High-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of diazepam, atropine and pralidoxime in human plasma

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Résumé en anglais
A high-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) procedure for the simultaneous determination of diazepam from avizafone, atropine and pralidoxime in human plasma is described. Sample pretreatment consisted of protein precipitation from 100 μl of plasma using acetonitrile containing the internal standard (diazepam D5). Chromatographic separation was performed on a X-Terra® MS C8 column (100 mm × 2.1 mm, i.d. 3.5 μm), with a quick stepwise gradient using a formate buffer (pH 3, 2 mM) and acetonitrile at a flow rate of 0.2 ml/min. The triple quadrupole mass spectrometer was operated in positive ion mode and multiple reaction monitoring was used for drug quantification. The method was validated over the concentration ranges of 1–500 ng/ml for diazepam, 0.25–50 ng/ml for atropine and 5–1000 ng/ml for pralidoxime. The coefficients of variation were always <15% for both intra-day and inter-day precision for each analyte. Mean accuracies were also within ±15%. This method has been successfully applied to a pharmacokinetic study of the three compounds after intramuscular injection of an avizafone–atropine–pralidoxime combination, in healthy subjects.

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