

Determination of fluoroquinolone antibacterial residues in milk by LC-MS/MS method

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Introduction

Fluoroquinolones (FQs) are a group of broad-spectrum antibiotics, derived from nalidixic acid. They are widely used to treat or prevent bacterial infections in veterinary and aquatic medicine. Also they can use as growth promoters. These residues may cause bacterial resistance, allergic hypersensitivity and toxicity effect (Junza et al., 2010). Due to their side effects in public health, the European Union has established maximum residue levels (MRLs) for most antibiotics in milk and animal tissues. Various analytical methods have been reported for analysis of FQs in food producing animals including screening and confirmatory methods. LC-MS/MS method have high sensitivity and selectivity which can allow for the simultaneously separation and confirmation of many antibiotics in a single run (Alija et al., 2016; Berendsen, 2013; Uzunov et al., 2019). The aim of this study was to develop and validation of LC-MS/MS method for determination of FQ drug residues Enrofloxacin (ENR) and Ciprofloxacin (CIP) in bovine milk and analysis of real bovine milk samples for detection of these residues.

Materials and Methods

Sample collection

In this study were analyzed a total of 250 bovine milk samples, collected from Macedonian farmers during 2016-2019.

Standards, chemicals and reagents

ENR, CIP, methanol, acetonitrile, water (LC-MS grade), trichloroacetic acid (TCA), disodium hydrogen phosphate dehydrate, disodium salt of ethylene diamine tetraacetic acid, formic acid, sodium chloride, citric acid monohydrate.

Preparation of standards

Individual stock standard solutions of 1.0 mg/mL were prepared in methanol. The range of the calibration curve was from 10 to 150 ng/mL.

Sample preparation and extraction procedure

On 5 mL of milk was added 2 mL of 20% trichloroacetic acid and the samples were shaken for 5 min. After that, 20 mL of McIlvaine buffer were added and samples were centrifuged at 4000 rpm,

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20 min, at +4 °C. The supernatant was immediately applied to an SPE cartridge (Oasis HLB, 3 cc, 60 mg), previously activated with 3 mL of methanol and 2 mL of water, washed with 4 mL of water, eluted with 3 mL of methanol. The samples were evaporated to dryness under stream of nitrogen at 35 °C. The dry residue was reconstituted with 500 µL of the mobile phase and filtered on a 0.22 µm microfilter. Into LCMS/MS system injected 10 µL.

Chromatographic conditions

The compounds were separated at 40 °C using Kinetex®C18 column (1.7 µm, 50x2.1 mm). The flow rate was 0.4 mL/min and total run time was 13 min. Mobile phase A was water with 0.1% formic and mobile phase B was acetonitril with 0.1% formic acid.

MS/MS conditions

The MS/MS measurements were performed with triple quadrupole mass detector. For identification and quantification of the residues were used positive electrospray ionization (ESI+) mode and the ions were monitored in the multiple reaction monitoring (MRM) mode.

Method validation

Validation of the method was performed according to Commission Decision 2002/657/EC. Linearity, decision limit (CC α), detection capability (CC β), accuracy and precision was determined.

Results and discussion

Each individual standard with a concentration of 10 µg/mL were directly infused into the MS/MS detector for determination of precursor and product ions. Also, fragmentary voltage and collision energy for each standard were optimized. Chromatographic separation providing satisfactory resolution with retention times between 3.43 min. (CIP) and 4.75 min (ENR). Na₂EDTA-McIlvain buffer and TCA solvents were found to be sufficiently for protein precipitation, which provided acceptable recoveries.

The linear regression analysis showed good correlation with R² from 0.9817 for ENR and 0.9946 for CIP. The CC β values ranged from 120.51 ng/mL

for ENR to 121.71 ng/mL for CIP, while the CC α values ranged from 110.58 ng/mL for ENR to 115.89 ng/mL for CIP. For determination of accuracy and precision (repeatability and reproducibility) of the method a blank milk samples were fortified with a mixture of FQs standards at three concentration levels (0.5 x MRL, 1x MRL and 1.5x MRL). The MRL for ENR and CIP is 100 ng/mL. The average recoveries for all three concentration levels varied from 88% to 109%. Precision was evaluated by CV, % (coefficients of variation). The CV for repeatability ranged from 5.23 to 20.38%, while the CV for reproducibility ranged from 4.11% to 20.80%. In the milk samples included in this study the residues of CIP and ENR above the MRL values weren't detected.

Conclusion

The LC-MS/MS method has been developed and successfully validated according to the EU requirements to determination of fluoroquinolone residues in milk. The method can be used in routine analyses of milk samples.

References

- Alija, Gj., Hajrullai-Musliu, Z., 2016. A review of analytical methods for the determination of antibiotics residues in food of animal origin. *Acta Medica Balkanica* 1(2), 55-62.
- Berendsen, B.J.A., 2013. LC-MS residue analysis of antibiotics, what selectivity is adequate? PhD thesis, Wageningen University, Wageningen, NL.
- European Commission. Decision 2002/657/EC of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and interpretation of results.
- Junza, A., Amatya, R., Perez-Burgos, R., Gokce, G., Grzelak, E., Barbosa, J., 2010. Residues of β -lactams and quinolones in tissues and milk samples. Confirmatory analysis by liquid chromatography–mass spectrometry. *Ovidius University Annals of Chemistry* 21(2), 109-122.
- Uzunov, R., Hajrulai-Musliu, Z., Stojkovski, V., Dimitrieska-Stojkovic, E., Stojanovska-Dimzoska, B., Sekulovski, P., Jankuloski, D., 2019. Development and validation of LC-MS/MS method for determination of ten beta agonists in bovine urine. *Kafkas Univ. Vet. Fak. Derg.* 25(1), 55-60.