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Analysis of veterinary drug residues in food: The nitrofurans issue

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Introduction

It is common practice in residues testing laboratories across the world to use a two-stage process to ensure efficient and cost-effective use of resources. Specific, yet high volume, low-cost screening methods are combined with low volume, high-cost confirmatory methods to ensure that samples that fail to comply with relevant national legislation are correctly identified. However, the availability of suitable analytical equipment is insufficient. Information concerning the appropriate marker residue is also needed for effective laboratory control. In the case of licensed substances, internationally accepted Maximum Residue Limits define both the sample type and the marker residue that can be analysed. In the case of banned substances, e.g. the nitrofurans, a definitive statement concerning the marker residue to be detected is not available.

Residue controls in the European Union

The EU has put in place a series of measures to protect the health of the consumer and to ensure that food of animal origin produced within the EU and produced in third countries to be exported into the EU is safe to eat. Licensed veterinary medicines are evaluated by the European Medicines Evaluation Agency. Based on their evaluation, a Maximum Residue Limit (MRL) is calculated for each medicine in the tissues of each animal species in which the product may be used. Large safety margins are built into the calculation of MRLs. This means that a violative concentration ($>$ MRL) in tissue does not necessarily pose a risk to human health. It does, however, mean that the product is not suitable for the purposes of international trade. All MRLs are listed in the Annexes to Council Regulation 2377/90 (1). Compounds listed in Annexe I have had a final MRL established (e.g. sulphonamides). Compounds listed in Annexe II require no MRL (e.g. vitamin A).

*Lecture presented at the 115th annual conference of the Swiss Society of Food and Environmental Chemistry in Berne on 12 September 2003

Compounds that are still under evaluation are granted a temporary MRL and are listed in Annexe III. A further annexe to this regulation (Annexe IV) lists those compounds for which no MRL can be set. These compounds cannot be used in food-producing animals. The nitrofurans are included in this group.

Use of nitrofuran drugs in the EU

The nitrofurans are a group of antibacterial compounds. The four main members of this group are furazolidone, furaltadone, nitrofurantoin and nitrofurazone (figure 1). They are relatively broad-spectrum bactericidal drugs. They are active against *Salmonella* spp., coliforms, *Mycoplasma* spp., *Coccidia* spp. and some other protozoa. The use of all nitrofurans, with the exception of furazolidone was banned in the EU in 1993 (2). The ban was introduced because of concerns over the carcinogenicity of these compounds. Two years later, the ban was extended to cover furazolidone. Again, the reasons for the ban were the carcinogenicity of the parent drug, the extensive metabolism of furazolidone and the lack of information concerning the safety of its metabolites (3). Since then, it has been forbidden to use any nitrofuran in any animal within the EU, or in any animal destined for export into the EU.

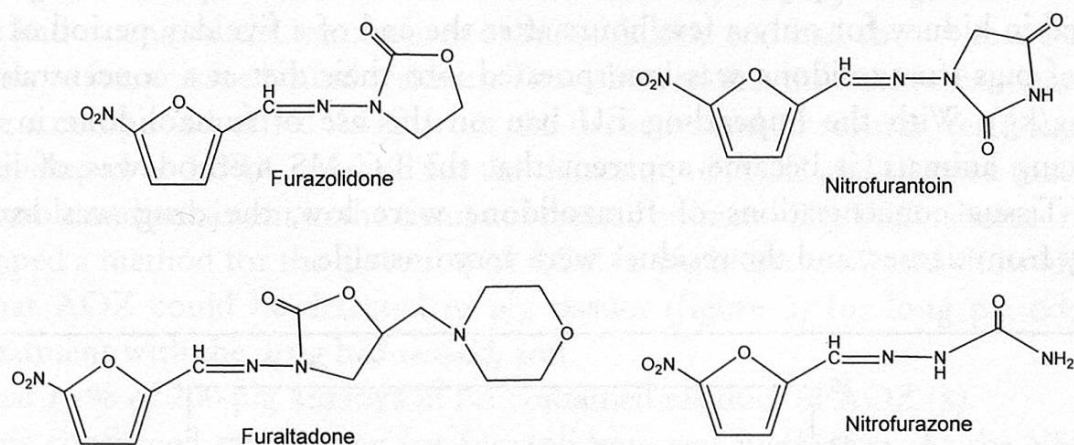


Figure 1 Structures of the nitrofurans

Monitoring for veterinary drug residues

The EU requires Member States (MS) to establish national residues surveillance programmes (4) to monitor food of animal origin for the presence of licensed medicines, Annexe IV compounds and for residues of the hormonal growth promoters and β -agonists, which are also banned in the EU (5). The EU requires MS to submit monitoring plans to the Commission for approval every year, and required MS to report results back to the Commission on an annual basis. The Commission has also established a rapid alert system to inform MS when potentially harmful residues are detected in products imported from third countries. This system is designed to speed up MS responses when such residues are detected. All EU MS are

therefore required to test domestic product for the presence of nitrofurans. This requirement has been in place since before the compounds were banned in the 1990s. When furazolidone was licensed in the EU, it had a provisional MRL (it was included at that time in Annexe III of Council Regulation 2377/90) of 5 µg/kg. The marker residue was defined as “any compound containing an intact 5-nitro group.” In practice, most MS developed analytical methods to detect the parent drug, usually based (at that time) on HPLC with UV detection.

Detection of nitrofurans

In common with most other EU laboratories, the laboratory of the Veterinary Science Division (VSD) developed an LC-MS assay for the detection of furazolidone in the early 1990s (6). Using this method, which had a detection limit of 2 µg/kg, several hundred pig kidneys were tested before the ban for the presence of furazolidone parent drug and positive results were never found. Either pig farmers in Northern Ireland (NI) were not using furazolidone or the test was ineffective. A series of animal experiments were carried out in this laboratory, which showed that furazolidone residues were very unstable in the tissues of treated animals (even when frozen). Furthermore, it was shown that the parent drug was very rapidly excreted from treated pigs. Figure 2 shows that furazolidone parent drug can be detected in kidney for only a few hours after the end of a five day period of treatment of pigs (furazolidone was incorporated into their diet at a concentration of 400 mg/kg). With the impending EU ban on the use of furazolidone in food-producing animals, it became apparent that the LC-MS method was of limited value. Tissue concentrations of furazolidone were low, the drug was excreted rapidly from tissues, and the residues were very unstable.

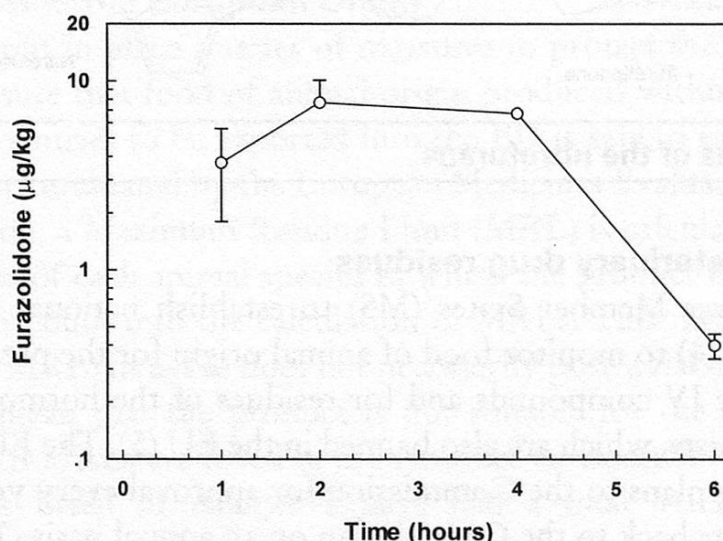


Figure 2 Depletion of furazolidone from the kidney of pigs treated with 400 mg/kg furazolidone for five days

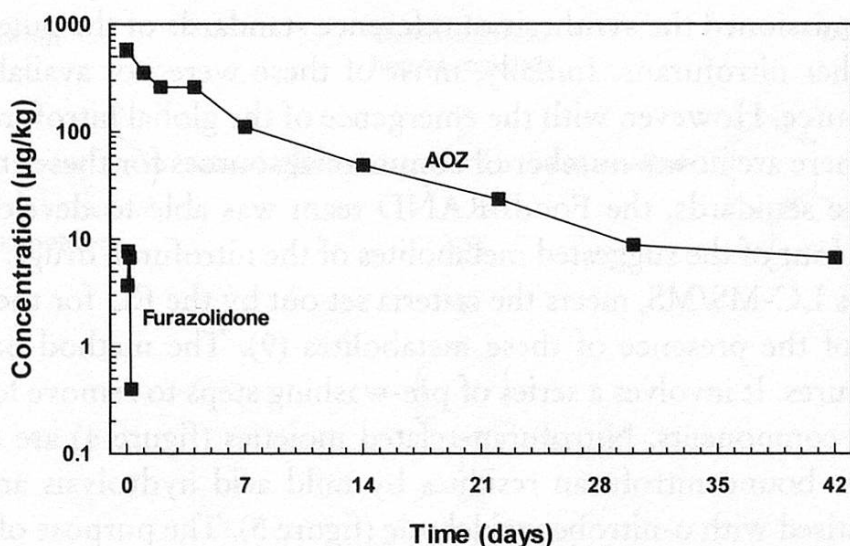


Figure 3 Depletion of furazolidone and AOZ from the kidney of pigs treated with 400 mg/kg furazolidone for five days

In the late 1980s, RIKILT (Institute of Food Safety in Wageningen, The Netherlands) had examined the metabolism of furazolidone and had shown that the drug was able to form tissue bound residues. They showed that mild acid treatment could release a compound called AOZ from the tissue bound nitrofurans residues. They had developed an HPLC method for the detection of AOZ and showed that it was both stable and persistent in the tissues of treated animals. The VSD laboratory then developed a method for the detection of AOZ in pig kidneys (7) and showed:

- That AOZ could be detected in pig tissues (figure 3) for long periods after treatment with the drug had ceased, and
- That 16% of 200 pig kidneys in NI contained residues of AOZ (8).

This confirmed that testing for furazolidone was ineffective. At the VSD, we never found any furazolidone residues in NI pigs, but were able to show that it was widely used in the pig industry. When the ban was introduced, this laboratory was in a position to monitor compliance of the ban on the use of furazolidone – but was unable to control any of the other nitrofurans drugs.

FoodBRAND and analytical methods for nitrofurans drugs

The FoodBRAND project (www.afsni.ac.uk/foodbrand) was funded by the European Commission to rectify this situation. FoodBRAND has four main aims:

- To develop screening tests for tissue bound nitrofurans residues,
- To develop a reference method for tissue bound nitrofurans residues,
- To assess prevalence of tissue bound residues in pork across the EU, and
- To disseminate results to the European consumer, the CRL/NRL network and to the scientific community.

This project, which has partners in five MS of the EU, Hungary and the Czech Republic commissioned the synthesis of reference standards of the putative metabolites of the other nitrofurans. Initially, most of these were not available from any commercial source. However, with the emergence of the global nitrofuran crisis over the last year, there are now a number of commercial sources for these standards.

Using these standards, the FoodBRAND team was able to develop a sensitive method for all four of the suggested metabolites of the nitrofuran drugs. The method, which employs LC-MS/MS, meets the criteria set out by the EU for the unequivocal confirmation of the presence of these metabolites (9). The method bases on early HPLC procedures. It involves a series of pre-washing steps to remove low molecular weight matrix components. Nitrofuran-related moieties (figure 4) are then released from the tissue bound nitrofuran residues by mild acid hydrolysis and are subsequently derivatised with o-nitrobenzaldehyde (figure 5). The purpose of the derivatisation step is two-fold. Firstly, it increases the solubility of the nitrofuran metabolites in organic solvents and, secondly, it increases their mass, reducing interference from the matrix. Two transition products are measured for each compound, ensuring that the four identification points required for confirmation are achieved. Single quadrupole LC-MS may also be used for the confirmation of illegal drugs, providing that four identification points are earned by the method. However, this is not readily

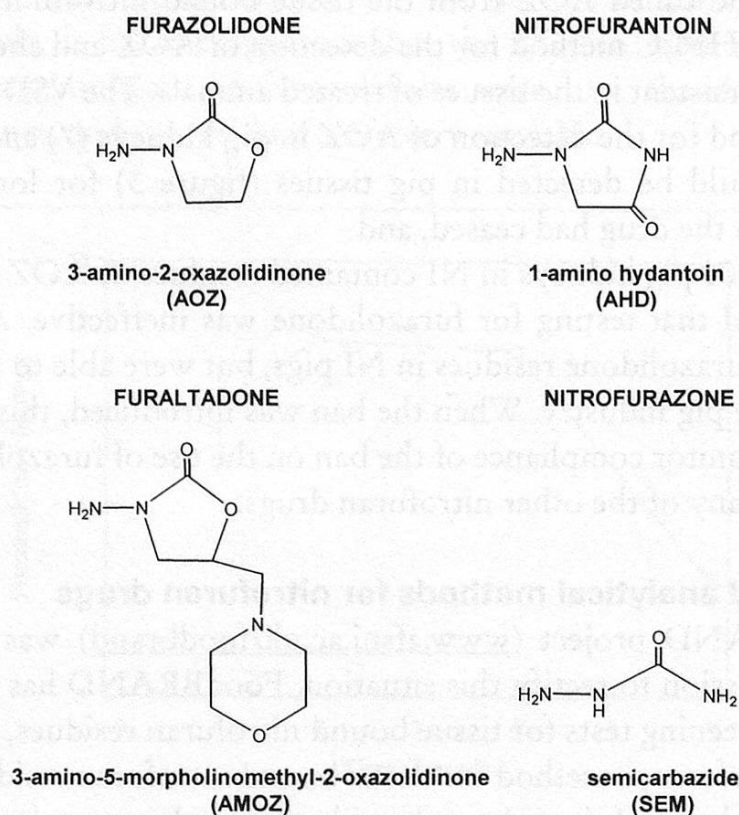


Figure 4 Structures of the nitrofuran metabolites

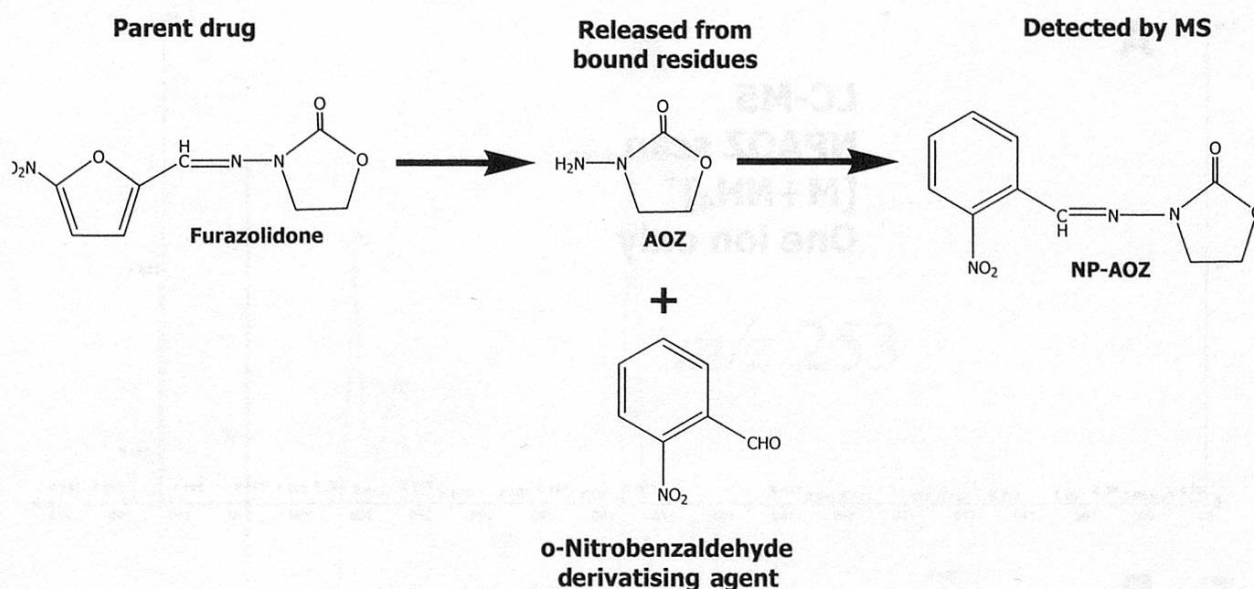


Figure 5 Derivatisation of AOZ with o-nitrobenzaldehyde

achievable. For example, figure 6A shows that the electrospray spectrum of NPAOZ contains only one diagnostic ion – corresponding to $[M+NH_4]^+$ at m/z 253. In contrast, at least two transition products (at m/z 134 and 104) derived from the nitrophenyl derivative of AOZ can be seen in its daughter ion spectrum shown in figure 6B. When the same sample (containing 5 $\mu\text{g}/\text{kg}$ AOZ) is analysed using both LC-MS and LC-MS/MS (figure 7), the LC-MS method cannot meet the EU criteria for analyte identification (scoring only one identification point for the pseudo-molecular ion) and has a signal to noise ratio of $\sim 60:1$, whereas LC-MS/MS, measuring two transition products, meets the identification criteria and is capable of much better sensitivity, having a signal to noise ratio of $\sim 800:1$.

Zero tolerance for banned substances

The EU has a policy of zero tolerance towards the use of nitrofurans in food-producing animals. Theoretically, any confirmed concentration of any of the metabolites is an offence. EU Member States are allowed to condemn a product when very low concentrations of the nitrofuran metabolites are detected and confirmed, providing that the competent authorities have sufficient statistical confidence in the analytical method. The EU has not, therefore, set “tolerance limits” for the nitrofuran metabolites. Rather it is in the process of setting a Minimum Required Performance Limit (MRPL) for methods aimed at detecting unauthorised substances. An MRPL for the nitrofuran metabolites in poultry and aquaculture products has been set at 1.0 $\mu\text{g}/\text{kg}$. This term has caused a lot of confusion, inside the EU and further afield. It does not mean that an AOZ concentration in poultry meat of 2 $\mu\text{g}/\text{kg}$ is unacceptable, while a concentration of 0.5 $\mu\text{g}/\text{kg}$ is acceptable. What it does mean is

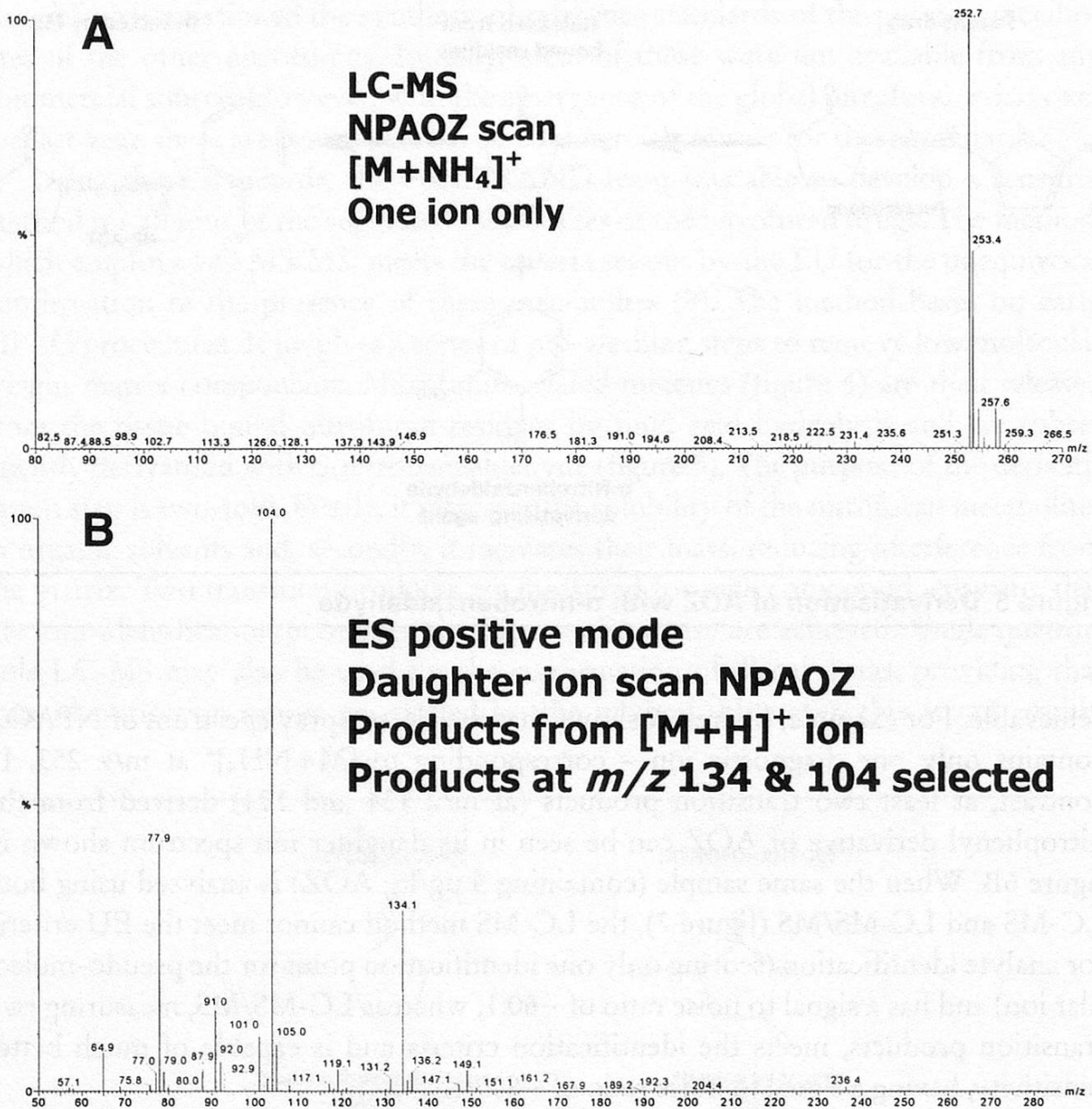


Figure 6 LC-MS scan (A) and LC-MS/MS daughter ion scans (B) of the nitrophenyl derivative of AOZ

that all Member States must be able to detect and confirm nitrofurantoin metabolites at the MRPL, or lower if they can. It was envisaged that the MRPL should be a target that the worst performing methods to achieve. Providing that the statistical control of the method is acceptable (as defined in Commission Decision 2002/657/EC (9)) there is no limit to the lowest concentration that can be reported positive. However, most Member States, including the United Kingdom, have adopted unofficial "Reporting Limits" for nitrofurans. Some Member States have selected the MRPL as the unofficial "Reporting Limit". This approach has recently been adopted by the United Kingdom, and is rapidly becoming the accepted practice throughout the EU.

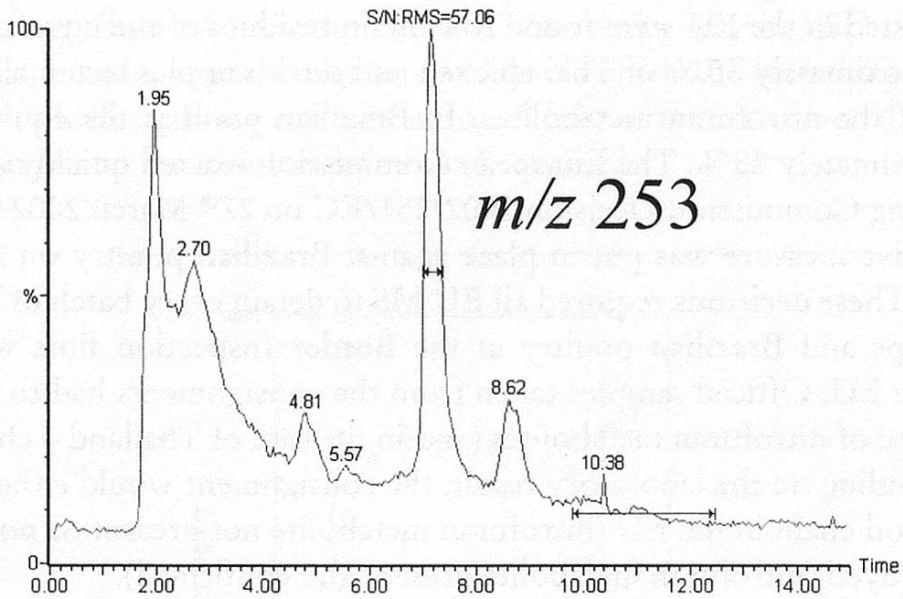
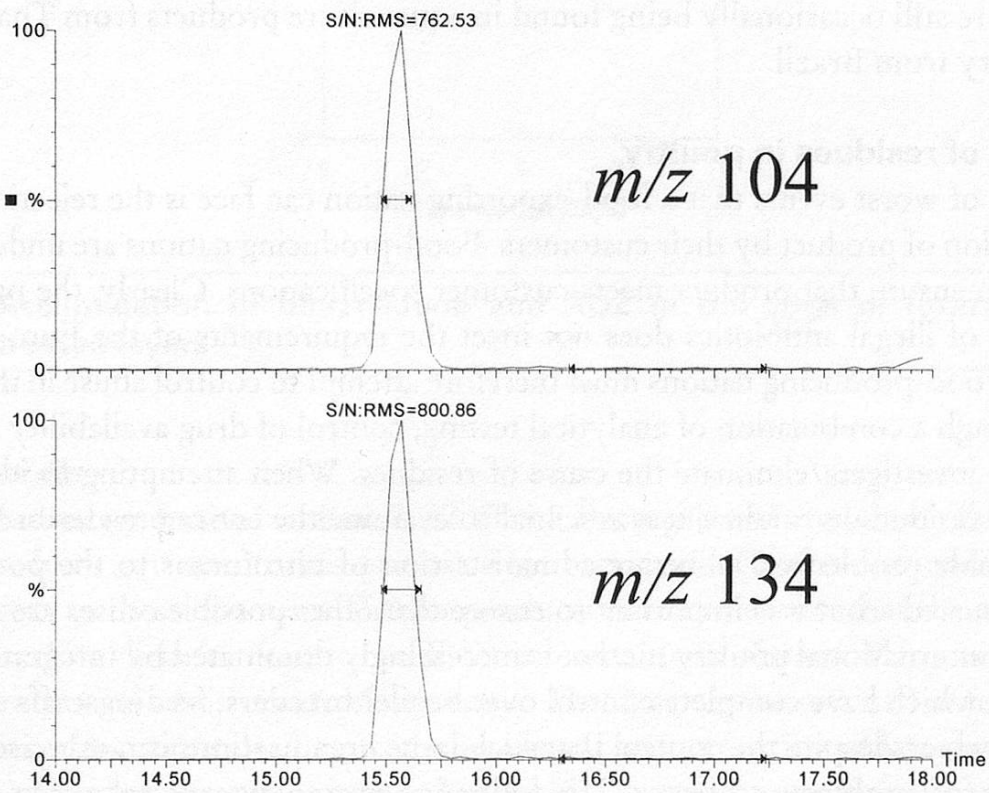
A**B**

Figure 7 Chromatograms showing the determination of AOZ (5 $\mu\text{g}/\text{kg}$) in an incurred shrimp sample using LC-MS (A) and LC-MS/MS (B)

EU protective measures against Thailand and Brazil

In early 2002, the VSD laboratory and RIKILT began to report positive results in a wide range of poultry and aquaculture products from a very wide range of countries, including Thailand and Brazil. At that time, more than 60% of Thai shrimps tested in the EU were found to contain residues of the nitrofurans metabolites. Approximately 10% of Thai chicken and duck samples tested also contained residues of the nitrofurans metabolites. In Brazilian poultry, the equivalent figure was approximately 20%. The European Commission reacted quickly against Thailand, passing Commission Decision 2002/251/EC on 27th March 2002 (10). A similar protective measure was put in place against Brazilian poultry on 27th October 2002 (11). These decisions required all EU MS to detain every batch of Thai poultry and shrimps and Brazilian poultry at the Border Inspection Post where it first entered the EU. Official samples taken from the consignments had to be tested for the presence of nitrofurans metabolites (and in the case of Thailand – chloramphenicol). Depending on the laboratory result, the consignment would either be allowed into the food chain in the EU (nitrofurans metabolite not present or not confirmed) or be destroyed (nitrofurans metabolite present and confirmed).

The official sampling and testing of Thai and Brazilian product has been implemented in all EU MS. Where MS do not have access to LC-MS/MS equipment, they sub-contract the work to another laboratory with the necessary equipment. In both countries, the violation rate has fallen dramatically. However, nitrofurans metabolites are still occasionally being found in aquaculture products from Thailand and in poultry from Brazil.

Causes of residues in poultry

One of the worst events that a food-exporting nation can face is the rejection and/or destruction of product by their customers. Food-producing nations are under an obligation to ensure that product meets customer specifications. Clearly, the presence of residues of illegal antibiotics does not meet the requirements of the European consumer. Food-producing nations must therefore attempt to control abuse in their country through a combination of analytical testing, control of drug availability and field-work to investigate/eliminate the cause of residues. When attempting to identify the cause of residues in poultry, it is essential to examine the entire breeder-broiler chain for possible problems. Deliberate administration of nitrofurans to the poultry may have occurred – but it is important to ensure that other possible causes are ruled out.

The international poultry market is increasingly dominated by integrated organisations, which have complete control over broiler breeders, feedingstuffs and broilers. However, despite the control that such large organisations can exercise, it is still possible for problems to occur. The following examples may not cover all of the potential problems. However, they serve as a starting point for further investigations. In some of these areas, work already done by VSD in pigs or being done by VSD in poultry may help to provide some of the answers.

Broiler breeders

It is recognised that nitrofurans can accumulate in eggs. Work carried out at VSD (12) has shown that furazolidone transfers readily from layers into eggs. One very unusual feature of the egg is that it is the parent drug (furazolidone) that accumulates in the egg (figure 8). Further work is now under way in VSD to see if the drug persists in breeder eggs through to the day-old chick and into the grown bird. These studies, which are being carried out for all four of the nitrofurans drugs, are at a very early stage and few results are currently available.

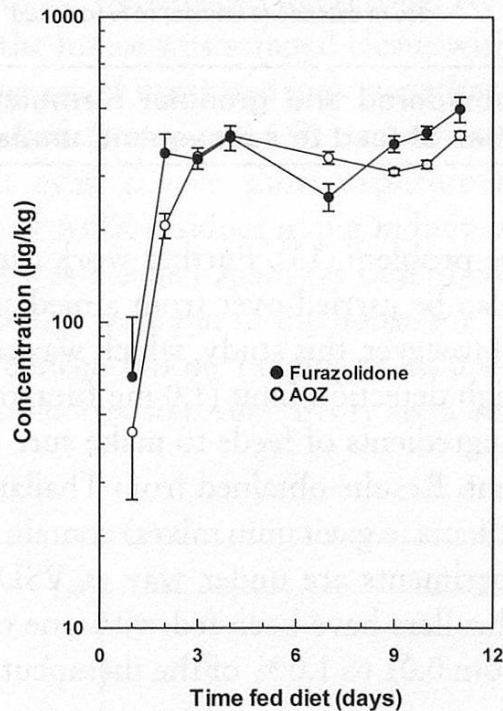


Figure 8 Accumulation of furazolidone and AOZ in the eggs of furazolidone-treated layers

Feedingstuffs

Nitrofurans are banned in Brazil, Thailand, Portugal, and elsewhere. Feed mills should not have any nitrofurans present. However, residues of nitrofurans metabolites do occur in these countries. Broiler producers need to ensure that the mill does not also produce diets for ornamental fish that contain nitrofurans. Many veterinary medicines and feed additives are electrostatic. The drug can be retained in feed mills at a number of locations, leading to the accidental contamination of later batches of feed. One of the best examples here is Lasalocid. Lasalocid was originally marketed in the EU as a powder. However, it could easily carry-over from a medicated batch (batch 0, figure 9) to the next nine four-tonne batches of feed. The manufacturers responded by introducing a granular form that was less electrostatic. This reduced

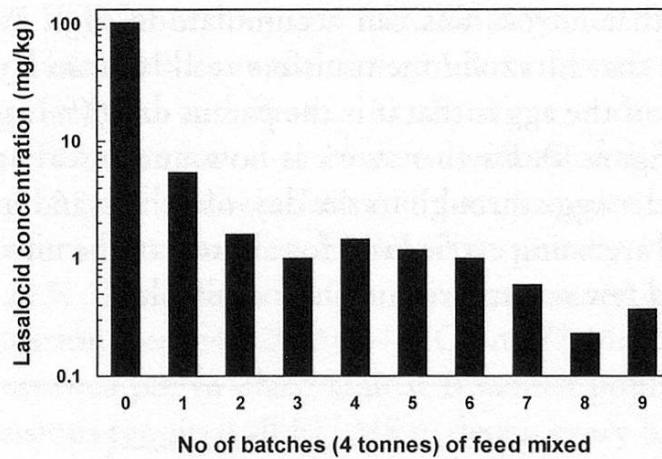


Figure 9 Carry over of powdered and granular formulations of Lasalocid from medicated batches of feed to subsequent, unmedicated batches of feed

(but did not eliminate) the problem (13). Further work carried out at VSD showed that furazolidone could also be carried over from a medicated batch of feed to the next two batches of feed. However, this study, which was carried out in 1995, used a method that had a very high detection limit (1.0 mg furazolidone per kg feed). Producers need to check all ingredients of feeds to make sure that nitrofurans (or their metabolites) are not present. Results obtained from Thailand suggest that some finished feeds and feed ingredients (e.g. vitamin mixes) contain low levels of nitrofurans.

Another series of experiments are under way at VSD to help to address this issue. Twenty groups of broilers have been fed with one of the four nitrofurans at concentrations ranging from 0.01 to 1.0% of the therapeutic dose. These diets were

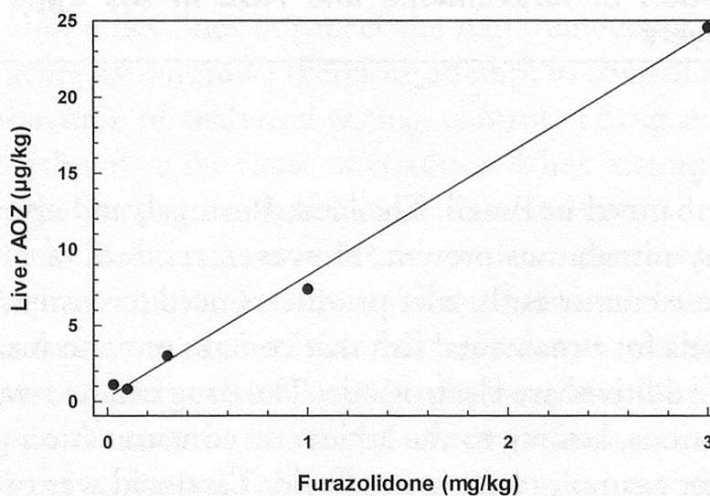


Figure 10 The relationship between furazolidone in poultry feed and AOZ in chicken liver

fed for eight days. Liver and muscle from the birds are being analysed for the presence of the appropriate metabolite. The picture that is emerging indicates that even very low concentrations in feed can cause residues above the MRPL in the chickens. Figure 10 shows that even 30 µg furazolidone per kg feed (approximately 0.01 % of the therapeutic dose) will cause residues in chicken liver at 1.0 µg/kg.

Contamination during transportation

A series of experiments, carried out at VSD, examined the ability of untreated pigs to accumulate AOZ in tissues as a result of brief exposure to contaminated housing (e.g. during transportation or in lairage) (8). Pigs with furazolidone in their diet (400 mg/kg for five days) were placed in housing. At the end of this period, the pigs were removed and the house was scraped clean, with residual feed, faeces and urine being removed. A group of untreated pigs were then put into the house. These were killed over a period of 24 hours. AOZ was measured in kidney. The results showed (figure 11) that even a very short exposure to contaminated housing resulted in the detection of AOZ residues in pig kidney. After this study was completed, the empty house was cleaned again (as described above) and then cleaned with running water. Two pigs were put in the house for 24 hr. AOZ was detectable in all tissues studied, at concentrations ranging from 0.4–23 µg/kg (8). These data confirmed that AOZ could transfer very easily as a result of brief exposure to contaminated housing.

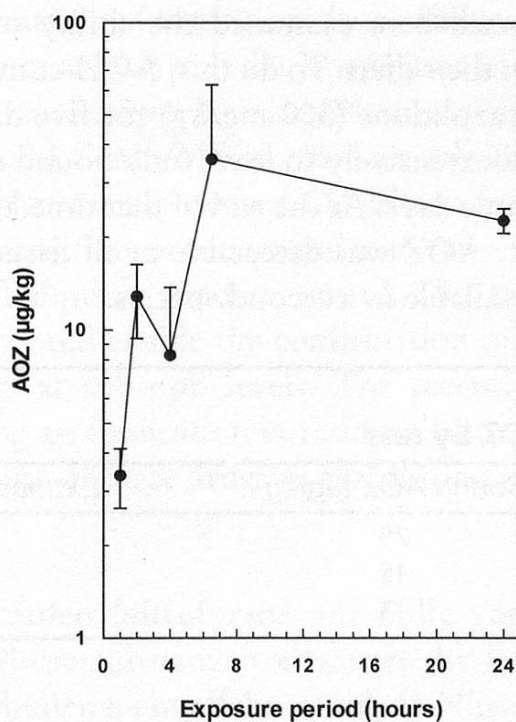


Figure 11 **Accumulation of AOZ in the kidney of pigs placed in housing that had previously been used to treat pigs with furazolidone**

The consumer

It does not matter to the consumer of chicken if AOZ residues are present because of contamination or abuse. The consumer wants food free from illegal residues. A series of studies were conducted at VSD to see if AOZ residues were stable after cooking and to see if consumers could absorb AOZ residues in food (14).

Stability after cooking

Consumers usually eat cooked meat. In many cases, it is useful to know if a veterinary residue in food is stable during the cooking process. It is well known that many veterinary drug residues are unstable when cooked. For example, penicillins and nitrofurans (parent drugs) disappear very rapidly. However, table 1 shows that AOZ was very stable after a range of cooking processes.

Table 1
Stability of AOZ after cooking

Tissue	Total AOZ concentration ($\mu\text{g}/\text{kg}$)			
	Cooking method			
	Uncooked	Fried	Grilled	Microwave
Liver	2226	2090	2047	1701
Kidney	1368	1415	1745	1373
Muscle	229	264	292	255

Absorption of AOZ from food

The final study reported here examined the ability of rats (to mimic human beings) to absorb AOZ in their diets. To do this, AOZ-containing pig liver was produced. A pig was fed furazolidone (300 mg/kg) for five days. The liver of the pig was collected and washed extensively to leave only bound nitrofurans residues. This was then fed to rats for three days. At the end of that time, the rat tissues were tested for the presence of AOZ. AOZ was detectable in all tissues studied, showing that AOZ residues were bioavailable in a second species.

Table 2
Absorption of dietary AOZ by rats

Tissue	Bound AOZ ($\mu\text{g}/\text{kg}$)	Extractable AOZ ($\mu\text{g}/\text{kg}$)
Liver	79	115
Kidney	48	175
Muscle	3	8

Conclusions

The development of new analytical methods to detect bound residues of the illegal nitrofurans antibiotics has revealed the widespread misuse of these compounds throughout South and South East Asia, Brazil and Portugal. The probability of mis-

use of these compounds is heightened because they are cheap, effective and (until recently) hard to detect. The finding of nitrofurans residues in prawn and poultry production worldwide has led the EU (to date) to take protective measures against Thailand and Brazil, subjecting produce from these countries to extensive testing at EU Border Inspection Posts. Competent authorities and primary producers in the affected countries are currently attempting to eradicate drug abuse from their production systems. However, they should also be aware that illegal residues in food can arise as a result of accidental contamination of feedingstuffs, vitamin mixes etc. Experimental work has demonstrated that even low-level contamination of feed can cause violative residues in chicken meat.

Summary

For many years, nitrofurans were detected using analytical methods that detect the parent drugs. However, it has been known for some time, that these methods are ineffective, since nitrofurans are excreted very rapidly from treated animals and are highly unstable *in vitro*. Work carried out during the 1980s and early 1990s showed that measuring tissue concentrations of a moiety (AOZ), released from tissue bound nitrofurans residues, could more effectively monitor furazolidone use.

An EU funded research project "FoodBRAND", set out to develop screening and confirmatory methods for tissue bound residues of all four of the nitrofurans drugs, resulted in the launch of two commercial immunoassay kits. The demands of the consumer, industry, and the European Union (EU) for increased testing for nitrofurans is now leading to the development of in-house biosensor tests for these compounds.

Traditionally, confirmation of veterinary drug residues by single quadrupole MS required the monitoring of at least four diagnostic ions and three ion ratios. In practice, this is impossible to achieve for the nitrofurans metabolites. However, the increasing availability of LC-MS/MS in residues laboratories; coupled with the inclusion of MS/MS in the recently revised EU criteria for the confirmation of drug residues (at least two transition products and one ratio) has greatly increased the applicability of this technique in routine control. This technique can readily meet EU identification criteria and enable the confirmation of tissue bound metabolites of the nitrofurans drugs at sub-ppb levels. The recently developed LC-MS/MS methods are now finding an application in residues laboratories worldwide to control the widespread misuse of these drugs in global food production systems.

Zusammenfassung

Über viele Jahre wurden Nitrofurane mit Hilfe von analytischen Methoden ermittelt, welche die Muttersubstanzen erfassten. Es ist jedoch seit einiger Zeit bekannt, dass diese Methoden nicht effektiv sind, da Nitrofurane von den behandelten Tieren sehr schnell ausgeschieden werden und sich *in vitro* äusserst instabil verhalten. In den 80er und den frühen 90er Jahren durchgeführte Arbeiten haben gezeigt, dass mit der Messung von Gewebekonzentrationen eines Metaboliten

(AOZ), freigesetzt aus gebundenen Nitrofuran-Rückständen, der Einsatz von Furazolidon effizienter überwacht werden kann.

Ein von der EU finanziertes Forschungsprojekt «FoodBRAND», bei dem Screening- und Bestätigungsmethoden für an Gewebe gebundene Rückstände aller vier Nitrofurane entwickelt werden sollten, führte zu der Einführung von zwei kommerziellen Immunoassay-Kits. Die Nachfrage der Verbraucher, Industrie und der Europäischen Union (EU) nach vermehrten Kontrollen auf Nitrofurane führen nun zu der Entwicklung von Biosensoren für diese Substanzen.

Traditionell erforderte der Nachweis von Tierarzneimittelrückständen durch single quadrupole MS eine Überprüfung von mindestens vier diagnostischen Ionen und drei Ionen-Verhältnissen. In der Praxis ist dies für die Nitrofuran-Metaboliten undurchführbar. Die zunehmende Verfügbarkeit von LC-MS/MS in Laboratorien, verbunden mit der Aufnahme von MS/MS in die kürzlich neu formulierten EU Kriterien für den Nachweis von Arzneimittelrückständen (wenigstens zwei Ionen und ein Verhältnis) hat jedoch die Anwendbarkeit dieser Technik bei Routinekontrollen erheblich gesteigert. Mit dieser Technik können die EU Identifikationskriterien einfach eingehalten und die an Gewebe gebundenen Metaboliten der Nitrofurane im sub-ppm Bereich bestätigt werden. Die kürzlich entwickelten LC-MS/MS Methoden finden weltweit Anwendung in Prüflaboratorien, um den weit verbreiteten Missbrauch dieser Medikamente in globalen Systemen der Nahrungsmittelproduktion zu kontrollieren.

Résumé

Pendant de nombreuses années, les nitrofuranes étaient détectés par le biais de méthodes analytiques qui décelaient le médicament mère. Toutefois, il est connu depuis un certain temps que ces méthodes sont inefficaces étant donné que les nitrofuranes sont excrétés très rapidement par les animaux en traitement et sont hautement instables *in vitro*. Les travaux effectués pendant les années 80 et le début des années 90 ont montré que mesurer les concentrations tissulaires d'un métabolite caractéristique (AOZ), libres de résidus de nitrofuranes liés aux tissus, pouvait permettre de contrôler de manière plus efficace l'utilisation de furazolidone.

Un projet de recherche financé par l'UE, «FoodBRAND», a entrepris de développer les méthodes de dépistage et de confirmation concernant les résidus liés aux tissus des quatre médicaments à base de nitrofurane, a entraîné le lancement de deux kits commerciaux de dosage immunologique. Les demandes des consommateurs, de l'industrie et de l'Union Européenne (UE) en faveur d'un accroissement des tests des nitrofuranes entraînent à présent un développement des tests de biosenseurs pour ces composés.

Traditionnellement, la confirmation des résidus de médicaments vétérinaires par spectrographie de masse quadrupolaire unique nécessitait le contrôle d'au moins quatre ions de diagnostic et trois taux d'ions. En pratique, cela est impossible à réaliser pour les métabolites de nitrofurane. Toutefois, la disponibilité croissante de LC-MS/MS dans les laboratoires de résidus ainsi que l'inclusion de MS/MS dans les

critères UE récemment mis à jour pour la confirmation des résidus de médicaments (au moins deux produits de transition et un taux) ont considérablement augmenté l'applicabilité de cette technique dans les contrôles de routine. Cette technique peut aisément satisfaire aux critères d'identification de l'UE et permettre la confirmation des métabolites liés aux tissus des médicaments à base de nitrofurane à des niveaux de concentration en dessous d'une partie par milliard. Les méthodes LC-MS/MS récemment mises au point sont maintenant mises en pratique dans des laboratoires de résidus à travers le monde entier pour contrôler l'emploi abusif répandu de ces médicaments dans les systèmes globaux de production alimentaire.

Key words

Nitrofurane, analysis, drug residues, LC-MS/MS, EC regulation

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