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Effective monitoring for ractopamine residues in samples of animal origin by SPR biosensor and mass spectrometry

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ABSTRACT

Ractopamine (RCT) is a member of the β-2-agonist (β-agonist) family. It is licensed for use as an animal growth promoter in more than 20 countries worldwide, including the United States and Canada, but is either not licensed or prohibited by over 150 others, including those within the European Union. The issue of the use of RCT in livestock bound for human consumption has risen to prominence recently following the decision by The People's Republic of China to ban the import of pork from a number of processing plants after finding traces of RCT in shipments from the U.S.A.

In order to monitor for the illegal use of such compounds within Europe, there is a requirement to have a robust and reliable testing scheme capable of the detection of low concentrations of RCT. In the present study an optical biosensor screening assay was developed. The developed assay was compared with a liquid chromatography/mass spectrometry/mass spectrometry (LC–MS/MS) confirmatory procedure. These methods were used to study the ability to detect RCT in pigs following treatment. Both testing procedures were capable of detecting low $\mu g\,kg^{-1}$ concentrations of the drug in urine and liver. Liver was found to be a less suitable sample matrix, with RCT residue levels being undetectable after 5 days withdrawal of the drug. Urine samples however still contained detectable RCT residues several weeks after withdrawal. The correlation (as measured by r^2) between the biosensor and LC–MS/MS methods was 0.99 and 0.97 for urine and liver samples, respectively.

It is concluded that testing regimes based on RCT analysis in liver are less likely to detect illegal administration of the drug than those based on urine analysis. Urine samples provide an excellent matrix for the detection of RCT residues for an extended period post withdrawal.

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1. Introduction

The pharmacological compound RCT belongs to the class of drugs known as β-agonists. These compounds, originally developed to treat respiratory diseases, were found to have the important side effect of causing a significant reduction in fat levels and a dramatic increase in the amount of muscling when administered to animals in high doses [1–4]. This effect, often referred to as repartitioning, can bring about significant financial gain to farmers employing this type of treatment to their animals. Though not licensed for animal production, the wide scale use of such drugs, especially in Europe, caused major problems to regulatory authorities as it was discovered that the presence of beta-agonist residues in foods resulted in many cases of acute food poisoning [5,6]. Subsequently, in Europe, the use of all β-agonists was banned from animal production [7].

RCT, although a member of the β-agonist family, has been shown to be safe to administer to animals with residue concentrations at a withdrawal period of 0 days in edible tissues lower than tolerance values established by the U.S. Food and Drug Administration (F.D.A.) Centre for Veterinary Medicine (C.V.M.) and MRL values listed by the Joint FAO/WHO Expert Committee on Food Additives (J.E.C.F.A.). It has subsequently received approval from the F.D.A. for use in pig (Paylean®) and cattle (Optaflexx™ 45) production [8]. The view in Europe is, however, very different. Consumers consider chemical growth promoter residues as unwholesome and unwelcome constituents in food and it is therefore illegal to use ractopamine within the European Union. Thus, because of the dual requirements of providing quality assurance for the consumer and of satisfying legal testing obligations, the ability to detect residues of the drug at low concentrations has become a very important issue.

While physicochemical procedures such as high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry/mass spectrometry (LC–MS/MS) can detect the drug at concentrations of approximately 2 $\mu g\,kg^{-1}$ [13–16], significant drawbacks can arise from employing these methods as a result of elaborate sample preparation and time consuming and expensive analytical protocols.

The most common testing procedures for β-agonists, since the early 1990s, have been screening immunoassays [9–12] followed by physicochemical confirmation when required. A number of commercial screening test kits have been produced which can detect residues of a range of β-agonists at less than 2 $\mu g\,kg^{-1}$ and are widely used in routine testing laboratories around the world. None of the multi β-agonist test kits available, however, can detect RCT. A project was therefore initiated to develop, validate and implement a RCT screening test based on surface plasmon resonance (SPR) technology in combination with a high affinity RCT antibody and led to the development of Qflex® Kit Ractopamine available from Biacore AB (Uppsala, Sweden).

A biosensor method had previously been developed for the detection of the β-agonist clenbuterol in bovine urine [17] and although this assay has now been modified as a multi β-agonist test capable of detecting a wide range of the β-agonist family, it is unable to detect RCT [18]. Shelver and Smith have shown good correlation between biosensor and HPLC technologies for the detection of RCT residues in the urine of sheep and cattle fed a diet containing 18 $mg\,kg^{-1}$ RCT for 7–8 consecutive days [19]. Qiang et al. have also reported on tissue and serum residues as well as urinary excretion in swine treated with the same recommended dietary level of RCT for 28 consecutive days by high performance liquid chromatography–fluorescence detection (HPLC–FLD) [20].

The purpose of this study was to develop suitable analytical procedures that would be capable of detecting low concentrations of RCT and its glucuronides which have previously been reported as the major excretory metabolites [21–23]. This developmental work was followed by a feeding trial to monitor residues post medication in pigs fed dietary levels of RCT for 10 consecutive days.

2. Materials and methods

2.1. Instrumentation

An optical biosensor (BIACORE®Q) was obtained from Biacore AB (Uppsala, Sweden). Instrument operation and data handling was performed using BIACORE®Q Control Software (Version 3.0.1).

An Alliance® 2690 HPLC pump (Waters®, Milford, MA, USA) was used. Reversed phase liquid chromatography was performed on octadecyl grafted silica Nucleosil® C_{18}AB (50 mm × 2 mm, 5 μm) stationary phase (Macherey-Nagel®, Düren, Germany) with a guard column (Nucleosil® C_{18}AB, 10 mm × 2 mm, 5 μm). Elution solvents were methanol (A) and 0.5% acetic acid in water (B). Mobile phase composition (A/B, v/v) was 95/5 at 0 min, 50/50 at 10 min, 10/90 at 20 min and 95/5 from 30 min to 40 min. Flow rate was 220 $\mu L\,min^{-1}$ and injected volume was 10 μL. Data were acquired in the positive electrospray mode using a Quattro LC® triple quadrupole analyser (Micromass®, Manchester, UK). Nitrogen was used as nebulisation and desolvation gas at flow rates of 90 $L\,h^{-1}$ and 600 $L\,h^{-1}$, respectively. Source and desolvation temperatures were 120 °C and 350 °C, respectively. Potentials applied on the capillary and on the sampling cone were 3.5 kV and 25 V, respectively. MS/MS experiments were performed using argon as collision gas at a pressure of 4.10^{-4} mBar and a collision energy varying from 15 V to 25 V. The signal acquisition was performed on the multiple reaction monitoring mode (MRM). For RCT and isoxsuprine (internal standard), the precursor ion was the pseudo-molecular ion ($m/z = 302$). For isoxsuprine, the two diagnostic product ions were $m/z = 302 > 284$ and 150 and for ractopamine, the six diagnostic product ions were $m/z = 302 > 284$, 164, 136, 121, 107, and 91.

2.2. Reagents and chemicals

HBS-EP buffer, CM5 sensor chips (certified grade) and an amine coupling kit containing *N*-ethyl-*N*′-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), *N*-hydroxy-succinimide (NHS) and 1 M ethanolamine pH 8.5 were all obtained from Biacore AB (Uppsala, Sweden). The RCT derivative was a

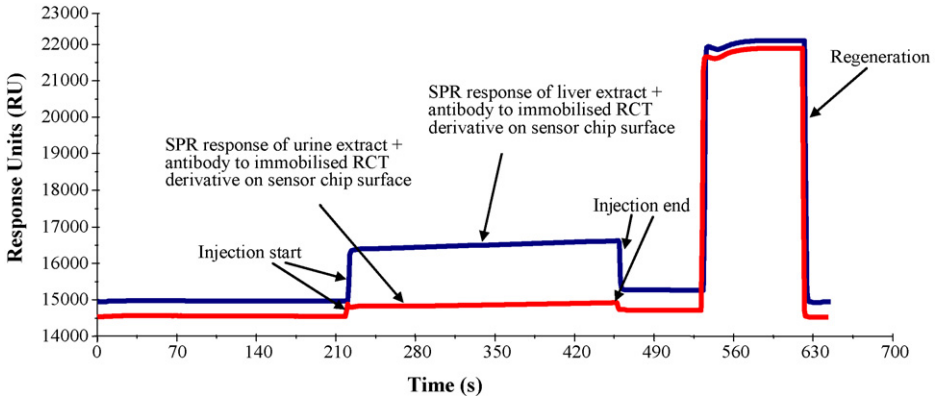

Fig. 1 – Typical sensorgrams obtained during optical biosensor analysis for RCT residues in porcine liver and urine samples.

gift from Xenosense Ltd. (Belfast, U.K.). RCT hydrochloride and salmeterol were gifts from Eli-Lilly & Co. (Indianapolis, U.S.A.) and GlaxoSmithKline (Uxbridge, England), respectively. The RCT glucuronides were gifts from the USDA. All other reference standards were obtained from Sigma–Aldrich Chemical Company Ltd. (Poole, Dorset, U.K.). Paylean® premix was obtained from Elanco Animal Health (Indianapolis, U.S.A.). "Sow and Weaner" pig meal was obtained from John Thompson & Sons Ltd. Animal Feed Compounders (Belfast, U.K.). β-Glucuronidase enzyme hydrolysis solution (from *E. coli* K12) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Isolute® Alumina-A solid phase extraction columns were obtained from Jones Chromatography Ltd. (Hengoed, Mid Glamorgan, U.K.).

Methanol (analytical and HPLC grade), hexane, diethylether, ethyl acetate, glacial acetic acid (all analytical grade) and 32% ammonium hydroxide were provided by Solvents Documentation Syntheses (Peypin, France). Sodium acetate and sodium phosphate were purchased from Merck (Darmstadt, Germany).

All other chemicals were of HPLC grade and were supplied by BDH (Poole, Dorset, U.K.).

2.3. *Immobilisation of RCT derivative*

A RCT derivative was immobilised onto the surface of a CM5 sensor chip using the Biacore Q instrument. The surface was activated with 1/1 (v/v) 0.2 M EDC/0.05 M NHS at a flow rate of 10 μL min^{-1} and the RCT derivative (2 mM in 50 mM borate buffer pH 8.5) immobilised onto the newly activated surface at a flow rate of 5 μL min^{-1}. The surface was washed with deionised water and all unreacted sites were deactivated with 1 M ethanolamine pH 8.5 at a flow rate of 5 μL min^{-1}.

The sensor chip surface was washed with water and dried under a stream of nitrogen gas. The chip was stored at 4 °C with a desiccant when not in use.

2.4. *Immunogen and antibody production*

A RCT-HSA immunogen was prepared and anti-RCT polyclonal antiserum produced in a goat according to the method outlined in a previously published paper [12].

2.5. *Collection of RCT free material*

Urine and liver samples were taken from pigs reared on a strictly controlled government farm where the use of ractopamine or any other beta-agonist based growth promoter was not permitted.

2.6. *Urine sample preparation (biosensor analysis)*

Negative pooled urine (20 mL), known to be free from RCT residues, and sample urines (5 mL) were passed successively through 0.45 μm and 0.22 μm filter units (Millipore, Cork, Ireland) into plastic universal bottles. The pH of all urine samples was adjusted to 6.25 ± 0.05 using either 1 M hydrochloric acid or 1 M sodium hydroxide and aliquots of either negative or sample urine (2.5 mL) were then transferred into test tubes. Calibrants (0.05, 0.25, 0.75 and 3.0 ng mL^{-1} RCT) were prepared by adding working standards (125 μL) to the negative urine aliquots and an equivalent volume of assay buffer was added to the sample urines. HPLC grade water (2.375 mL) was added to all tubes. β-Glucuronidase (12.5 μL) was added to all tubes, which were vortexed gently for 5 s and then placed in an incubator (47 °C) for 1 h. All deconjugated urines were then centrifuged at 3000 rpm for 10 min at 10 °C. Aliquots of

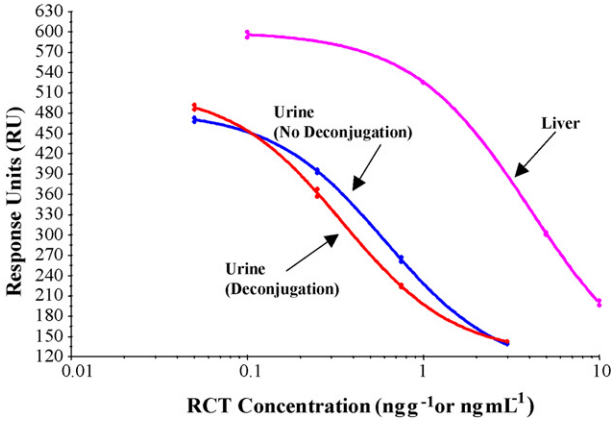

Fig. 2 – Typical calibration curves obtained following biosensor analysis of RCT residues in porcine liver and urine.

deconjugated calibrant and sample urines (3 mL) were transferred into glass universals and 2 M carbonate/bicarbonate buffer pH 9.6 (7 mL) added to each. Ethyl acetate (10 mL) was added to all of the universals, which were then spiral mixed for 30 min. Following centrifugation at 2500 rpm for 10 min at 10 °C, the aqueous layers were frozen using aluminium freezing blocks and the ethyl acetate poured off into test tubes. Solid phase extraction (SPE) was performed under gravity using Alumina-A SPE columns. Columns were conditioned with ethyl acetate (3 × 4 mL). Calibrants and samples were loaded onto the columns in aliquots (2 × 5 mL) and each column washed with ethyl acetate (2 × 2.5 mL). Eluants were collected from the columns by the addition of methanol (2 × 3 mL). A 2 min 'soak-step' was observed between the addition of the two elution aliquots. These were then evaporated to dryness using a sample concentrator at 60 °C under a stream of

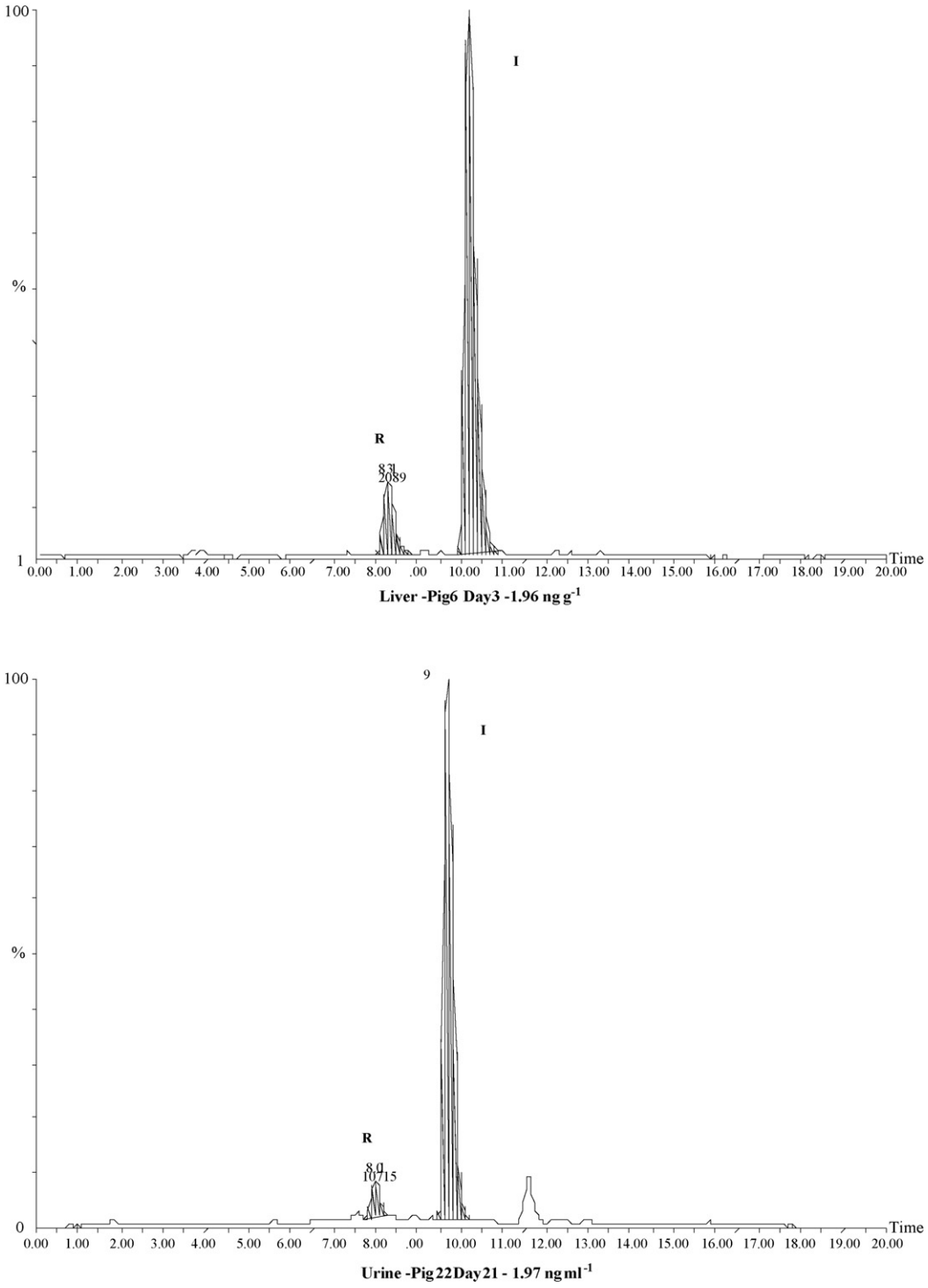

Fig. 3 – Characteristic ion chromatograms (ESI^+, $MS^2[M+H]^+$, MRM) obtained for incurred liver and urine samples at different days after treatment. I: isoxsuprine (internal standard), R: ractopamine (analyte).

nitrogen gas. The resulting extracts were reconstituted immediately in HBS-EP buffer (250 μL) by vortexing for 30 s and aliquots (105 μL) transferred, in duplicate, to the wells of a microtitre plate.

2.7. Liver sample preparation (biosensor analysis)

Negative pooled liver (20 g), known to be free from RCT residues, and sample livers (5 g) were homogenised. Negative and sample liver aliquots (2.5 g) were weighed into glass universal bottles. Calibrants (0.1, 1.0, 5.0 and 10.0 $\mu g\,kg^{-1}$ RCT) were prepared by adding working standards (125 μL) to the negative liver aliquots and assay buffer (125 μL) was added to the sample livers.

Acetonitrile (10 mL) was added to each glass universal and vortexed for 10 s. The universals were mixed end-over-end for 15 min and centrifuged at 3500 rpm for 15 min at 10 °C. Supernatant (9 mL) was transferred from each universal into glass test tubes and evaporated to dryness using a sample concentrator at 60 °C under a stream of nitrogen gas. The resulting extracts were reconstituted by adding HBS-EP buffer (1 mL) to each tube and vortexing for 30 s. The tubes were placed in a water bath at 37 °C for 10 min and vortexed again for 30 s. The extracts were then transferred to micro-centrifuge tubes and centrifuged at 10,000 rpm for 10 min. The extracts were carefully removed (avoiding any fatty deposits on the sides of the micro-centrifuge tubes) and passed through 0.22 μm filter units. Aliquots of each filtrate (120 μL) were transferred, in duplicate, to the wells of a microtitre plate.

2.8. Biosensor assay

The biosensor method used was designed as an inhibition assay. A known concentration of drug-specific antibody was mixed with the sample and injected over the surface of a sensor chip immobilized with RCT derivative. Any RCT present in the sample was bound to the antibody. This RCT antibody was therefore inhibited from binding to the derivative on the sensor chip surface (Fig. 1). The higher the concentration of drug in the sample, the higher the level of inhibition and hence the lower the response measured by the biosensor instrument. All measurements were made using a Biacore®Q instrument. Unknown concentrations were calculated with reference to a calibration curve prepared in negative matrix (Fig. 2).

Briefly, urine extract (60/40, v/v) or liver extract (50/50, v/v) was mixed with RCT antibody using HBS-EP buffer. These were then injected over the chip surface at a flow rate of 20 $\mu L\,min^{-1}$ for 4 min. Report points were recorded before and after each injection to determine the relative response units for each injection. The chip surface was regenerated with an injection of 20/80 (v/v) acetonitrile/250 mM sodium hydroxide at a flow rate of 20 $\mu L\,min^{-1}$ for 1 min.

The extraction and analysis of 20 urine and 20 liver samples can be achieved within 19 h and 17 h, respectively.

2.9. Sample preparation (LC–MS/MS analysis)

Urine and liver samples were extracted and purified according to a previously published method [14]. Briefly, fresh liver samples (15 g) were freeze-dried, ground and extracted by a methanol/acetate buffer mixture. After enzymatic hydrolysis of the conjugated metabolites with *Helix pomatia* Type H5 (Sigma, St. Louis, USA), the extract was purified successively on ChromP© and Screen DAU SPE columns (Supelco, St. Quentin, Fallavier, France). Urine samples (10 mL) were hydrolysed and purified according to the same procedure.

2.10. Animal study

2.10.1. Preparation of RCT incurred material

2.10.1.1. Experimental animals. Thirty pigs (Large White/Landrace Cross), with a mean body weight of 40 ± 5 kg, known to be free from any anabolic and β-agonist treatment were randomly divided into four groups. Group A (controls) consisted of three pigs which were fed non-medicated meal throughout the duration of the trial and were housed in a separate building from the medicated animals. Groups B, C and D consisted of the remaining 27 animals which were randomly divided into 3 groups of 9 each. The three medicated groups were housed in separate, adjacent, concrete-floored, straw-bedded pens separated by a 1 m high concrete partition. Animals within each group were fed from communal troughs.

2.10.1.2. Animal feed preparation. Paylean® premix was added to "Sow and Weaner" pig meal at the approved 18 $mg\,kg^{-1}$ level [8] in a rotating mixer and allowed to blend thoroughly for 1 h. The medicated meal was then sub-divided into batches (30 × 9 kg).

2.10.1.3. Feeding protocol. All animals used in the trial were fed 1 kg non-medicated "Sow and Weaner" pig meal per head per day for a two-week period prior to commencement of the trial. RCT medicated meal (1 kg per head) was offered to groups B–D daily for 10 consecutive days. The meal was added to the troughs between 8:00 am and 8:30 am each day and consumption observed on each occasion. The control group received non-medicated meal (1 kg per head) daily throughout the duration of the trial. Following medication, groups B–D were moved to clean pens and returned to non-medicated meal for the remainder of the trial.

2.10.1.4. Sampling procedure. On the morning of days 1, 2, 3, 5, 7, 10, 14, 21 and 28 post withdrawal of ractopamine from the diet, 1 pig from each of groups B–D was removed at random, sacrificed and experimental samples taken. The 3 control animals from group A were sacrificed and samples collected on day 28. All samples taken during the trial were stored at −20 °C until analysed.

3. Results and discussion

3.1. Antibody characterisation

The polyclonal antibody produced was assessed for cross-reactivity against a range of β-agonists prepared in buffer solutions and in both negative urine and liver matrix. The results (Table 1) showed that the only significant cross-reactivity was found against the RCT glucuronides and this

Table 1 – Cross reactivity profile for the RCT polyclonal antibody

Analyte	Buffer (%)	Urine (%)	Liver (%)
Ractopamine	100.0	100.0	100.0
Ractopamine glucuronides	78.0	84.7	21.8
Fenoterol-HBr	4.2	1.1	3.7
Isoxuprine-HCl	2.7	1.3	<1.0
Ritodrine-HCl	<1.0	<1.0	<1.0
Salmeterol	<1.0	<1.0	<1.0
Clenbuterol-HCl	<1.0	<1.0	<1.0
Salbutamol	<1.0	<1.0	<1.0
Terbutaline-hemisulfate	<1.0	<1.0	<1.0

varied depending on the type of matrix used in the assay. The antibody was then injected over the RCT chip surface and a high R_{max} of >15,000 RU was detected. When an irrelevant antibody was injected over the same surface, an R_{max} of <200 RU was found thus indicating the interaction detected between the chip surface and the RCT antibody was of a high affinity and high specificity.

3.2. *Biosensor assay validation*

The biosensor analytical method was validated according to the requirements of Commission Decision 2002/657/EC [24].

Twenty known RCT free porcine urine and liver samples were extracted and analysed as previously described. The limit of detection (LOD) was calculated by determining the mean relative response – 3 sd and substituting this value into the extracted standard curve using BIAevaluation software. This gave values of 0.34 $\mu g L^{-1}$ and 0.19 $\mu g kg^{-1}$ for urine and liver, respectively. To determine CCβ, the same blank samples were spiked with 0.40 $\mu g L^{-1}$ and 0.50 $\mu g kg^{-1}$ RCT and in both cases all 20 spiked samples gave concentrations above their LOD thus indicating CCβ <0.40 $\mu g L^{-1}$ and <0.50 $\mu g kg^{-1}$ for urine and liver, respectively.

3.3. *LC–MS/MS assay validation*

The LC–MS/MS analytical method was validated according to the requirements of Commission Decision 2002/657/EC [24]. The details of the validation procedure have already been published [14]. In summary, for tissue samples, the decision limit (CCα) and the detection capability (CCβ) were found to be as low as 9 $ng kg^{-1}$ and 28 $ng kg^{-1}$, respectively. The linearity of the procedure was proven using spiked samples at 25, 50, 100 and 500 $ng kg^{-1}$ for which the obtained correlation coefficient (r^2) was determined as 0.99. The repeatability was estimated on the basis of 20 spiked samples at 50 $ng kg^{-1}$ which were analysed in two different batches on separate days. The obtained relative standard deviations were 17.3% and 18.1% for the two monitored diagnostic MRM transitions, respectively.

Fig. 3 shows the total ion chromatograms (ESI^+, $MS^2[M+H]^+$, MRM) obtained for RCT incurred liver and urine samples for which residues had been identified at low $\mu g kg^{-1}$ and $\mu g L^{-1}$ concentrations. The withdrawal patterns of RCT from these matrices were appreciably different. In liver samples taken one week after drug withdrawal no residues could be detected, whereas in the urine samples taken up to three weeks after withdrawal residues were still detectable.

Fig. 4 shows the 6 RCT diagnostic MRM transitions for liver samples collected 1 and 10 days after treatment. The presence of the 6 signals allowed, on each of the chromatograms, for the unambiguous identification of the analyte.

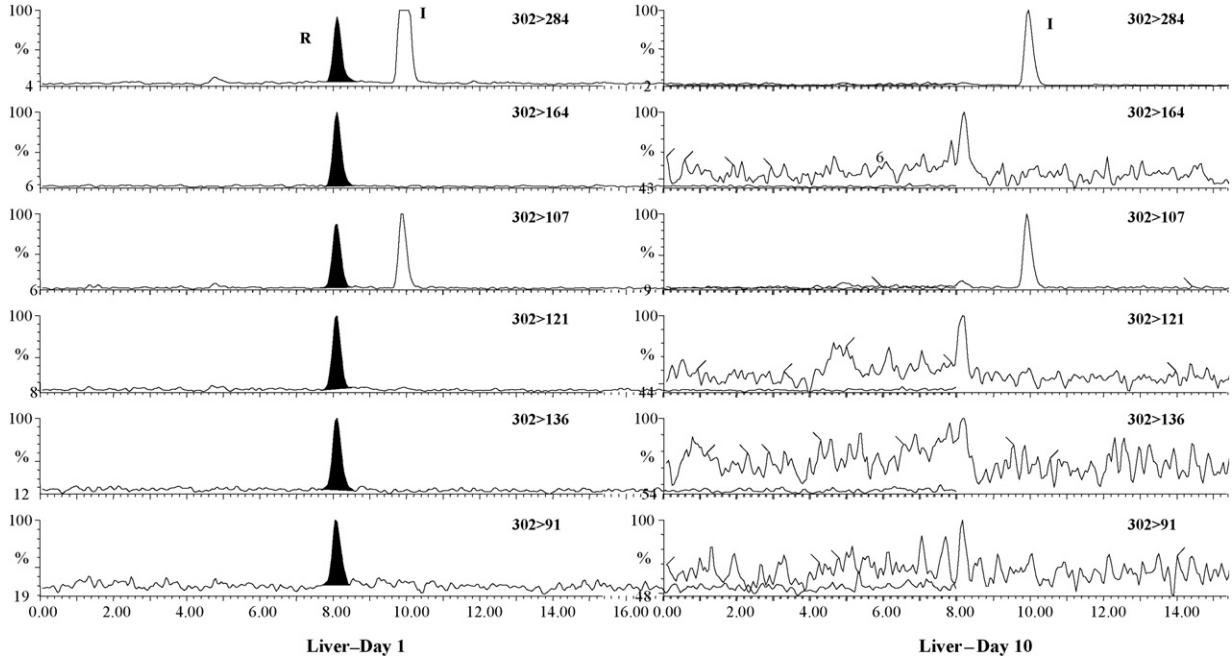

Fig. 4 – Six ractopamine diagnostic MRM transitions monitored in liver samples collected 1 and 10 days after RCT treatment (I: isoxsuprine, R: ractopamine).

Table 2 – Concentrations of RCT detected in experimental urine samples by biosensor analysis (with and without sample deconjugation) and LC–MS/MS

Sample number	Withdrawal period (days)	RCT concentration ($\mu g L^{-1}$)		
		Biosensor		LC–MS/MS
		No deconjugation	Deconjugation	
1	1	>250.0	1720.0	945.9
2	1	>250.0	2360.0	1131.6
3	1	>250.0	671.0	473.6
4	2	4.1	77.9	67.5
5	2	1.6	35.5	[a]
6	2	4.6	82.4	74.7
7	3	4.0	66.5	30.2
8	3	[a]	[a]	[a]
9	3	1.3	[a]	[a]
10	5	1.6	23.2	[a]
11	5	1.5	28.2	21.3
12	5	1.4	19.7	15.6
13	7	[a]	[a]	[a]
14	7	0.6	15.8	3.4
15	7	0.5	8.9	6.2
16	10	[a]	9.4	4.0
17	10	0.4	3.5	4.4
18	10	0.9	9.9	[a]
19	14	0.1	4.4	3.4
20	14	0.4	6.3	6.2
21	14	0.3	4.8	3.1
22	21	0.1	2.4	2.0
23	21	0.0	1.6	1.1
24	21	0.8	1.7	1.4
25	28	0.0	0.6	0.5
26	28	0.0	0.4	0.2
27	28	[a]	[a]	[a]

[a] Insufficient urine obtained for analysis.

3.4. *Results from animal study*

3.4.1. *Urine*

Urine samples were analysed by biosensor with and without the addition of glucuronidase. A summary of all results has been included in Table 2. Results obtained by biosensor were substantially higher in the deconjugated samples even though the RCT antibody had been shown to cross-react significantly with the glucuronide metabolites. It would seem likely that these compounds were not extracted efficiently during the sample clean-up procedure and it was therefore deemed necessary that the deconjugation procedure be included prior to urine analysis. The concentrations of RCT detected in urine samples collected immediately after withdrawal of the drug from the diet were high (>500 $\mu g L^{-1}$). Though the concentrations of residue found in samples decreased over the withdrawal period, detectable concentrations of RCT were still found in all but one urine sample by biosensor analysis up to day 28 post withdrawal. A coefficient of correlation ($r^2 = 0.99$) for the screening and confirmatory results in urine was calculated (Fig. 5).

3.4.2. *Liver*

A summary of all results has been included in Table 3. Concentrations of RCT residues found in liver were considerably lower than in the urine samples taken from the same animals. Confirmed positive samples were found in all animals up to 3 days after being withdrawn from RCT medication, with 1 animal out of 3 being confirmed positive on each of days 5 and 7 post withdrawal. Several samples were screened positive by biosensor when taken at later stages of the withdrawal experiment but these were not confirmed by LC–MS/MS. Comparison between the results obtained by biosensor and LC–MS/MS (Fig. 6) showed a coefficient of correlation of $r^2 = 0.97$.

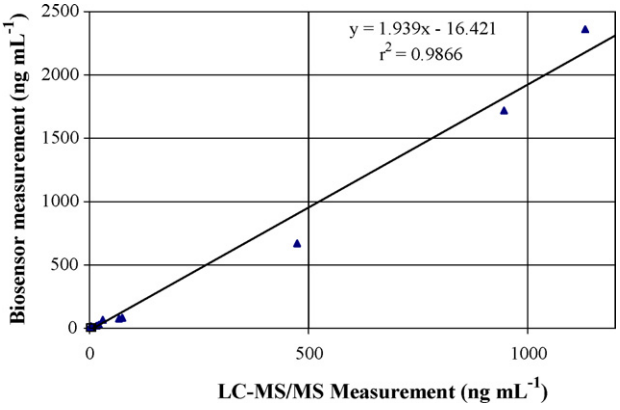

Fig. 5 – Comparison of biosensor and LC–MS/MS technologies for detection of RCT residues in porcine urine.

Table 3 – Concentrations of RCT detected in experimental liver samples by biosensor analysis and LC–MS/MS

Sample number	Withdrawal period (days)	RCT concentration ($\mu g\,kg^{-1}$)	
		Biosensor	LC–MS/MS
1	1	>10.0	11.3
2	1	5.8	18.3
3	1	6.4	6.9
4	2	1.9	0.7
5	2	1.7	0.9
6	2	2.5	2.0
7	3	1.9	0.5
8	3	0.9	1.1
9	3	1.9	1.9
10	5	0.5	0.5
11	5	1.2	ND
12	5	0.7	ND
13	7	0.7	0.5
14	7	0.9	ND
15	7	0.5	ND
16	10	ND	ND
17	10	ND	ND
18	10	ND	ND
19	14	ND	ND
20	14	0.8	ND
21	14	ND	ND
22	21	ND	ND
23	21	ND	ND
24	21	1.2	ND
25	28	ND	ND
26	28	0.5	ND
27	28	ND	ND

ND: concentrations detected less than 0.5 $\mu g\,kg^{-1}$ RCT.

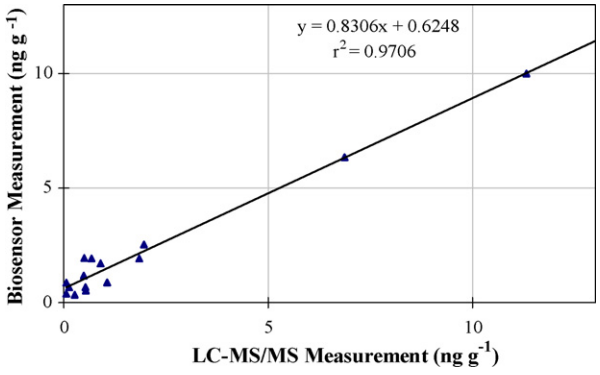

Fig. 6 – Comparison of biosensor and LC–MS/MS technologies for detection of RCT residues in porcine liver.

4. Conclusions

A screening procedure based on optical biosensor technology has been developed and this has been compared with a confirmatory assay based on LC–MS/MS. While the confirmatory method is the more sensitive of the two, the screening method is also capable of detecting RCT residues well below the Minimum Required Performance Levels (MRPLs) of 3 $\mu g\,L^{-1}$ and 3 $\mu g\,kg^{-1}$ for urine and liver, respectively. As well as being capable of detecting low concentrations of RCT in porcine urine and liver samples, excellent correlation has been shown between the two technologies.

When applied to experimental samples, the analysis of urine was shown to be the most suitable matrix for detecting RCT residues for an extended period following withdrawal of drug from the diet. Residues in this matrix were still detectable, by both screening and confirmatory methods, until day 21 post withdrawal. In contrast, analysis of liver only yielded detectable RCT residues in animals sampled up to 5 days following drug withdrawal. While Qiang et al. [20] had previously reported similar results for RCT depletion in liver, they found levels of the drug falling rapidly in urine even prior to withdrawal of the drug from the diet, with residues post withdrawal detectable until day 9 but undetectable at day 14. The pigs in the study by Qiang et al. were fed RCT for a 28-day period as opposed to a 10-day period in the current study, however it would seem unlikely for this factor to be responsible for the marked difference in urinary concentration during withdrawal. Urinary concentrations recorded in the current study, even after the removal of fortified feed for a 24 h period, were higher than those recorded by Qiang et al. after 7 days feeding (the point at which these workers recorded the maximum residue concentration) as levels fell dramatically throughout the remainder of the feeding and withdrawal periods.

These findings have significance in laboratories that seek to control the unauthorised use of RCT. In many laboratories, based on the experience of detecting other β-agonist drugs, such as clenbuterol, salbutamol and mabuterol, liver has proven to be a good matrix for the detection of drug abuse. From the results obtained in the present study, however, it is concluded that it is highly unlikely that RCT residues will be detected in animals that are given a 1-week withdrawal period if liver is used as the matrix for analysis.

It is therefore recommended that urine is selected as the matrix of choice for analysis in control laboratories because of both the extended withdrawal period during which RCT abuse can be detected and also because of the option this sample type offers to authorities in terms of on-farm and show pig testing regimes.

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