analyzed using Phoenix WinNonlin (Version 7.0; Pharsight Corporation, Mountain View, CA) with adjustment for lag time after oral administration. The maximum concentration (C_{max}) and time corresponding to the C_{max} (T_{max}) were determined from the plasma concentration versus time data. The area under the plasma concentration–time curve from time 0 to infinity ($AUC_{0-\infty}$) was determined using the linear trapezoidal rule. The terminal half-life ($t_{1/2}$), was calculated using ln 2/k_{el}, with k_{el} as the terminal rate elimination constant estimated from the slope of the linear regression of the log plasma concentration versus the time curve during the terminal phase. Oral clearance (CL/F) was calculated by dividing the oral dose by $AUC_{0-\infty}$ and the oral volume of distribution (Vz/F) during the terminal elimination phase was calculated by dividing CL/F by k_{el}.

3. Results

3.1. Method development and qualification

In a preliminary test for the separation of caffeine, theacrine, and methylliberine, analytes were applied to different UHPLC columns using various mobile phase compositions. The tested columns were Acquity UPLC HSS T3 (100 mm × 2.1 mm I.D., 1.8 μm), UPLC BEH C18 (100 mm × 2.1 mm I.D., 1.7 μm), and UPLC BEH C18 (50 mm × 2.1 mm I.D., 1.8 μm). Considering the shorter run time, the separation and peak shape, a 50 mm × 2.1 mm UPLC BEH C18 column gave the best results. Optimal chromatographic separation was observed with solvent composition of acetonitrile with 0.1% formic acid (*v/v*) and water containing 0.1% formic acid as the mobile phase. Caffeine, theacrine and methylliberine were baseline-separated within 2 min.

In ESI positive ion mode, capillary and cone voltages of mass spectrometer (MS) for each analyte were tuned to achieve maximal sensitivity. All analytes produced protonated molecular ions $[M+H]^+$ as the major species. Fragmentation patterns of $[M+H]^+$ were evaluated by elevating the collision energy from 2 to 80 eV. Two Molecular Reaction Monitorings (MRMs) were chosen to be quantifier and qualifier, respectively, for each analyte. The most intense peaks, which were used for quantification of corresponding analyte, were observed at *m/z* 138.01 for caffeine, 140.07 for caffeine-$^{13}C_3$ (IS), 168.02 for theacrine, and 167.95 for methylliberine (Fig. 1). The MS parameters of quantifier and qualifier ions such as cone voltage and collision energy for all analytes are listed in Table 1.

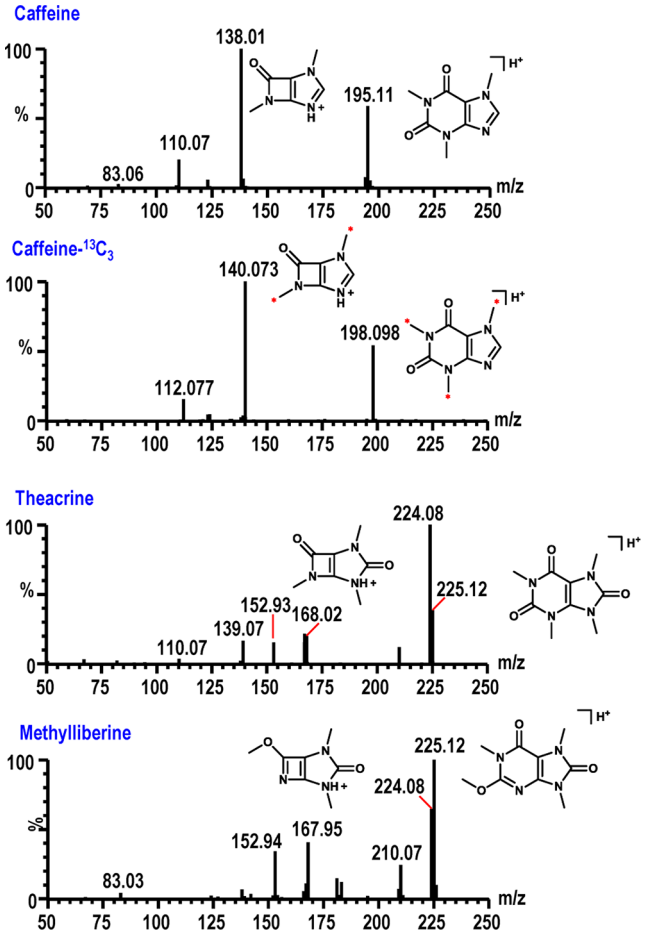

Fig. 1. MS/MS spectra of caffeine, caffeine-$^{13}C_3$, theacrine, and methylliberine.

Table 1
Settings of MS parameters for the quantification of caffeine, theacrine, and methylliberine.

Compound Name	Target Ions (*m/z*)	Quantifier Ions (*m/z*)	Cone (V)	Collision Energy (eV)	Qualifier Ions (*m/z*)	Cone (V)	Collision Energy (eV)
Caffeine	195.11	138.01	22	16	112.92	22	18
Theacrine	225.11	168.02	42	18	152.94	42	24
Methylliberine	225.12	167.95	44	20	152.94	44	24

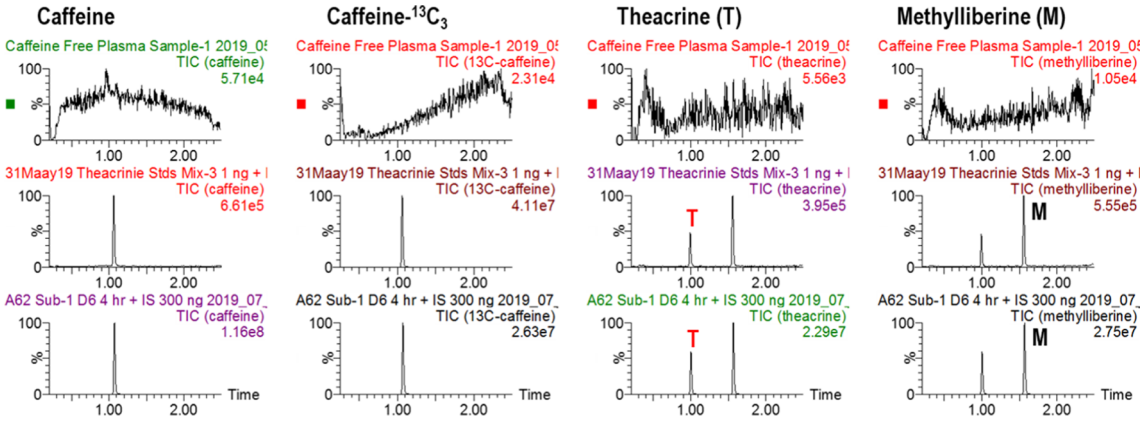

Fig. 2. MRM chromatograms of caffeine, caffeine-$^{13}C_3$, theacrine, and methylliberiine in drug free plasma, plasma sample spiked with each analyte at 0.67 ng/mL, and a plasma sample at 4 h after co-adiministration.

3.2. Selectivity

No interfering peaks were observed at the elution times of theacrine (t_R 0.98 min), caffeine (1.06 min), or methylliberine (1.55 min). Caffeine-$^{13}C_3$ (IS) had the same retention time as caffeine, but the IS mass transition was 198.1 → 140.07, which differed from that of caffeine (195.11 → 138.01). Though theacrine and methylliberine showed the same MS/MS pattern, elution times of theacrine and methylliberine were different. Representative chromatograms of drug-free human plasma, a plasma QC sample (0.67 ng/mL), and a plasma sample obtained at 4 h after co-administration of caffeine, theacrine, and methylliberine are shown in Fig. 2.

3.3. Linearity and sensitivity

To minimize matrix effects, standard solutions containing caffeine-$^{13}C_3$ (IS) were added to drug-free human plasma and extracted using the optimized method. Calibration curves were established using standards at 0.67, 1.33, 3.33, 6.67, 13.3, 33.3, 53.3, 66.7, 133, 333, 533, 667, and 1333 ng/mL for all analytes. During validation in human plasma, the coefficient of determination (r^2) for each analyte was above 0.99 (n = 12). The calibration curves, linear ranges, and LOQs for all analytes are shown in Table 2.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy of the developed method were evaluated by analyzing QC samples (6.67, 66.7, and 667 ng/mL for three analytes) on three different days (Tables 3 and 4). Both precision and accuracy were well within the 20% acceptance range. The relative standard derivation (RSD, %) for intra- and inter-day precision were below 5%. The intra- and inter-day accuracies were 98.0–105.4% for all analytes.

3.5. Stability

Stock solutions of caffeine, theacrine, methylliberine, and caffeine-$^{13}C_3$ in methanol stored at −20 °C were found to be stable for up to three months. No changes were observed with UV spectra, peak areas, and the appearance of any extra peaks. The stability of short-term storage and post-treatment storage for caffeine, theacrine, and methylliberine in human plasma were satisfied. No significant degradation (within ± 15% deviation between the predicted and nominal concentrations) was noted for the tested samples (data not shown).

3.6. Pharmacokinetics

The above developed LC-MS/MS method was used to characterize methylliberine, caffeine, and theacrine oral pharmacokinetics in humans. Mean plasma concentration (± standard deviation) time profiles for methylliberine, caffeine, and theacrine are shown in Figs. 3–5. Pharmacokinetic parameters for methylliberine, caffeine, and theacrine are shown in Table 5. Methylliberine was found to have a short terminal half-life (1.5 ± 0.8 h), a rapid clearance (158 ± 88.9 L/h), and a high volume of distribution (Vd) (283 ± 109 L). Caffeine was found to have a high terminal half-life (21.2 ± 18.1 h), a low clearance (16.9 ± 9.80 L/h), and a high Vd (328 ± 105 L). Theacrine was found to have a very high terminal half-life (30 ± 12 h), a very low clearance (9.10 ± 2.70 L/h), and high Vd (371 ± 117 L).

Table 2
Calibration curve, coefficient of determination, linearity range, LOD and LOQ of caffeine, theacrine, and methylliberine.

Compound Name	Calibration Curve	R^2	Linearity Range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Caffeine	Y = 1.03157X + 0.470465	0.998	0.67–333.33	0.1	0.33
Theacrine	Y = 0.259937X + 0.0437431	0.999	0.67–1333.33	0.2	0.67
Methylliberine	Y = 0.501858X + 0.0545777	0.999	0.67–666.67	0.1	0.33

Table 3
Intra-day assays of plasma samples.

Analyte	Nominal Conc. (ng/mL)	Intra-Day								
		Day-1 (n = 3)			Day-2 (n = 3)			Day-3 (n = 3)		
		Detected Conc. (ng/mL)	Precision (RSD, %)	Accuracy(%)	Detected Conc. (ng/mL)	Precision (RSD, %)	Accuracy (%)	Detected Conc. (ng/mL)	Precision (RSD, %)	Accuracy
Caffeine	6.67	6.83	0.8	102	6.91	0.6	104	7.03	0.5	105
	66.7	68.8	1.2	103	69.2	0.4	104	69.0	0.3	103
	667	660	4.7	99.0	678	0.4	102	707	0.8	106
Theacrine	6.67	6.98	1.2	105	6.88	0.3	103	6.61	1.3	99.1
	66.7	69.5	1.2	104	69.3	0.7	104	67.0	1.0	101
	667	653	1.1	98.0	656	0.0	98.4	654	1.8	98.1
Methylliberine	6.67	6.78	1.0	101.6	6.79	1.2	102	6.81	0.6	102
	66.7	68.1	1.1	102.1	67.9	0.8	102	67.7	0.4	102
	667	669	0.5	100.3	672	0.5	101	689	0.9	103

Table 4
Inter-day assays of plasma samples.

Compounds	Inter-Day (n = 9)			
	Norminal Conc. (ng/mL)	Detected Conc. (ng/mL)	Precision (RSD, %)	Accuracy (%)
Caffeine	6.67	6.93	1.5	104
	66.7	69.0	0.3	104
	667	681	3.5	102
Theacrine	6.67	6.82	2.8	102
	66.7	68.6	2.0	103
	667	654	0.2	98.1
Methylliberine	6.67	6.79	0.2	102
	66.7	67.9	0.2	102
	667	676	1.6	101

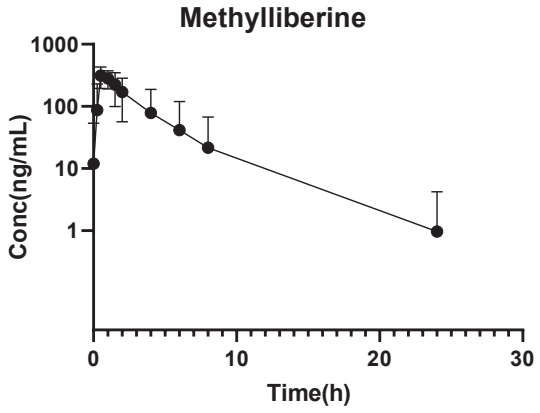

Fig. 3. Plasma concentration of methylliberine after a single oral dose of methylliberine 100 mg, theacrine 50 mg, and caffeine 150 mg. Data represent mean ± SD.

4. Conclusions

Scientific publications in the field of nootropic (cognitive enhancement) pharmacology have doubled over the past twenty years. A major driver of this expansion is the quest for pharmacologic agents to replace or augment the energy, mood, and focus (EMF) effects of caffeine. The methylurates theacrine and methylliberine represent unique nootropics that have similar, yet distinct, pharmacokinetic and pharmacodynamic properties compared to caffeine. Consequently, methylurates have been utilized to augment caffeine's EMF-related properties, which creates the potential for pharmacokinetic and/or pharmacodynamic interactions. For example, we have recently documented a pharmacokinetic interaction between theacrine and caffeine in which

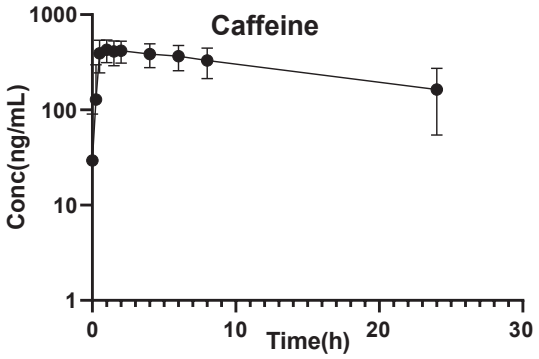

Fig. 4. Plasma concentration of caffeine after a single oral dose of methylliberine 100 mg, theacrine 50 mg, and caffeine 150 mg. Data represent mean ± SD.

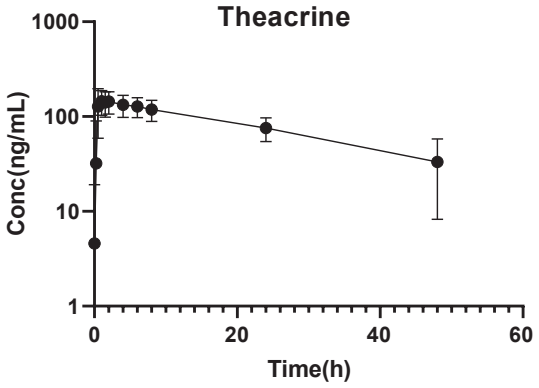

Fig. 5. Plasma concentration of theacrine after a single oral dose of methylliberine 100 mg, theacrine 50 mg, and caffeine 150 mg. Data represent mean ± SD.

Table 5
Methylliberine, caffeine, and theacrine pharmacokinetics.

Parameters	Methylliberine	Caffeine	Theacrine
C_{max} (ng/mL)	365 ± 137	473 ± 128	167 ± 56.5
T_{max} (hours)	0.8 ± 0.4	1.1 ± 0.7	1.4 ± 1.0
$t_{1/2}$ (hours)	1.5 ± 0.8	21 ± 18	30 ± 12
AUC (h × ng/mL/mg)	10.3 ± 10.5	90.1 ± 69.3	122 ± 47.8
CL/F (L/h)	158 ± 88.9	16.9 ± 9.80	9.10 ± 2.70
V_d/F (L)	283 ± 109	328 ± 105	371 ± 117
MRT (hours)	2.4 ± 1.3	29 ± 27	43 ± 17

caffeine enhances the oral bioavailability of theacrine [9]. There is increasing market penetration of dietary supplements that leverage the unique pharmacodynamics of methylurates and caffeine when combined. Thus, there is a critical need to develop new bioanalytical methodologies to evaluate human PK/PD interaction potential.

To address the aforementioned need, a rapid and robust LC–MS/MS-based bioanalytical method was developed and validated for the simultaneous quantification of methylliberine, caffeine, and theacrine in human plasma. The chromatographic separation conditions provided short retention times as well as finely resolved peaks for each of the three analytes. Moreover, the application of different collision energies led to minimization of background noise and interference peaks without adversely affecting sensitivity. Optimization of chromatographic separation coupled with fine-tuning of mass spectrometric parameters yielded short HPLC run times. The novel method was successfully employed to examine caffeine, methylliberine, and theacrine pharmacokinetics in humans following simultaneous oral administration. The single-arm pharmacokinetic study was not designed to assess interaction potential, however, the observed pharmacokinetic parameters for caffeine and theacrine appear quite different than literature reports [9]. Studies designed to rigorously examine the pharmacokinetic interaction potential between methylxanthines and methylurates are in progress.

CRediT authorship contribution statement

Yan-Hong Wang: Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing. **Goutam Mondal:** Methodology, Software, Validation, Formal analysis, Writing - original draft, Writing - review & editing. **Matthew Butawan:** Investigation. **Richard J. Bloomer:** Conceptualization, Investigation, Writing - review & editing. **Charles R. Yates:** Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

RJB and CRY have received research funding from Compound Solutions, Inc., including this study. These contracts paid for direct and indirect costs, as well as salary. This study was funded by Compound Solutions, Inc., who was consulted in the design of the study. All other authors have no competing interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2020.122278.

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