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Journal of Chromatography B, 688 (1997) 265–274

JOURNAL OF CHROMATOGRAPHY B

Determination of caffeine and its metabolites by micellar electrokinetic capillary electrophoresis

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Received 26 March 1996; revised 11 June 1996; accepted 13 June 1996

Abstract

The determination of caffeine and its analogues is important for a wide variety of analyses and is performed in an assortment of matrices ranging from food to clinical samples. While reversed-phase HPLC has become the standard analysis protocol in most laboratories, capillary electrophoresis has the advantages of higher separation efficiency and shorter separation time. The micellar capillary electrophoresis (MECC) separation of caffeine and its metabolites, theobromine, paraxanthine, theophylline and 1,3,7-trimethyluric acid was investigated using sodium dodecyl sulphate (SDS) as the micellar phase. The effects of pH, micelle concentration, buffer concentration, ionic strength, buffer salts, applied voltage and injection time were studied to select the optimum conditions for the determination of caffeine and its four analogues in drugs, foods and body fluids. Caffeine and its three analogues were resolved within 120 s with detection limits less than 1 μg/ml. Samples could be analyzed utilizing direct injection with satisfactory resolution and reproducibility.

Keywords: Caffeine; Theobromine; Paraxanthine; Theophylline; 1,3,7-Trimethyluric acid

1. Introduction

Capillary electrophoresis (CE) has been developed as a technique for the analysis of charged molecules, including proteins, peptides and oligonucleotides. Compared to conventional separation methods such as HPLC, CE provides higher efficiency (approaching or exceeding 1 000 000 theoretical plates), shorter analysis time, smaller sample volume requirements and low running cost. The application of this method was extended to neutral molecules by the introduction of micellar capillary electrophoresis (MECC) as first developed by Terabe et al. [1]. A psuedophase is formed by adding a reagent such as sodium dodecyl sulfate (SDS) to the electrophoresis run buffer at concentrations sufficient to form micelles. In MECC, analytes are separated on the basis of both their hydrophobicity and electrophoretic mobility. Solutes can differentially partition between the hydrophobic interior of the micelles and the aqueous phase leading to different migration rates in the MECC system. MECC has been applied to the separation of both neutral and charged organic molecules, including derivatized amino acids [2], water-soluble vitamins [3], pharmaceuticals [4], and nucleic acids [5].

Caffeine and its metabolites are widely found in the human diet and pharmaceutical formulations. The xanthines have a wide range of therapeutic activity. Theophylline is a bronchodilator used in the treatment of chronic asthma, caffeine is a central nervous system stimulant, theobromine and theophylline are

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PII S0378-4347(96)00278-2