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Pharmacochemistry

Determination of Metabolites of Nitrofurans in Chicken Eggs by Liquid Chromatography with Diode-Array Detector

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Nitrofuran antibiotics have been banned for use in food-producing animals in many countries, including the European Union, owing to the threat they pose to human health. In the paper an improved liquid chromatography method using a diode-array detector, to detect parent compounds, furazolidone (AOZ), nitrofurantoin (AHD), furaltadone (AMOZ) and nitrofurazone (SEM), in chiken eggs is presented. Considering the requirement of MRPL (Minimum Required MRPL Performance Limit of 1 µg/kg), we achieved the required detection limit using high performance liquid chromatography by solid phase purification of the sample after extraction. For solid-phase purification, instead of expensive cartridges (columns), columns with silica gel of our own production were used. The method complies with the requirements of the European Commission Resolution 2002/657/EC. The validation of the method was conducted following the European Union criteria for the analysis of veterinary drug residues in foods. The decision limits (CCα) were 0.16-0.34 µg/kg, and the detection capabilities (CCβ) 0.20-0.35 µg/kg. The advantage of the method is that with relatively less financial costs it is possible to determine the amount less than the minimum working limit for nitrofuran metabolites set by the EU. *© 2023 Bull. Georg. Natl. Acad. Sci.*

LC/DAD, nitrofuran metabolites, furazolidone, furaltadone, nitrofurazone, nitrofurantoin, chicken eggs

Nitrofurans are a group of drugs related by chemical structure to 5-nitro-2-furfulidenhydrozones. Nitrofurans, except for furazolidone, have a small molecular weight, due to which they easily penetrate the walls of blood and lymphatic vessels, as well as the blood-brain and placental barriers. Their antimicrobial activity is manifested both against gram-positive bacteria, bartonella, pathogens of fungal infections and a number of large viruses. Nitrofurans are effective against antibiotic- and sulfanilamide-resistant strains of pathogens and also as a growth factor [1-3]. These medicines are rapidly metabolized within a few hours after ingestion, and residual metabolites can remain in the organism for several weeks and possibly months in the form of protein connected [4,5]. It was confirmed that these metabolites of nitrofurans represent a potential risk to human health due to their carcinogenic, teratogenic and mutagenic effects [6]. Metabolites of nitrofurans disrupt the electrolyte balance in the human body, inhibit the activity of liver enzymes, cause cardiomyopathy

and have a carcinogenic and mutagenic effect. They are thermostable and remain in products after culinary processing.

The use as veterinary drugs of the four main nitrofurans furazolidone, furaltadone, nitrofurantoin and nitrofurazone is limited in the EU, USA, Brazil, Thailand and other countries [7]. Considering the requirements of the European Union, the use of nitrofurans in animal husbandry is also regulated in Georgia [8]. However, due to their low cost and significant effectiveness, the use of nitrofurans is allowed or used illegally as a veterinary drug in some developing countries [9].

As of today, in the EU, in poultry and seafood, marginal permissible norm of the mentioned four nitrofurans (MRPL – Minimum Requried Performance Regulation Limit) is 1 μg/kg [7, 10].

In the interest of exports, third countries are forced to adopt the MRPL set by the Council of Europe and thus reach the same threshold as EU laboratories [11]. Given the strict regulations and validation requirements of analytical methods set by the Council of Europe, the development of highly sensitive and specific analysis methods for the determination of nitrofuran residues in foodstuffs is becoming an increasingly difficult task [12-15].

Many analytical methods for the determination of nitrofuran metabolites have been developed in various matrices.

But not all of these methods meet the requirements due to the inappropriate detection threshold required for the substances under study. Therefore, HPLC-MS/MS is mainly used because of the highest sensitivity and accuracy. The application of this method of analysis is limited due to the high cost of equipment and maintenance of this equipment, and therefore it is impossible to implement this method in economically developing countries. The liquid chromatographic method using a diode-array detector is relatively less expensive and convenient than other detection methods, but there is little data on its use for this purpose due to the fact that it is difficult to achieve the required detection limit using a diode detector.

Therefore, the aim of this work was, to develop a method for purifying an extract of chicken eggs, which makes it possible to increase the sensitivity of the determination of nitrofuran metabolites in the extract by a liquid chromatographic method, taking into account the MRPL and validation of the developed method.

The effectiveness of the method developed by us, is confirmed by the results of professional testing, implemented in the testing laboratory "GlobalTest" accredited according to ISO 17025 by the Accreditation Agency of Georgia.

Chiken eggs were chosen as the study matrix, as chiken egg control ensures the safety of products derived from controlled the chiken eggs. As a result, we obtained sensitive, fast and relatively inexpensive method that meets the requirements of the established MRPL.

Materials and Methods

Research sample. Chicken eggs were taken from different poultry farms located in Georgia.

Standard solutions. Individual standard stock solutions of 1 mg/mL were prepared in acetonitrile. Working solutions of 10 ng/mL were diluted with water. All standard stock soutions were stored – 20ºC, and the working solutions were stored in refrigerator.

The concentration and content of mix standard solution were used to spiked samples with AMOZ, AOZ, AHD and SEM at a 8, 16, 20, 28 and 36 ng/mL respectively.

Preparation of silica gel columns. The silica gel column was prepared by the dry method (20 x 400 mm). The prepared column was washed with 5 ml of hexane, dried under vacuum, then washed with 10 ml of acetonitrile, and again dried under vacuum.

Instrumentation. LC/DAD (liquid chromatography with diode-array detector). The LC/DAD system consisted of an Agilent Series 1260 HPLC system (Agilent Technologies, Germany) whith DAD detector.

The chromatography was performed in a C18 column 3 μm x 2 mm x 150 mm (Phenomenex, USA), connected to a C18 precolumn 3 μm x 2 mm x 4 mm (Phenomenex, USA). The mobile phase was Acetonitrile: 0.01 M sodium acetate buffer pH 6.0 – 250:750, λ 376 nm, flow rate of 1 mL/min, Injection volume was 50 μL. The column was thermostated at 30^0C.

Results

The study was conducted on 170 samples of chicken eggs taken from various poultry farms, located on the territory of Georgia.

Specificity/Selectivity. Specificity/selectivity were evaluated via analysis of blank matrix samples fortified with standards of nitrofuran metabolites (concentration of 1 μg/kg each).

According to analysis for the studied substances of the nitrofuran group, the specific wavelength is 376 nm, no significant peaks with an S/N (signal to noise) ratios of 3 or more and chromatographic interference were being observed at the retention times of the targeted nitrofuran metabolites;

The coefficient of variation of the specificity of the results obtained during the working day and during the working week <2%, which indicates satisfactory, required by Decision 2002/657/EC [1].

LOD (Limit of detection) and LOQ (Limit of quantitation). For furazolidone LOD – 0.32 μg/kg, LOQ – 0.91μg/kg; For furaltadone LOD – 0.35μg/kg, LOQ – 0.95 μg/kg; For nitrofurantoin LOD – 0.29 μg/kg, LOQ – 0.91 μg/kg; For nitrofurazone LOD – 0.38 μg/kg, LOQ – 0.99 μg/kg.

Linearity. To construct calibration graphs, we used 5 concentrations: 0.4, 0.8, 1, 1.4 and 1.8 μg/kg for all four metabolites. The graphs are linear in the indicated range and are acceptable as long as the correlation coefficient r^2 is above 0.999.

Recovery. The method recoveries and RSDs were determined from 6 replicates at four concentration levels spiking blank samples over three days. The recovery results were observed in acceptable range of 70-110%. mean recoveries ranging and CV% values were satisfactory, required by Decision 2002/657/EC [1].

Decision limit (CCα) and detection capability (CCβ). The decision limits (CCα) were 0.16-0.34 μg/kg, and the detection capabilities (CCβ) 0.20-0.35 μg/kg.

Conclusion

Taking into account the requirement of MRPL (Minimum Required MRPL Performance Limit 1 μg/kg), we have improved a high-performance liquid chromatographic method for the determination of nitrofuran metabolites by solid-phase purification of the sample after extraction. For solid-phase purification, instead of expensive cartridges (columns), columns with silica gel of our own production were used. The method complies with the requirements of European Commission Resolution 2002/657/EC.

The obtained results of the validation of the method developed by us testify to the compliance with Decision 2002/657/EC [1]. CCα and CCβ are below the MRPL of 1 μg/kg.

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ქათმის კვერცხის ნიმუშში ნიტროფურანების მეტაბოლიტების ნარჩენების რაოდენობრივი განსაზღვრისთვის სრულყოფილი და ვალიდირებულია მგრძნობიარე, ზუსტი, ეფექტური და შედარებით იაფი სითხოვანი ქრომატოგრაფიული მეთოდი დიოდური დეტექტორის გამოყენებით. მსზ-ის (მინიმალური სამუშაო ზღვარი, 1 მკგ/კგ) მოთხოვნის გათვალისწინებით, სითხოვანი ქრომატოგრაფიული მეთოდის მგრძნობელობის გასაზრდელად, ექსტრაქტის გასასუფთავებლად, ნაცვლად ძვირადღირებული სვეტებისა, გამოვიყენეთ ჩვენ მიერ მომზადებული სილიკაგელის სვეტები. კვლევა ჩატარდა საქართველოს ტერიტორიაზე არსებულ, მეფრინველეობის ფაბრიკებიდან აღებულ, ქათმის კვერცხის 170 ნიმუშზე. მეთოდი შეესაბამება ევროკომისიის 2002/657/EC დადგენილებას. ვალიდაციის შედეგად დადგინდა მეთოდის სრული შესაბამისობა საერთაშორისო მოთხოვნებთან შემდეგი ვალიდაციური მახასიათებლების მიხედვით: სპეციფიკურობა, აღმოჩენის ზღვარი, რაოდენობრივი განსაზღვრის ზღვარი, სწორხაზოვნება, სიზუსტე, ზღვრული მნიშვნელობა (CCα) და გამოვლენის შესაძლებლობა (CCβ). მეთოდი წარმატებით შეიძლება იქნეს გამოყენებული ქათმის კვერცხში ნიტროფურანების ოთხი მეტაბოლიტის ერთდროული განსაზღვრისთვის და იძლევა განვითარებად ქვეყნებსა და რეგიონებში ქათმის კვერცხისა და, შესაბამისად, ამ კვერცხისგან მომზადებული პროდუქტების უსაფრთხო გამოყენების გარანტიას. მეთოდის ეფექტურობას ადასტურებს ISO 17025-ით აკრედიტებული, გლობალტესტის საგამოცდო ლაბორატორიის პროფესიული ტესტირების შედეგები.

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