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UHPLC-QTOF-MS method development for assessment of thymol metabolite profile in plasma and tissues

Jana Pisarcikova[1, 2]
[1]Institute of Animal Physiology-Slovak Academy of Sciences, Slovakia
[2]Auburn University Harrison School of Pharmacy, USA

Due to the fact that the use of antibiotics as growth promoters in the European Union has been prohibited since 2006, the application of essential oils in food preservation, animal production and in agriculture is growing in last two decades. The main expected consequence of the ban is to minimize the amount of antibiotics used in animal production owing to concerns about consumer safety. In our experiment, the thymol metabolites – thymol sulfate was quantified and thymol glucuronide was identified in plasma, liver and duodenal wall of broiler chickens after feeding with a Thymus vulgaris L. essential oil at different concentrations (0.01%, 0.05%, 0.1% w/w) using UHPLC coupled with accurate-mass QTOF-MS. The aim of this work was to develop and validate a method in a direct way without previous enzymatic hydrolysis of thymol metabolites. The validation was performed according to the ICH guidelines and the parameters for validation were fully determined for each biological matrix; plasma, liver and duodenal wall as follows: selectivity, linearity, accuracy and precision, matrix effect, recovery, stability, limit of detection and limit of quantification. UHPLC-QTOF-MS was shown to be highly sensitive and selective for metabolite profiling and thymol metabolites were confirmed by MS software according to molecular formula, score, mass error and double bond equivalent.

janka.pisarcikova83@gmail.com

Analysis of topiramate in human plasma using liquid chromatography – tandem mass spectrometry and its application to pharmacokinetic study

Kashyap N Thummar
Saurashtra University, India

A simple, sensitive and reliable liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of topiramate (TOP) in human plasma by using the liquid-liquid extraction method. The method was developed and validated over the linearity range 15-3000 ng/mL with 0.2 mL of human plasma using niclosamide (IS) as an internal standard. The MS-MS ion transitions were monitored at m/z 338.20-78.20 and m/z 325.20-171.20 for TOP and IS in negative mode respectively. Chromatographic separation was achieved on Gemini C18 (150 mmx4.6 mm, 5 µm) column with an isocratic mobile phase composed of 2 mM ammonium acetate and acetonitrile in the ratio of 15:85 (v/v), at a flow-rate of 0.5 mL/min. This method demonstrated intra- and inter-day precision within 3.61-9.35% and 4.85-9.61% and accuracy within 93.09-105.24% and 92.46-104.40%. The extraction recoveries of TOP were over 89%. TOP was found to be stable throughout three freeze–thaw cycles, bench top and postoperative stability studies. The method is proved to be accurate and specific, and was successfully applied to the pharmacokinetic study in epileptic patients.

kasu_patel5@yahoo.co.in