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**An automated homogeneous liquid-liquid microextraction based on deep eutectic solvent for the HPLC-UV determination of caffeine in beverages**

**Andrey Shishov\***, Natalia Volodina, Daria Nechaeva, Svetlana Gagarinova, Andrey Bulatov

*Department of Analytical Chemistry, Institute of Chemistry, St. Petersburg State University, SPbSU, SPbU, 7/9 Universitetskaya nab., St. Petersburg, 198504 Russia*

\*Corresponding author. Tel.: +7 9119835320.

*E-mail address: [a.y.shishov@spbu.ru](mailto:a.y.shishov@spbu.ru)*

**Abstract**

This work presents an automated homogeneous liquid-liquid microextraction procedure for separation of caffeine from beverages. The microextraction procedure involved mixing sample and water-miscible deep eutectic solvent (DES, choline chloride with phenol at a 1:3 molar ratio) into a mixing chamber of a flow system resulting formation of homogeneous solution. Injection of aprotic solvent (tetrahydrofuran) into homogeneous sample solution promoted the fine DES droplets formation and caffeine extraction into DES phase separated. The automated homogeneous liquid-liquid microextraction procedure was coupled with a HPLC-UV system. The conditions of caffeine microextraction and its HPLC-UV determination in DES phase were studied and optimized. Under optimal experimental conditions the linear detection range and limit of detection, calculated from a blank test, based on  $3\sigma$  were found to be  $0.1 - 200 \text{ mg L}^{-1}$  and  $0.03 \text{ mg L}^{-1}$ , respectively. The application of the developed procedure was demonstrated in the determination of caffeine in commercial soft drinks samples.

Keywords: Homogeneous liquid-liquid microextraction, deep eutectic solvent, automation, flow analysis, HPLC-UV, caffeine, soft drinks

**1. Introduction**

Caffeine (1,3,7-trimethyl-1H-purine-2,6 (3H,7H)-dione) (ESM Fig. 1) is a natural alkaloid widely used in food industry as the most favorable psychostimulant in beverages or foods for motor activation, mood changes, information processing and cognitive/motor performances [1]. Caffeine can have both positive and negative health effects. On the one hand, it is powerful stimulant of the central nervous system and also stimulates the cardiac muscle. On the other hand, its high amounts have noticeable irritation of gastrointestinal tract [2] and might cause the “caffeinism” syndrome

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