

Abstract

A new method was developed and fully validated for the quantification of hydrochlorothiazide and chlorthalidone, both diuretic and anti-hypertensive agents, in human serum. This liquid chromatography-tandem mass spectrometry method (LC/MS/MS) is rapid, simple, accurate and selective. The analytes and the internal standard were extracted by liquid-liquid extraction (LLE) with ethylacetate-dichloromethane (80:20, v/v). The chromatographic separation was performed on a reversed-phase column C8 with a mobile phase acetonitrile-formic acid (0,2 %), the elution was gradient. The analytes were quantitated by linear ion trap mass spectrometry with an electrospray ionization interface (ESI). The diuretics were analysed in negative ion monitoring mode, the internal standard in positive ion monitoring mode. The assay exhibited a linear dynamic range of 0,5-200 ng/mL for hydrochlorothiazide and 0,5-500 ng/mL for chlorthalidone in human serum.

Acceptable precision and accuracy were obtained for QC samples with concentration over the standard curve ranges. This developed and validated method has been used in the toxicological laboratory in the Institution of Clinical Biochemistry and Diagnosis in Hradec Králové.