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ACCEPTED MANUSCRIPT

**An automated salting-out assisted liquid-liquid microextraction approach using 1-octylamine: On-line separation of tetracycline in urine samples followed by HPLC-UV determination**

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## **Abstract**

An automated salting-out assisted liquid-liquid microextraction (SALLME) procedure based on a flow system was developed as new approach for pretreatment of complex sample matrix. In this procedure 1-octylamine was investigated as novel extractant for the SALLME. The procedure involved aspiration of the 1-octylamine and sample solution into a mixing chamber of a flow system followed by their air-bubble mixing resulting to isotropic solution formation. To provide phase separation a salting-out agent solution was added into the mixing chamber. After phase separation, the micellar 1-octylamine phase containing analyte was mixed with methanol and transported to a HPLC-UV system. To demonstrate the efficiency of the suggested approach, the automated procedure was applied for the HPLC-UV determination of tetracycline as a proof-of-concept analyte in human urine samples. Under the optimal conditions, the detector response of the analytes was linear in the concentration ranges of 0.5-20 mg L<sup>-1</sup>. The limit of detection, calculated from a blank test based on 3 $\sigma$ , was 0.17 mg L<sup>-1</sup>. The results demonstrate that the developed approach is highly cost-effective, simple and rapid.

*Keywords:* salting-out assisted liquid-liquid microextraction; flow analysis; HPLC-UV; 1-octylamine; tetracycline; urine.

## **1. Introduction**

Determination of target analytes in complex matrix (biological liquids, foods) is an important challenge of analytical chemistry. Complex sample matrix and presence of various

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