Quantitative determination of zearalenone and its major metabolites in animal plasma using LC-MS/MS and (U)HPLC-HR-MS detection

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A sensitive and specific method for the quantitative determination of zearalenone (ZON) and its major metabolites (alpha-zearalenol (α-ZOL), beta-zearalenol (ß-ZOL), alpha-zearalanol (α-ZAL), beta-zearalanol (ß-ZAL) and zearalanone (ZAN)) in animal plasma using liquid chromatography combined with heated electrospray ionization (h-ESI) tandem mass spectrometry (LC-ESI-MS/MS) and high-resolution mass spectrometry ((U)HPLC-HR-MS) is presented. The sample preparation was simple and fast and consisted of a deproteinization step using acetonitrile. Chromatographic separation of the analytes of interest was achieved on a Hypersil Gold column (50 mm x 2.1 mm i.d., dp: 1.9 µm) using 0.01 % acetic acid in water (A) and acetonitrile (B) as mobile phases. A gradient elution was performed at a flow rate of 300 µL min^{-1} and a column temperature of 45 °C.
To obtain a sensitivity as high as possible, both mass spectrometers were operated in the negative h-ESI mode. For the LC-MS/MS analysis, analytes were detected in the selected reaction monitoring mode (SRM), whereas for the (U)HPLC-HR-MS analysis, the instrument was operated in the MS scanning mode (m/z 80 to 800).
Using the LC-MS/MS instrument, the method was in-house validated for all analytes, according to EU guidelines (2002/657/EC): matrix-matched calibration curves were prepared and good linearity ($r \geq 0.99$) was achieved over the concentration range tested (1 - 200 ng mL^{-1}). Limits of quantification (LOQ) were between 1 and 5 ng mL^{-1} for all compounds. Limits of detection ranged from 0.004 to 0.07 ng mL^{-1}. The results for the within-day and between-day precision and accuracy fell within the ranges specified.
The method has been successfully used for the quantitative determination of ZEA in plasma samples from broiler chickens and pigs. α-ZOL was the only metabolite that could be detected, but the concentrations were below the LOQ.
In order to investigate the presence of glucuronide conjugates of ZON and its metabolites, plasma samples were also analysed using a (U)HPLC-HR-MS instrument. In addition, the applicability of the (U)HPLC-HR-MS technique for quantitative purposes was evaluated. A good correlation ($r^2 = 0.9957$) was obtained between the results obtained with the LC-MS/MS and (U)HPLC-HR-MS instruments.
The results prove the usefulness of the developed method for application in the field of toxicokinetic analysis and for exposure assessment of mycotoxins.

Reference

Commision Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performances of analytical methods and the interpretation of results, Official Journal of the European Communities, N° L221, 17/08/2002, Decision of 12 August 2002, European Commision, Directorate General for Public Health and Consumers Protection.