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METHYLLIBERINE AS A SALIVARY BIOMARKER UNDER POSTPRANDIAL CONDITIONS

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ABSTRACT

The biomarker, therapeutic pharmacokinetic potential, and stimulating properties of methylliberine, a naturally occurring purine alkaloid, are currently being investigated. Saliva is a preferred non-invasive biological matrix for biomarker characterization and therapeutic drug monitoring due to its ease of collection and strong correlation with systemic drug exposure. Postprandial evaluations are crucial due to the fact that methylliberine distribution, absorption, and salivary excretion are influenced by food intake. This investigation will evaluate the salivary concentration profile and its correlation with systemic exposure in order to determine the efficacy of methylliberine as a salivary biomarker subsequent to consumption.. For pharmacokinetic studies, therapeutic monitoring, and clinical research, methylliberine may be a more viable and patient-friendly salivary biomarker than blood samples.

Keywords: *Methylliberine; Salivary biomarker; Postprandial conditions; Saliva; Pharmacokinetics; Non-invasive monitoring; Therapeutic drug monitoring.*

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1. INTRODUCTION

Methylliberine, or O(2),1,7,9-tetramethyluric acid, is present in coffee and other types of Camellia assamica var. kucha. Methylliberine has garnered scientific interest as a distinctive bioactive chemical that has the potential to improve cognitive function, alertness, mood, and physical endurance due to its structural closeness to theacrine and coffee. Methyliberine exhibits unique pharmacological characteristics, continuous systemic exposure, and rapid absorption following oral administration, as indicated by pharmacokinetic research. Monitoring its biological availability, absorption, and excretion in humans is necessary due to its increasing use in functional beverages and nutritional supplements. Recent research suggests that methylliberine may serve as a beneficial non-invasive salivary biomarker for postprandial physiological reactions and gastric emptying, thereby expanding its application beyond pharmacokinetic evaluation..

Saliva is a critical diagnostic biofluid due to its frequent sampling, minimal risk of infection, non-invasiveness, and ease of collection in clinical and nutritional research. Salivary sample collection is more tolerable than venous blood collection and allows for serial monitoring without the need for trained personnel or discomfort. The unbound, pharmacologically active fraction of specific xenobiotics and endogenous metabolites in plasma is displayed in saliva, which is significant because it reveals systemic exposure. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) have improved the sensitivity, selectivity, and repeatability of quantifying saliva purine alkaloids at low concentrations. The significance of salivary biomarker research has increased in the fields of clinical pharmacology, therapeutic medication monitoring, nutritional sciences, and precision medicine as a substitute for intrusive biological samples.

The physiologically dynamic state of postprandial chemical assimilation, distribution, metabolism, and excretion is significantly influenced by food consumption. The pharmacokinetics of bioactive chemicals can be influenced by splanchnic blood flow, luminal pH, dietary composition, gastrointestinal motility, and stomach emptying. As an outcome, salivary chemical concentrations may fluctuate. The oral bioavailability, temporal

distribution, and relationship to gastrointestinal transit of methylliberine can be ascertained through postprandial salivary indicators.

2. MATERIALS AND METHODS

Compression-Coated Tablets

In order to prevent saliva contamination during sample collection, compression-coated tablets were developed, which contained 25 mg of caffeine and 100 mg of methylliberine. Caffeine, colloidal silicon dioxide (Aerosil®), hydroxypropyl methylcellulose (Methocel® E4M), croscarmellose sodium (Vivasol®), silicified microcrystalline cellulose (Prosolv® SMCC 90), microcrystalline cellulose (Avicel® PH 102), magnesium stearate, sodium saccharin, and black iron oxide were supplied by authorized pharmaceutical suppliers. The formulation was established on the basis of a compression-coated tablet design.

The production of tablets was divided into two halves. An eccentric tablet press is employed to compress squeezed methylliberine and caffeine-infused tablet cores into 7-mm spherical, flat tablets. The final dosage form was subsequently produced by compressing the outer-layer excipient mixture onto each core tablet in a 10 mm biconvex die. Each tablet was heat-sealed in a metal pouch to ensure stability.

Quality control tests were conducted to assess the content uniformity (n = 10), hardness (n = 6), tablet height (n = 6), friability, disintegration time (n = 6), dissolution profile (n = 3), and stability under long-term (25°C/60% RH) and accelerated (40°C/75% RH) storage conditions. All evaluations were conducted in accordance with the European Pharmacopoeia guidelines.

An in vitro dissolution test was performed in 300 mL of pepsin-free simulated stomach fluid (pH 1.2) at 25°C with a paddle rotation speed of 75 rpm using a USP Apparatus II (paddle method). In order to preserve sink conditions, 1 mL samples were collected every 30 seconds during the 10-minute dissolve test and replaced with fresh dissolving liquid. The HPLC-UV/Vis analytical method was employed to measure drug concentrations. Three dissolutions of compression-coated tablets and tablet cores were implemented.

Table 1. Composition of the Tablet Cores (Theoretical Composition per Tablet)

Ingredient	Quantity (mg)	Quantity (%)
Methylliberine	100	58.8
Caffeine	25	14.7
Saccharin sodium	10	5.9
Avicel® PH 102	18	10.6
Vivasol®	8.5	5
Black iron oxide	3.4	2
Aerosil®	3.4	2
Magnesium stearate	1.7	1
Total	170	100

Table 2. Composition of the Outer Compression-Coating Layer (Theoretical Composition per Tablet)

Ingredient	Quantity (mg)	Quantity (%)
Prosolv® SMCC HD 90	207.74	94
Methocel® E4M	8.84	4
Magnesium stearate	4.42	2
Total	221	100

In Vivo Study

A three-period crossover study included 12 healthy men and women. Participants took the compression-coated pill fasting, after a little meal, and after a high-fat meal. Between research phases, 72-hour washouts were required. Daylong saliva collection was maintained at -80°C until analysis. After Institutional Ethics Committee approval, the German Clinical Trials Register registered the trial.

Sample Preparation and Analysis

After saliva centrifugation and cooling, D9-caffeine was the internal reference. Acetonitrile with 1% formic acid separated proteins. We used validated LC-MS/MS to evaluate treatment samples in MRM mode.

Statistics

Saliva concentration data was analyzed using GraphPad Prism 5.0 to evaluate pharmacokinetic properties such Cmax, Tmax, AUC0-1440, and t^2. We used one-way ANOVA and Tukey's post hoc test on normal distribution data. Non-parametric data analysis using Friedman-Dunn post hoc tests. P-values below 0.05 are significant.

3.METHODOLOGY

Study Design

In a randomized three-way crossover trial of twelve healthy persons, methylliberine was examined during fasting, light, and high-fat meals. Washout between probes took 72 hours.

Preparation of the Study Formulation

Uniformity, hardness, friability, disintegration, solubility, and stability were examined on compression-coated 25 mg caffeine/100 mg methylliberine tablets.

In Vitro Dissolution Study

USP II paddle apparatus measured drug breakdown in pH 1.2 artificial gastric juice, while HPLC-UV/Vis monitored release.

Participant Preparation

Participants fasted for 72 hours and abstained from alcohol for 48 hours before each study. Patients ate and drank before therapy.

Experimental Procedure

High-fat, fasting, and light diet patients were directed to take the tablet with 240 ml water.

Saliva Sample Collection

Saliva samples were taken without stimulation at the start and throughout the day using the Salivary Tracer Technique. The materials were stored at -80°C until analysis.

Sample Preparation and Chemical Analysis

Centrifuged saliva samples were processed with an internal standard and analyzed by LC-MS/MS to meet EMA bioanalytical validation standards.

Pharmacokinetic Analysis

From saliva concentration–time data corrected for baseline caffeine, Cmax, Tmax, AUC, and half-life were determined.

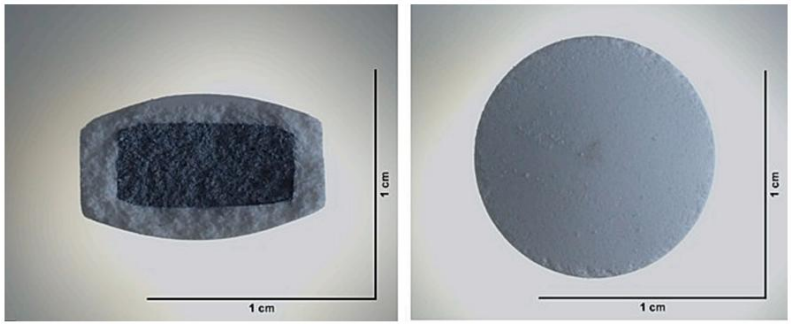
Statistical Analysis

We utilized ANOVA or Friedman tests after normality testing. Significant P-values were under 0.05.

4. RESULTS

Quality Control of the Formulations

Completed compression-coated pills. Figure 1 displays full and half-cut wafers. Table 5 shows core and compression-coated tablets. The masses were consistent, with less than 2% deviation. All six pill cores contained the active ingredient. Core capsules were 170 mg and pressed-coated tablets 412 mg. The difference is 1 mg. Content consistency analysis showed 94.4–103.2 mg methylliberine and 23.5–26.5 mg caffeine per pill. Friability tests cut the tablet's weight by 0.1%, confirming its durability.



In vitro Dissolution

Compression-coated and paddle-generated tablet core degradation is shown in Figure 2. Every formulation comprises 100 mg methylliberine and 25 mg caffeine. Both model pharmaceuticals were easily removed with compression-coated tablets and centers. Tablet cores released 85% methylliberine and caffeine in 120 seconds. 85% of compression-coated pills released in 180 seconds. After 240 seconds, caffeine and methylliberine appeared.

Figure 3 shows the three-month accelerated stability investigation results. Late-released compression-coated tablets from three months ago. After three months of stability testing, post-production methylliberine and caffeine released 85% of compounds after 180 and 210 seconds.

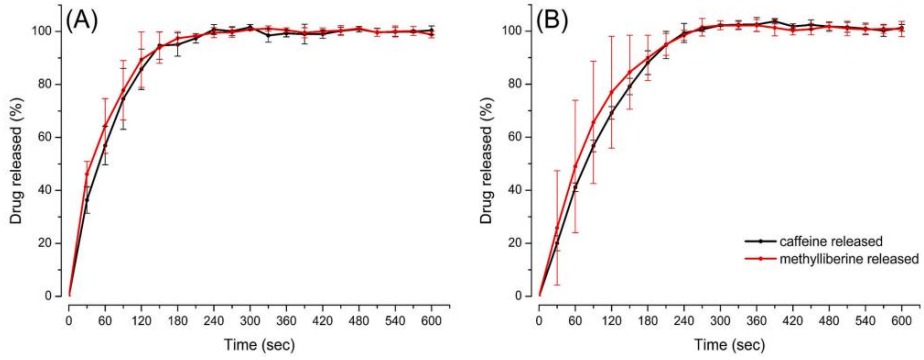


Fig. 2. Dissolution profiles of tablet cores and compression-coated tablets containing caffeine and methylliberine (mean ± SD, n = 3).

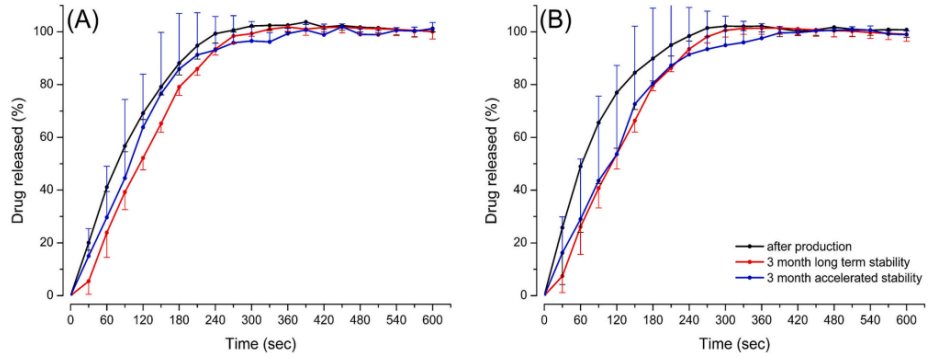


Fig. 3. Dissolution profiles of compression-coated tablets after production and 3-month stability testing (mean ± SD, n = 3).

Table 5. Physical Properties of Compression-Coated Tablets and Core Tablets

Parameter	Compression-Coated Tablet	Core Tablet
Average mass (mg)	412 ± 7	170 ± 1
Height (mm)	6.2 ± 0.1	3.8 ± 0.1
Hardness (N)	86 ± 10	20 ± 2
Disintegration time	Within 52 s	Within 43 s

Study Population

Table 6 provides a summary of the demographic characteristics of the study participants. The study protocol's inclusion and exclusion criteria were met by each participant. The study's design was approved by all 12 volunteers, and they effectively completed the three trial days. No accidents occurred.

Table 6. Demographic Characteristics of Study Participants (n = 12)

Parameter	Median (Range)	Mean ± Standard Deviation
Sex	Male: 6, Female: 6	—
Age (years)	23 (19–36)	23.7 ± 1.2
Height (cm)	178 (167–200)	177.7 ± 11.0
Weight (kg)	65 (51–93)	71.5 ± 13.6
BMI (kg/m²)	22.1 (18.3–26.9)	22.4 ± 2.0

Study results

Mean Profiles

Figure 4 illustrates the median concentrations of caffeine and methylliberine in saliva from research arms A–C. The Supplementary File contains the salivary concentration time patterns of each of the twelve study participants (Figs. S1 and S2). The compression-coated pills' early disintegration did not result in the contamination of the mouths of the trial participants. Salivary caffeine levels were detected in participants 10 and 12 in all research arms, 8 in C, and 9 in B and C. In these cases, the caffeine levels of the initial saliva sample were eliminated from subsequent results as a baseline.

Regardless of their dispositions, all three research groups (A–C) exhibited a substantial increase in salivary drug concentrations following a meal. In response to the consumption of additional calories, the research groups' methylliberine exposure (AUC0–1440min) decreased, while their caffeine exposure remained constant. The tmax was higher in arms A and B following a supper than in arm C, which was administered without food, in both drug experiments. The research arms' cumulative exposure was determined over a 24-hour period due to the short half-life of methylliberine. Table 7 contains an exhaustive list of all pharmacokinetic parameters.

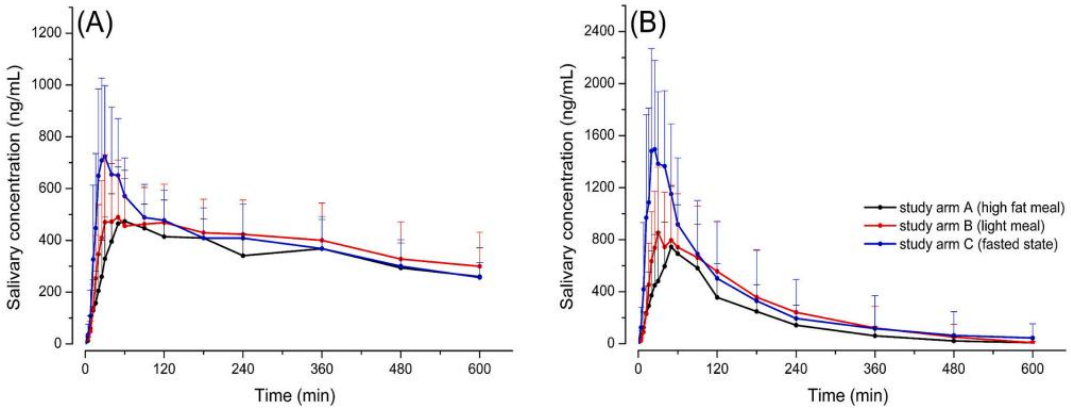


Fig. 4. Mean salivary concentration profiles of caffeine and methylliberine under fed and fasted conditions (mean ± SD, n = 12).

Statistics

The AUC0–1440min, t0.5, tmax, and cmax of methylliberine and caffeine were statistically compared by the three research groups (A–C) in Figures 5 and 6. In contrast to fasting, caffeine and methylliberine exhibited statistically significant increases in tmax and decreases in cmax in fed state experiments. None of the analytes exhibited a statistically significant variation in their elimination half-life (t0.5). There was no statistically significant difference in the AUC0–1440min of caffeine between trial arms A–C. Methylliberine, on the other hand, varied significantly between research arms A (high-fat meal) and C (fasted administration).

Table 7. Pharmacokinetic parameters of caffeine and methylliberine under fed and fasted conditions (n = 12, mean ± SD).

Parameter	Study Arm	Caffeine (Mean ± SD)	Methylliberine (Mean ± SD)
Tmax (min)	High-fat meal	69 ± 46	48 ± 17
	Light meal	65 ± 35	50 ± 27
	Fasted state	29 ± 12	26 ± 11
Cmax (ng/mL)	High-fat meal	534 ± 179	867 ± 397
	Light meal	596 ± 201	1033 ± 440
	Fasted state	842 ± 251	1894 ± 573
$AUC_{0–1440}$ (ng·h/mL)	High-fat meal	5954 ± 1878	1873 ± 1133
	Light meal	6915 ± 2813	2622 ± 1952
	Fasted state	6952 ± 1731	3362 ± 3164
t½ (h)	High-fat meal	15.50 ± 9.22	1.34 ± 0.51
	Light meal	14.73 ± 6.57	1.29 ± 0.35
	Fasted state	15.56 ± 6.03	1.36 ± 0.75

5. CONCLUSION

Methylliberine is an excellent saliva biomarker due to its low baseline saliva levels, brief elimination half-life, and ease of absorption. However, it is more susceptible to physiological changes caused by food than caffeine, as its systemic exposure significantly diminishes as the calorie content of a meal increases following consumption. Methylliberine can be employed to evaluate the early release of medication and the emptying of the stomach; however, it cannot replace coffee in salivary biomarker studies when the subject is being fed. It is advantageous for investigating the impact of meals on individuals and the absorption of drugs, as it can also detect modifications in the body subsequent to a meal.

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