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## Determination of caffeine, theobromine and theophylline in Mate beer and Mate soft drinks by high-performance thin-layer chromatography

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### ABSTRACT

Mate beer and Mate soft drinks are beverages produced from the dried leaves of *Ilex paraguariensis* (Yerba Mate). In Yerba Mate, the xanthine derivatives caffeine, theobromine and theophylline, also known as methylxanthines, are important active components. The presented method for the determination of caffeine, theobromine and theophylline in Mate beer and Mate soft drinks by high-performance thin-layer chromatography with ultraviolet detection (HPTLC–UV) offers a fully automated and sensitive determination of the three methylxanthines. Filtration of the samples was followed by degassing, dilution with acetonitrile in the case of Mate beers for protein precipitation, and centrifugation before the extracts were analyzed by HPTLC–UV on LiChrospher silica gel plates with fluorescence indicator and acetone/toluene/chloroform (4:3:3, v/v/v) as the mobile phase. For quantitation, the absorbance was scanned at 274 nm. Limits of detection and quantitation were 1 and 4 ng/zone, respectively, for caffeine, theobromine and theophylline. With recoveries close to 100% and low standard deviations reliable results were guaranteed. Experimental Mate beers as well as Mate beers and Mate soft drinks from the market were analyzed for their concentrations of methylxanthines.

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### 1. Introduction

Yerba Mate (*Ilex paraguariensis*) is a plant of the Aquifoliaceae family that typically grows in South America [1,2] and comprises several constituents which are known to be anti-carcinogenic and anti-oxidative [2]. *Ilex paraguariensis* is widely known as the source of Yerba Mate tea, also simply called Mate. Mate and further Mate beverages are infusions made from green or dried leaves or other plant parts of *Ilex paraguariensis* [1,2]. The infusion Mate is known as stimulating beverage with health promoting properties that has been traditionally consumed in South America [1]. The extract of the infusion is used as ingredient in alcoholic and non-alcoholic soft drinks. Recently also a flavored beer, the Mate beer, was designed, when smoked and not smoked Yerba Mate is added during the beer brewing.

Among others the plant alkaloids caffeine, theophylline, and theobromine are important active components in Yerba Mate [2], which are derivatives of xanthine and also are known as methylxanthines [3]. They all have in common the stimulating effect on the central nervous system, with caffeine as the most potent repre-

sentative. Methylxanthines also affect the cardiovascular system, with theophylline generating the strongest effect [2,3]. Thus, the concentration of caffeine, theophylline, and theobromine in Mate beverages is generally of great interest. In this context, for the manufacturing of Mate beer, the influence of different procedures of the Yerba Mate addition during the beer brewing on the quantity of the active components is an aspect, which has to be evaluated.

For the determination of caffeine, theobromine, and theophylline in different foods, high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) were suggested. For coffee, tea and coca samples, HPLC with ultraviolet (UV) detection or mass selective detection was often reported [4–7]. HPTLC was suggested for the analysis of caffeine and other active components in herbal products, power drinks [8], and energy drinks [9]. A TLC approach for the determination of caffeine and theobromine in Mate tea was mentioned by Bojić et al. [10]. Cimpoi et al. [11] presented a TLC method for the analysis of caffeine, theobromine and theophylline in different types of tea, and Badea [12] described a TLC/GC/MS method for tea and coffee. However, a fully automated and validated HPTLC method for the simultaneous determination of the three methylxanthines, caffeine, theobromine and theophylline, in Mate beer and Mate soft drinks is not available.

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**Table 1**  
Conditions of the Yerba Mate addition and caffeine concentrations in seven experimental Mate beers.

Sample	Hops	Yerba Mate [g/L]	Method of Yerba Mate addition	Time of Yerba Mate addition	Caffeine [mg/L] $\pm$ SD <sup>a</sup> (n = 3)
1	Bitter and aroma	8	Loose	30 min after the starting of the wort boiling	125 $\pm$ 2
2	Bitter	8	Loose	30 min after the starting of the wort boiling	107 $\pm$ 1
3	Bitter and aroma	8	Loose	During the washes	78 $\pm$ 3
4	Bitter	8	Tea towel	During the washes	90 $\pm$ 4
5	–	8	Tea towel	5 min after the starting of the wort boiling	78 $\pm$ 4
6	–	16	Tea towel	5 min after the starting of the wort boiling	87 $\pm$ 7
7	Bitter and aroma	10	Tea strainer	5 min after the starting of the wort boiling	110 $\pm$ 4

<sup>a</sup> Standard deviation.

Thus, the aim of this work was to develop a sensitive, selective and automated planar chromatographic method for the simultaneous determination of caffeine, theobromine and theophylline in Mate beer. The method should further be applicable to investigate the methylxanthines in Mate soft drinks. With the plenty separation possibilities in HPTLC, an efficient separation of the methylxanthines from Mate matrix compounds should easily be possible. The highly automated HPTLC devices guarantee well repeatable procedures for a rapid, simple and reliable determination of the methylxanthines and additionally offer the analysis of many samples in parallel.

## 2. Material and methods

### 2.1. Chemicals and materials

Caffeine ( $\geq 99\%$ ), theobromine ( $\geq 98\%$ ) and theophylline, anhydrous ( $\geq 99\%$ ) were obtained from Sigma-Aldrich (Steinheim, Germany). Acetone (Rotisolv pestilyse) was purchased from Carl Roth (Karlsruhe, Germany). Toluene (for pesticide residue analysis), acetonitrile (LC–MS, Chromasolv) and chloroform (for pesticide residue analysis,  $>99.8\%$ ) were obtained from Sigma-Aldrich. Ultrapure water ( $>18\text{ M}\Omega\text{ cm}$ ) was supplied by a Synergy System (Millipore, Schwalbach, Germany). Folded paper filter (diameter 185 mm) and filter tips (0.45- $\mu\text{m}$ ) were purchased from Macherey-Nagel (Düren, Germany). HPTLC silica gel LiChrospher F<sub>254</sub>s plates from Merck (Darmstadt, Germany) were used without pre-washing. Experimental Mate beer samples were obtained from the Department of Yeast Genetics and Fermentation Technology, University of Hohenheim (Stuttgart, Germany). The used Yerba Mate Pajarito Selección Especial was from Lauro Raatz S.A., Itapúa, Paraguay. Commercially available Mate beers and Mate soft drinks were provided from different producers.

### 2.2. Standard solutions

For the preparation of standard stock solutions, methylxanthines generally were weighted into 100-mL volumetric flasks, dissolved in 60 mL of hot water (60 °C), and filled up with water at ambient temperature.

For HPTLC–UV method development, individual stock solutions of caffeine, theobromine, and theophylline (100 mg/L) were prepared. A standard-mix solution (33.3 ng/ $\mu\text{L}$ ) was obtained by pipetting 500  $\mu\text{L}$  of the individual standard stock solutions together in an autosampler vial. For the determination of limits of detection and quantitation (LOD/LOQ), a combined standard stock solution (40 mg/L) was prepared, from which a working standard solution (1 ng/ $\mu\text{L}$ ) was achieved by dilution 1:40 with methanol. As calibration standards for recovery experiments and the analysis of Mate beer samples, individual standard solutions were prepared at a concentration of 60 mg/L caffeine, 40 mg/L theobromine and 40 mg/L theophylline. For the analysis of Mate soft drink samples, individual standard solutions were prepared for calibration at a concentration

of 150 mg/L caffeine, 100 mg/L theobromine and 100 mg/L theophylline.

### 2.3. Sample preparation

#### 2.3.1. Mate beer

Ten mL of Mate beer were filtered through a folded filter paper and were degassed in an ultrasonic bath (for 45 min). A 1-mL aliquot of the degassed sample was transferred into a 15-mL plastic centrifuge tube equipped with a screw cap, containing 3 mL of acetonitrile. The tube was vigorously shaken for 2 min and centrifuged at 3200  $\times$  g at ambient temperature for 10 min (Biofuge primo R, Heraeus, Hanau, Germany). A 1-mL aliquot of the supernatant was transferred through a 0.45- $\mu\text{m}$  filter tip into an HPTLC vial.

For the determination of recovery rates, spiked samples were prepared by dissolving 10 mg of caffeine, theophylline and theobromine in 100 mL of a degassed hop beer sample (100 mg/L) as described in Section 2.2 for the standard stock solutions. Sample preparation was performed as described for the Mate beer samples.

#### 2.3.2. Mate soft drink

Ten mL of Mate soft drink was degassed in an ultrasonic bath (for 45 min) and directly transferred through a 0.45- $\mu\text{m}$  filter tip into an HPTLC vial.

### 2.4. High-performance thin-layer chromatography–ultraviolet detection (HPTLC–UV)

Application was performed by an Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland). Samples and standards were applied as 6-mm bands with the following settings leading to 21 tracks on a 20 cm  $\times$  10 cm plate: 8 mm distance from the lower edge, 15 mm distance from the left edge, and 8.5 mm track distance. For the application parameters, the predefined settings for methanol were used. Methanol was used as the rinsing solvent with 1 rinsing cycle and 1 filling cycle. The application volume for LOD/LOQ determinations was 2.5–25  $\mu\text{L}$  of the working standard solution, resulting in 2.5–25 ng/zone for caffeine, theobromine and theophylline. For recovery experiments, calibration was performed by application of 1.3–15  $\mu\text{L}$  of the individual standard solutions of caffeine, theobromine and theophylline in the overspray mode. Sample application volume for recovery experiments was 10  $\mu\text{L}$ . After the application, a drying step with a hair dryer followed for 5 min, and chromatography was performed without chamber saturation in the Automatic Developing Chamber (ADC2, CAMAG) with a 20 cm  $\times$  10 cm twin-trough chamber (CAMAG). The plate activity was controlled to 33% relative humidity by saturated magnesium chloride solution (5 min). As mobile phase, a mixture of acetone/toluene/chloroform (4:3:3, v/v/v) was used up to a migration distance of 60 mm (developing time 9 min), and a drying step followed for 2 min. Plate images were captured with the TLC Visualizer (CAMAG) under UV 254 nm illumination. The plate was scanned in the absorption mode at UV 274 nm (deuterium lamp)

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