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NITROFURANS AND THEIR METABOLITES IN FOOD

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რეზიუმე

ნიტროფურანები (ფურაზოლიდონი, ფურალტადონი, ნიტროფურანტოინი, ნიტროფურაზონი), მიეკუთვნება ფართო სპექტრის სინთეზურ ანტიბიოტიკებს, რომლებიც გამოიყენებოდა მეცხოველეობაში, აქვაკულტურაში და მეფუტკრეობაში გასტრო-ინტესტინალური ინფექციების პრევენციისთვის. მათ იყენებდნენ აგრეთვე ზრდის სტიმულაციის მიზნით საკვები დანამატების სახით. 1995 წელს ევროკავშირში ნიტროფურანების გამოყენება ვეტერინარიაში აიკრძალა საკვებ პროდუქტებში მათი ნარჩენების კანცეროგენური და ადამიანის ორგანიზმზე პოტენციური მავნე გავლენის საშიშროების გამო. ევროკავშირში ნიტროფურანების უკანონო გამოყენება კონტროლდება ოფიციალური საინსპექციო და ანალიზური სამსახურების მიერ.

ევროსაბჭოს 96/23/EC დირექტივების მოთხოვნების შესაბამისად, ნიტროფურანების შემცველობა ცოცხალ ცხოველებსა და ცხოველური წარმოშობის სურსათში კონტროლდება საქართველოს მთავრობის დადგენილებითაც, თუმცა ნიტროფურანებისა და მათი ნარჩენების მონიტორინგის მიზნით დღეისათვის ძირითადად გამოიყენება ანალიზის სკრინინგული, იმუნოფერმენტული მეთოდი. ევროსაბჭოს მიერ დადგენილი ახალი, მკაცრი რეგულაციების და ანალიზური მეთოდების ვალიდაციის მიმართ განსაზღვრული მოთხოვნების გათვალისწინებით, აუცილებელია საკვებ პროდუქტში ნიტროფურანების ნარჩენების განსაზღვრისთვის მაღალმგრძნობიარე სპეციფიკური ანალიზის მეთოდების შემუშავება, შემუშავებული მეთოდის საშუალებით სხვადასხვა მატრიცაში ნარჩენების განსაზღვრისთვის ექსტრაგირების კოეფიციენტების გამოთვლა და შემუშავებული მეთოდის ვალიდაცია.

Nitrofurans, namely furazolidone (AOZ), furaltadone (AMOZ), nitrofurantoin (AHD), and nitrofurazone (SEM), are broad-spectrum synthetic antibiotics containing the characteristic 5-nitrofuran ring. Nitrofurans are commonly used in livestock (poultry, pigs, cattle), aquaculture (fish, shrimp), and in beekeeping to prevent and treat agastrointestinal infections caused by Escherichia coli, Salmonlella spp., Coccidia spp., Oliforms and protozoa, Which are found in animal products and water [13,14,15,31,32,37]. They were also used as food supplements to stimulate growth.

In 1995, the use of nitrofurans in livestock was banned in the EU [17] due to the risk of carcinogenic and potentially harmful effects of their waste in food [51,67]. In the EU, the use of four major nitrofurans - furazolidone, furaltadone, nitrofurantoin and nitrofurazone as veterinary drugs is restricted due to their toxic, carcinogenic and mutagenic properties. According to EU rules, products intended for EU countries are subject to EU rules and products imported into these countries should not contain nitrofurans [11]. The use of nitrofurans in livestock is also banned in countries such as Australia, the US, the Philippines, Thailand and Brazil.

Despite the complete ban on the use of nitrofurans in livestock, these drugs are freely available for veterinary and human therapy: Nitrofurazone is used in infected burns and skin infections, furazolidone - for the oral treatment of cholera [62], as well as for the treatment of bacterial diarrhea and giardiasis [61] and nitrofurantoin - for the treatment of urinary tract infections [43].

Studies on the metabolism and toxicity of furazolidone have shown that monitoring of nitrofuran residues based only on the determination of the initial structures did not provide adequate data on the actual contamination of tissues and their damage to health [69,70]. Therefore, certain

metabolic structures of drugs have been identified as marker residues to control the illicit use of nitrofuran antibiotics by determining waste in tissues.

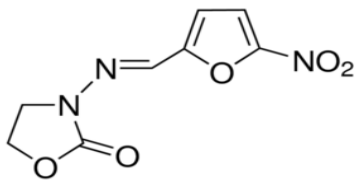
The development of highly sensitive and specific analysis methods for the determination of nitrofuran residues in food is becoming an increasingly difficult task, given the new, stringent regulations and validation requirements set by the Council of Europe [10,11,72].

A key role in the development of sensitive methods for monitoring nitrofuran metabolites was played by the EU multinational project FoodBRAND (2000–2003), organized by the Department of Veterinary Sciences of the Royal University of Belfast. This project provided the European Commission with analytical methods, analytical standards and the study and training of new instrumental methods. The project developed the first immunoassays to identify nitrofuran metabolites in food.

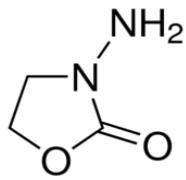
FoodBRAND additionally studied data from the monitoring of nitrofuran waste residues in pork in European countries. It played a crucial role in detecting the global nitrofuran crisis in food production [7].

According to the 2003 MRPL (Minimum Requried Performance Regulation 1442/95), the maximum permissible limit for above mentioned four nitrofurans in the EU in poultry and seafood is 1 μg / kg (Commission Decision 2003/181 / EC) [11,16].

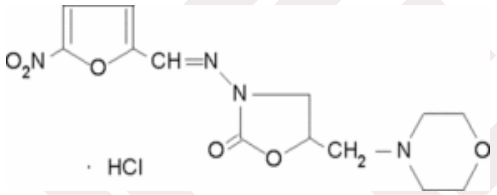
Studies have shown that nitrofurans are rapidly metabolized in the animal and their in vivo stability does not exceed several hours. Unlike basic molecules, the protein-binding complex in the body is stable and remains constant. Release of residues from proteins is possible by acid hydrolysis. Nitrofurans can be determined by identifying not bonded molecules:



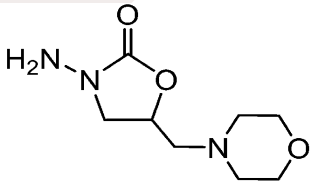
Furazolidone



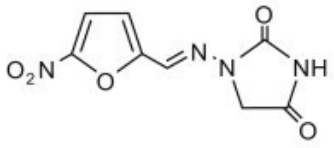
3-Amino-2-oxazolidinone (AOZ)
Stable metabolite



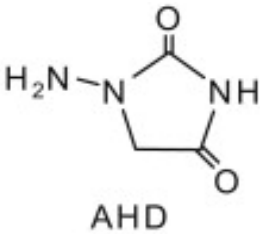
Furaltadone



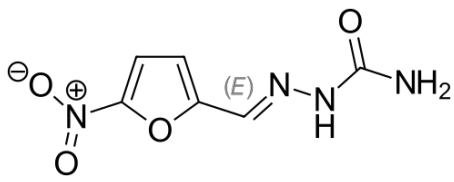
AMOZ
Stable metabolite



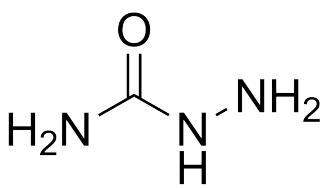
Nitrofurantion



AHD
Stable metabolite



Nitrofurazone



Semicarbazide (SEM)
Stable metabolite

Studies on the bioavailability of nitrofuran metabolites have shown that it is possible to transfer the waste to a secondary organism, in particular, when rats were fed radioactive, isotope-indicated furazolidone (14C) tissue, 41% of the total isotope content was in the rats organism [6,28,40].

Bioavailability is also high from other contaminated products such as, eggs. Experiments have also demonstrated the stability of metabolites after storage or heat treatment of meat [12,19-26,37]. In particular, the concentration of nitrofurans did not change after 8 months of storage of pork and liver samples. The authors showed that after cooking, roasting, grilling, or microwave the samples, 67–100% of the pre-quantified nitrofurans in the samples were determined. Other studies have shown that AOZ in eggs is stable for 12 months at 4°C and 78% of it accumulates in the heart of the egg [56]. It was also found that 50% of SEM accumulates in the egg shell [53,54,55,57,58].

The mutagenicity and toxicity of nitrofurans are considered not only because of their misuse in livestock, but also with particular attention paid to the toxicology of semicarbazide found in foods for which nitrofurazone was not used.

Mutagenicity studies in the 1970s and 1980s revealed the potential effects of nitrofurans on bacterial and mammalian cells. It has been suggested that endogenous nitroreductase is responsible for the in vitro recovery of nitrofurans in E. coli, which causes DNA cell damage in the stationary phase of bacterial growth [52]. The formation of DNA additives (adducts) after bacterial replication mistakenly induces the process of DNA replication, which indicates the mutagenic activity of the drug [48,50,51].

In vitro toxicity and mutagenicity in mammalian cells have been poorly studied. Studies show that irreversible DNA damage to human epithelial cells (HEp-2) as well as hormonal disorders manifested by endocrine dysfunction occur predominantly under the influence of furazolidone [5]. Most of the available information describes in vivo studies in which mice or rats were used as a model to study the effects of furazolidone and its major metabolites, nitrofurazone and semicarbazide.

The main studies in 1988 were devoted to the F344 / N and B6C31 groups of mice of both sexes that received nitrofurazone for 14 days, 13 weeks and 2 years. The results clearly showed carcinogenic activity as a direct result of nitrofurazone intake, which was demonstrated by increased breast adenoma in female rats as well as tumor formation in the ovaries. Other common signs of toxicity in mice of both species and both sexes were seizures, osteoporosis, degenerative atrophy, and usually coarse fur and lethargy, as well as reduced dose-related food demand [48].

Other studies in the B6C3F1 group of mice administered nitrofurazone daily for 14 days at various low doses did not show any significant changes by testing immunological resistance parameters. Significant reproductive disorders were observed in Swiss 1 CD group mice given nitrofurazone for 15 weeks. Overall, all experimental mice showed reduced fertility and lower weight in the offspring compared to the control group. Epidemiological studies showed that the sperm concentration decreased by 20-98%, and the number of abnormal spermatozoa tripled compared to the control group. The researchers concluded that the reproductive effects were due to exposure to nitrofurazone [41].

Major studies of toxicity and carcinogenicity have focused on the introduction of "SAM" into mice. Adittionally to above mentioned side effects, Lethargy [65], fetus death, and developmental delay [70], tissue abnormalities such as brain, liver, intestinal hemorrhage, abnormal bone formation, and poorly developed ovaries were also present [28,29]. Two studies have shown that mice exposed to low doses of "SAM" were significantly more likely to develop lung tumors [50,51], with some researchers reporting high mortality from nitrofurans [66].

The opinion of the EU Food Safety Almanac and European Food Safety Agency (EFSA) on the presence of semicarbazide and nitrofurazone metabolites in food has been published [73,35]. Due to the large differences between the experimental animals and the human, as well as the use of a sensitive methodology for the study (the drug was administered intraperitoneally to the experimental animals, resulting in a direct effect on the uterus), the EFSA concluded that nitrofuran carcinogenic effects on humans are not significant. However, it should be noted that nitrofurazone, nitrofurantoin, furaltadone, and furazolidone were included in the list of carcinogens by the State of California [67].

The global nitrofuran crisis of 2002–2003 revealed quite frequent imports of nitrofuran-containing animal products and aquaculture to EU countries from Thailand, China, Taiwan, India, Vietnam, Ecuador and Brazil. [7]

In addition, nitrofurans have been reported in poultry and pork produced in EU countries such as Portugal, Italy, Greece, Romania and Bulgaria. Later nitrofurans were found in products such as shrimp, honey, and canned meat. Particularly high concentrations were observed in products imported from countries such as India, China, Bangladesh and Thailand [34,36,38,39].

Although the use of nitrofurans in animal feed is strictly prohibited in the EU, they are still used because of their effectiveness and availability, as evidenced by the European Commission's Rapid Notification System (RASFF). This system has been in operation since 1979 and is a very effective tool for food safety control [27]. The high concentrations of SEM in infant food have also been great concern and have led to the development of methods for the determination of nitrofuran residues in food [30,41].

Azodicarbonamide, a foaming agent used to make gaskets, has been found to decompose mainly by nitrogen and carbon dioxide as a result of heat treatment and may leave traces of substances such as urea, urazole, cyanic acid, [33] further more as a side effect of gasket production (for jars) was "SAM" [64]. Potentially sensitive products to such gaskets are jams, honey, fruit juice, marinades, sterilized products, mayonnaise, mustard, and ketchup. Azodicarbonamide is currently banned in the EU [16].

The formation of "SAM" has also been observed in the process of baking bread [8,9] and making flour purees [42] in cases of adding azodiacarbonamide to flour [44,45]. "SAM" has also been found in nature in some crabs, shrimp, which questioned their use as food [60]. Nitrofurazone has been found to accumulate over time in the eyes of birds, in the retina of pig eyes, and these organs have been suggested as markers for "SAM" [20].

In 2008, Samsonova and co-authors [63] isolated proteins from rat liver and investigated the content of SEM binding metabolites. Albumin and glutathione-S-transferase contained relatively high concentrations of bound SEM, allowing them to be used as biomarkers to study the effects of nitrofurans. In 1995, nitrofuran antibiotics were included in Annex IV to Regulation (EC) 1442/95 [17] as substances prohibited for use in livestock. The EU has also set a minimum threshold (MRPL) for animal feeding of 1 μg / kg [33].

This days, the illicit use of nitrofurans in the EU is controlled by official inspection and analysis services in accordance with the requirements of EU Directive 96/23 / EC.

According to this document, plans to monitor nitrofurans in live animals and food of animal origin should be developed by EU Member States [34]. Due to the fact that laboratories have to perform a large amount of work in a relatively short time in monitoring process, they can use screening methods to increase the efficiency of the work, however, in case of a positive result with the screening method, the result must be confirmed by appropriate instrumental methods.

Despite MRPL being 1 μg / kg, the application and interpretation of this legislation in relation to exports from third countries in Europe has been quite difficult. The implementation of the new legislation has led to various restrictions for these countries and required large investments to purchase new and sensitive instrumentation analysis equipment. In the interest of exports, third countries are forced to adopt the Council of Europe MRPL to reach the same threshold as EU laboratories [49].

The European Commission Decision (2003) establishing the MRPL excludes the use of unsatisfactory methods by which very low concentrations of nitrofuran metabolites can not be determined, however, the MRPL concept does not include any provision on the maximum standard for methods [49]. In other words, the method is needed to quantify the concentration of 1 μg / kg of nitrofuran metabolites, but the lowest concentration is not specified. This value is called the decision-making margin CCα and is determined by many laboratories in accordance with the requirements of the Validation Guidelines established by the Council of Europe. However, fluctuations in CCα magnitude between different instrumental methods and different laboratories are a regulating factor.

To ensure the quality and comparability of analysis results obtained in laboratories, general efficiency criteria for waste analysis have been incorporated into European legislation. Decision 2002/657 / EC sets out guidelines for both screening methods and certification methods. The Commission resolution implements Council Directive 96/23 / EC on the use of the method and the interpretation of the results [18].

In accordance with this decision, in order to evaluate the screening method used for quantification, it is mandatory to define working characteristics such as detection capacity (CCβ),

accuracy, selectivity, usability and stability. Accuracy for quantitative screening is achieved by determining the coefficient of variation. Testing for variation between analyzes gives an idea of the accuracy of the analysis for a longer period of time. Selectivity is the ability of a method to distinguish between analytical and other substances. For screening methods used for qualitative analysis, the stability of the standard analysis in solution and matrix should be studied, only CCβ and selectivity should be determined.

Any positive results detected by the screening method should always be repeated and verified by the confirmatory method. In order for a method to be classified as an affirmative method, the decision-making margin (CCα) and accuracy must be determined [18]. CCα is defined as the limit at which a positive decision on the determination of a substance is made α = 1%. Probably.

Validation / confirmation of the developed method shows that the analysis method meets the criteria of the relevant working characteristics. For the validation / approval procedures of the analysis methods, the technical regulation approved by the Resolution №499 [4] of the Government of Georgia shall apply - the rules for the implementation of the analysis methods and the interpretation of the results of the analysis methods and results for the examination of certain substances and substances of animal origin and waste.

It is possible to use other methods to demonstrate the compliance of the working criteria of the analysis method with the working characteristics, provided that the same level and quality of information is achieved. Validation / confirmation can also be performed by conducting an interlaboratory examination as established by the Code Alimentarius, ISO or IUPAC IUPAC [46], or by an alternative method such as a separate laboratory testing or internal validation / confirmation [42, 47].

In accordance with EU requirements, the use of nitrofurans in livestock is regulated by Georgian law, in particular Article 56 first part of the Product Safety and Free Circulation Code, Article 58 - second part, and the Food / Animal Safety, Veterinary and Plant Protection Code 75 According to Part 2:

Resolution №567 of the Government of Georgia on the Maximum Permissible Limit for Some Contaminants (Contaminants) in Food [1], Resolution 39639 of the Government of Georgia approves the Technical Regulation on the maximum limit for the release of pharmacologically active substances, their classification and food content of animal origin [2], Resolution №22 of the Government of Georgia approves the "Technical Regulation - Rules for Monitoring Certain Substances and Their Residues in Live Animals and Food of Animal Origin" [3] and Resolution №499 of the Government of Georgia Rule of implementation of analysis methods and interpretation of results for examination of substances and their waste [4].

At present days, in accordance with the Resolution №22 of the Government of Georgia, some substances and their residues in live animals and food of animal origin are monitored. Due to the fact that Georgia does not have much experience in such monitoring, the analysis screening, immunoenzymatic method is mainly used in the monitoring process. However, in accordance with Article 15 point 2 of the above-mentioned decree, "in case of a positive result in a laboratory examination using a routine method, the result obtained shall be verified by the reference method".

It is clear that in order to comply with Article 15 point 2 of Article 22 of the Government of Georgia, it is necessary to develop a highly sensitive instrumental method for the determination of nitrofuran waste in animal products, calculation of extraction coefficients for the determination of waste in different matrices by means of the developed method and validation of the developed method in accordance with the requirements of Resolution №499 of the Government of Georgia.

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НИТРОФУРАНЫ И ИХ МЕТАБОЛИТЫ В ПИЩЕВЫХ ПРОДУКТАХ

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РЕЗЮМЕ

Нитрофураны (фуразолидон, фуралтадон, нитрофурантоин, нитрофуразон) относятся к широкому спектру синтетических антибиотиков, используемых для профилактики желудочно-кишечных инфекций в животноводстве, аквакультуре и пчеловодстве. Их также использовали в качестве пищевых добавок для стимуляции роста.

В 1995 году использование нитрофуранов в ветеринарии было запрещено в Европейском союзе из-за канцерогенного и потенциально вредного воздействия на человека. Незаконное использование нитрофуранов в ЕС контролируется официальными службами инспекции и анализа.

В соответствии с требованиями Директивы Совета Европы 96/23/ЕС, содержание нитрофуранов в живых животных и продуктах питания животного происхождения также контролируется Правительством Грузии. Скрининг, иммуноферментный метод анализа в настоящее время используется для мониторинга нитрофуранов и их отходов.

В соответствии с новыми строгими правилами и требованиями к валидации аналитических методов, установленными Советом Европы, необходимо разработать высокочувствительные и специфические методы анализа для определения остатков нитрофурана в пищевых продуктах. Рассчитать коэффициенты извлечения для определения отходов в различных матрицах с использованием разработанной методики и валидировать разработанную методику.

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NITROFURANS AND THEIR METABOLITES IN FOOD

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SUMMARY

Nitrofurans (furazolidone, furaltadone, nitrofurantoin, nitrofurazone) belong to a wide range of synthetic antibiotics used in the prevention of gastrointestinal infections in livestock, aquaculture and beekeeping. They were also used as food supplements to stimulate growth. In 1995, the use of nitrofurans in veterinary medicine was banned in the European Union because of the carcinogenic and potentially harmful effects in humans. Illegal use of nitrofurans in the EU is controlled by official inspection and analysis services.

In accordance with the requirements of Council of Europe Directive 96/23/EC, the content of nitrofurans in live animals and food of animal origin is also controlled by the Government of Georgia. Screening, immunoenzymatic method of analysis is currently used to monitor nitrofurans and their wastes.

In accordance with the new, strict regulations and validation requirements of analytical methods set by the Council of Europe, it is necessary to develop highly sensitive and specific analysis methods for the determination of nitrofuran residues in food. Calculate extraction coefficients for waste determination in different matrices using the developed method and validate the developed method.

Keywords: nitrofurans, metabolites, food.