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CE determination of the Fe (III) complex of 1-(2′-carboxyethyl)-2-methyl-3-hydroxypyridin-4-one (CP38)

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Capillary electrophoresis (CE) is being increasingly applied to the analysis of metal ion complexes (Macka and Haddad 1997). However, it remains a challenge to directly analyse metal complexes in biological samples, due to their stability being critically dependent on the type of biological matrix. In the present study, a CE method was developed to analyse the major iron complex of 1-(2'-carboxyethyl)-2-methyl-3-hydroxypyridin-4-one (CP38) in rat bile, urine, blood and liver homogenate. A fused-silica capillary with 70 cm total length (55 cm effective length) and 75 μm I.D. was utilised. The carrier electrolyte consisted of cetyltrimethylammonium bromide (CTAB, 0.5mM) and phosphate buffer (100mM, pH7.4). Samples were introduced hydrodynamically for 2 or 5 s and the temperature of the capillary was maintained at 25°C. A reversed field (-30kV) was applied for the separations and a built-in diode array detector was utilised for monitoring the analytes. The ML_3 complex is formed by CP38 with iron(III) in phosphate buffer at pH7.4 (λmax 459nm). This complex has a migration time of about 10 min under the CE conditions employed (Figure 1). The method permitted resolution of the complex from the free ligand CP38 which possesses a migration time of about 11 min (Figure 1). The calibration curve was linear in the concentration range of 50-1000 μM (calculated as iron concentration and monitored at 460 nm). The limit of detection (LOD) of the ML_3 complex was 11.2 μM at 460 nm and 1.3 μM at 285 nm. The limit of quantification (LOQ) was 37.2 μM at 460 nm and 4.4 μM at 285 nm. The within-day coefficients of variation (CV) for 100 μM and 1000 μM were 1.9% and 2.3%, respectively. The inter-day CVs for 100 μM and 1000 μM were 6.7% and 2.2%, respectively. These results indicated that the present CE method was reliable, sensitive, and repeatable for analysis of the ML_3 complex of Fe (III)-CP38.

CP38 is the major metabolite present in bile and urine after rats are orally administered with 1-(3'-hydroxypropyl)-2-methyl-3-hydroxypyridin-4-one (CP41). After normal rats were orally administered with CP41 (450 μmole/kg), the bile, urine, blood plasma and liver homogenate were analysed using the CE method. The Fe-CP38 (ML_3) complex was found in the bile samples (Figure 2) at much higher concentration than in urine, whilst it was undetectable in plasma and liver homogenate.

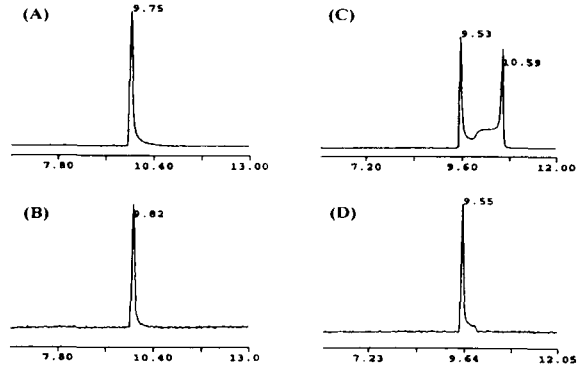

Figure 1. The electropherograms of Fe(III)-CP38 complex (ML_3) in phosphate buffer (100mM, pH7.4). The molar ratios of Fe(III) : CP38 were (A) & (B) 1:3; and (C) & (D) 1:8. The monitoring wavelengths were (A) & (C) 285 nm; and (B) & (D) 460 nm.

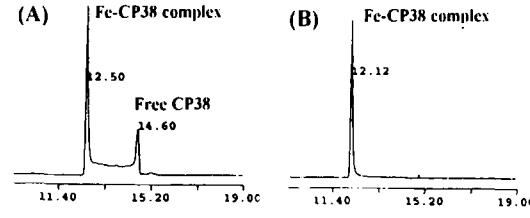

Figure 2. The electropherogram of Fe(III)-CP38 complex (ML_3) in rat bile monitored at (A) 285 nm and (B) 460 nm.

Macka, M., Haddad, P.R. (1997) Electrophoresis 18: 2482-2501