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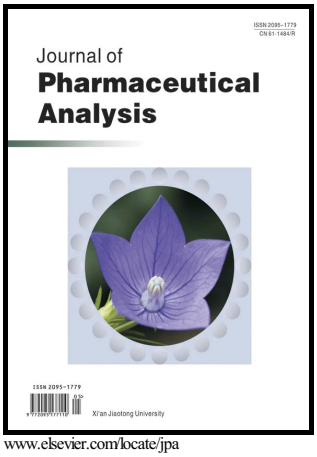

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Simultaneous quantification of amiloride and hydrochlorothiazide in human plasma by liquid chromatography-tandem mass spectrometry

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Abstract

A selective, sensitive and precise assay based on solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed for the simultaneous determination of amiloride (AMI) and hydrochlorothiazide (HCTZ) in human plasma. Sample clean-up with 250 µL of plasma was done on Phenomenex Strata^{TM}-X extraction cartridges using their labeled internal standards. Chromatography was performed on Hypersil Gold C_{18} (50 mm × 3.0 mm, 5 µm) column using acetonitrile with 4.0 mM ammonium formate (pH 4.0, adjusted with 0.1 % formic acid) (80:20, v/v) as the mobile phase. Detection was carried out on a triple quadrupole API 5500 mass spectrometer utilizing an electrospray ionization interface and operating in the positive ionization mode for AMI and negative ionization mode for HCTZ. Multiple reaction monitoring was used following the transitions at *m/z* 230.6/116.0, *m/z* 233.6/116.0, *m/z* 296.0/204.9 and *m/z* 299.0/205.9 for AMI, AMI-15N3, HCTZ and HCTZ-13C,d2 respectively. Calibration curves were linear ($r^2 \geq 0.9997$) over the concentration range of 0.050-50.0 and 0.50-500 ng/mL for AMI and HCTZ, respectively with acceptable accuracy and precision. The signal to noise ratio at the limit of quantitation was ≥ 14 for both the analytes. The mean recovery of AMI and HCTZ from plasma was 89.0 % and 98.7 %, respectively. The IS-normalized matrix factors determined for matrix effect ranged from 0.971 to 1.024 for both the

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