

# Sequential liquid-liquid extraction coupled to LC-MS/MS for simultaneous determination of amlodipine, olmesartan and hydrochlorothiazide in plasma samples: Application to pharmacokinetic studies.

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## Abstract

A recent fixed combination of amlodipine (AML) besylate, olmesartan (OLM) medoxomil and hydrochlorothiazide (HCT) has been marketed for the treatment of hypertension. The bioanalysis of this mixture is a challenge due to the diverse physicochemical properties. The aim of this study is to develop a method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous bioanalysis of the mixture in human plasma.

A mobile phase of 10 mM ammonium formate containing 0.1% formic acid: methanol: acetonitrile (35:50:15, v/v/v) was recruited for the chromatographic separation of the mixture on a Zorbax SB-Aq (150 4.6 mm, 5" m) column. The deuterated analogues of AML, OLM and HCT were used as the internal standards (IS). Polarity switching SRM transitions in positive-mode for AML, OLM, and negative-mode for HCT were applied for detection.

Sequential double liquid-liquid extraction (SdLLE) was adopted for sample preparation. The developed method was fully validated and applied for the determination of AML, OLM and HCT following a single oral administration of 5/20/12.5 mg of AML, OLM and HCT, respectively tablets to healthy volunteers (n = 30).

The developed method was linear ( $r^2 > 0.99$ ), accurate (105 - 90%), precise (CV% < 11.92) and specific for the determination of AML, OLM and HCT over the concentration range of 0.1615, 561200 and 26150 ng/mL. The adopted SdLLE resulted into reproducible extraction recoveries of 75%, 63% and 83% for AML, OLM and HCT, respectively. The pharmacokinetic analysis revealed uniform profile in the subjects which were comparable to previously reported results in other populations.

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