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Multi-residue determination of phenolic and salicylanilide anthelmintics and related compounds in bovine kidney by liquid chromatography–tandem mass spectrometry

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ABSTRACT

This paper describes an analytical method for four phenolic and salicylanilide anthelmintics authorised for use within the EU (nitroxinil, oxyclozanide, rafoxanide and closantel) in bovine kidney, and the extension of this procedure to include a number of related compounds; ioxynil, niclosamide, salicylanide and 3-trifluoromethyl-4-nitrophenol (TFM). The method comprises a solvent extraction with 1% acetic acid in acetone and clean-up using a mixed-mode anion-exchange solid phase extraction column. Determination is by reversed phase LC–MS/MS. The method was validated to the latest EU requirements (Commission Decision 2002/657/EC) using both spiked and incurred tissues and was subject to second laboratory evaluation.

1. Introduction

Compounds contained within the chemical grouping of phenolic and salicylanilide anthelmintics include nitroxinil, oxyclozanide, rafoxanide and closantel. All of these compounds are active against liver fluke and are used extensively to treat against fasciolosis and haemonchosis in sheep and cattle. Oxyclozanide is principally active against adult flukes, whereas nitroxinil, rafoxanide and closantel have activity against both adult and immature flukes [1]. The chemical structures and EU Maximum Residue Limits (MRLs) [2] for these compounds in bovine tissues are shown in Table 1. In each case, the marker residue for monitoring purposes is the parent compound. For nitroxinil, literature states that the most persistent residues are found in the kidneys and at the injection site [3]. High residue concentrations for the other three compounds have been found in the kidneys of animals used in controlled depletion studies [4–6], hence kidney is the matrix of choice when monitoring for their usage.

Historically, single-residue methods have been mainly used to detect these compounds. For example, there are a number of methods available for the determination of nitroxinil in bovine milk and/or tissues utilising determination by HPLC-UV [7], HPLC-electrochemical detection [8], LC–MS [9] and GC [10]. LC-electrochemical detection has been used to determine five fasciolides, including nitroxinil and oxyclozanide, in bovine milk [11]. Closantel determination has been undertaken using HPLC-fluorescence in bovine milk [12] and in plasma and tissues [13] and a method has been reported for the analysis of closantel and rafoxanide in ovine plasma using HPLC-UV [14]. In addition LC–MS has been used for the determination of rafoxanide in various tissues [15]. Few, if any of these methods meet the method validation criteria of EU Legislation for confirmatory methods (Commission Decision 2002/657/EC [16]) and, since all pre-date 2002, none has been validated to this standard. Bound residues are also of particular concern; nitroxinil has been shown to undergo binding to plasma proteins [17]. Protein binding is also an issue with the salicylanilides [1].

A number of structurally related compounds also exist, which have a variety of uses, i.e. herbicides, anthelmintics, molluscicides, fungicides and piscicides. In order to test the applicability of this method to monitor for other potential contaminants, four such compounds were selected (see Table 1 for structures). Those compounds chosen included the herbicide ioxynil (structurally related to nitroxinil), the piscicide 3-trifluoromethyl-4-nitrophenol (TFM, structurally related to nitroxinil), and the salicylanilides niclosamide (a molluscicide and anthelmintic, used in conjunction with TFM) and salicylanilide (a fungicide). Methods for determination of these compounds in animal tissues, although somewhat limited, are available; for example, both TFM [18] and niclosamide [19] have been determined in trout and catfish tissues using HPLC-UV. The aim of this work was therefore to:

- Develop a multi-residue method for phenolic and salicylanilide anthelmintics and related compounds in bovine kidney.

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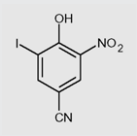
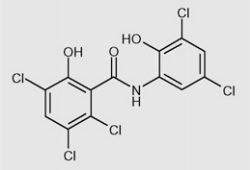
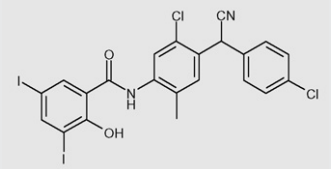
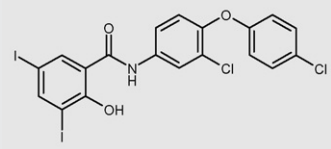
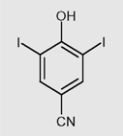
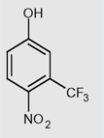
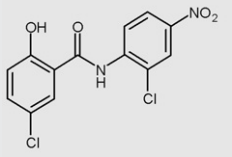
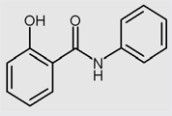
Table 1
Compound structures and MS/MS transitions monitored.

Compound	Structure	Bovine MRLs[a] ($\mu g\,kg^{-1}$)	Quantification (Q) transition	Confirmation (C) transition	Typical ion ratio (Q/C)
Nitroxinil		400 muscle 400 kidney	288.9 > 126.9	288.9 > 162.0	2.1
Oxyclozanide		20 muscle 100 kidney	397.9 > 175.8	399.9 > 363.9	1.2
Closantel		1000 muscle 3000 kidney	660.8 > 126.9	662.8 > 126.9	1.6
Rafoxanide		30 muscle 40 kidney	623.8 > 126.9	625.8 > 126.9	2.1
Ioxynil		None	369.8 > 126.9	369.8 > 214.9	5.7
TFM		None	206.0 > 176.0	206.0 > 160.0	1.8
Niclosamide		None	325.0 > 170.9	325.0 > 289.0	2.6
Salicylanide		None	212.1 > 92.0	212.1 > 93.0	3.6

[a] European Commission, Regulation 2377/90, as amended.

- Optimise the extraction of bound residues via the use of incurred tissues.
- Validate the developed method to the standard of Commission Decision 2002/657/EC [16].

2. Materials and methods

2.1. Chemicals and reagents

Closantel, ioxynil, nitroxinil, oxyclozanide and rafoxanide were purchased from Riedel-de-Haan (Dorset, UK). Salicylanilide was purchased from Fluka (Dorset, UK). Niclosamide was obtained from Sigma (Dorset, UK). 3-Trifluoromethyl-4-nitrophenol (TFM) was purchased from Aldrich (Dorset, UK). Solvents were HPLC grade or equivalent. Oasis MAX cartridges (150 mg/6 cm^3) were purchased from Waters (Elstree, UK). All other chemicals were of analytical grade.

2.2. Standard solutions

Stock standards were prepared in methanol for all compounds (except closantel which was prepared in acetone) at 1000 $\mu g\,mL^{-1}$. These stock solutions were diluted in methanol to give a 'mixed spiking standard solution' containing nitroxinil (8 $\mu g\,mL^{-1}$), rafox-