Technical Note:

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Determination of clenbuterol from bovine urine samples using Thermo Scientific Multiskan FC

Key Words:
- Clenbuterol
- β-agonist
- ELISA
- EIA

Clenbuterol is one of a group of drugs called beta 2-agonists. The use of these β-agonists as feed additives is not permitted in the European Union (EU). Therefore it is important to be able to reliably determine the β-agonists from several types of sample matrices. This note describes how to determine clenbuterol from bovine urine samples using the Thermo Scientific Multiskan FC microplate photometer. The method used is a competitive enzyme immunoassay.

Introduction

Clenbuterol belongs to the family of β-agonists. It is used for the treatment of allergic respiratory disease in horses and cattle. However, its ability to increase the muscle-to-fat body ratio has led to its illegal use as a growth promoter in livestock production. Due to acute food poisoning cases and negative consequences to human health, the use of β-agonists as feed additives is not permitted in the EU and most other countries[1].

The maximum residue limits (MRL) set by the EU are 0.1 mg/kg for muscle and 0.5 mg/kg for liver[2]. However, the most frequently used sample material for this test is urine.

The test described here is a microplate-based competitive enzyme immunoassay for analysis and screening of urine, feces, feed, bile, tissue, plasma, hair and choroid/retina samples for the presence of several β-agonists. The concentration range of the kit is 0.062 - 2.0 ng/ml.

The principle of the assay is described in Figure 1.

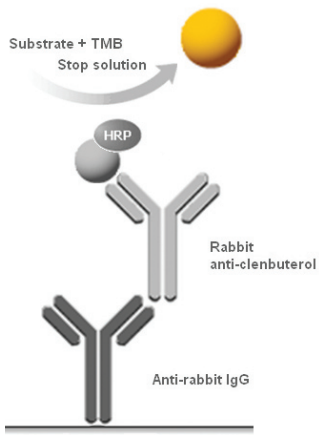

Figure 1. The assay principle

The microplate wells are precoated with sheep anti-rabbit IgG.

1. Incubation Step

Rabbit anti-clenbuterol and anti-salbutamol antibodies, HRP-labeled salbutamol (enzyme conjugate) and salbutamol standards or samples are added. The specific antibodies are bound to the rabbit antibodies and the free β-agonist and enzyme-labeled salbutamol compete for the specific antibody binding sites. The incubation time is 1h. The non-bound reagents are removed by washing.

2. Incubation Step

The substrate chromogen TMB is added. Bound enzyme transforms the chromogen into a colored product. The reaction is stopped with sulphuric acid and the color intensity is measured 450 nm. The color intensity is inversely proportional to the β-agonists concentration in the sample.

Materials and methods

- β-agonist EIA (cat. code 5061BAG1p) from EuroProxima B.V.
- Wellwash microplate washer (Thermo Fisher Scientific code 5165000)
- Multiskan FC microplate photometer (Thermo Fisher Scientific code 51119000)
- Bovine urine samples

The assay was performed by the Chemistry and Toxicology Unit of the Finnish Food Safety Authority at Viikki, Helsinki (EVIRA). This kit is also used at EVIRA for analyses of water samples. The cut-off value used is 1 µg/l for urine.

Four clenbuterol control samples were spiked to the concentration of 1 ng/ml and assayed together with the unknown samples in replicates. The reference material was acquired from The National Institute for Public Health and the Environment (RIVM), The Netherlands.

The urine samples were enzymatically hydrolyzed prior to the liquid-liquid extraction. After this, a liquid-liquid extraction was made. No dilution factor is needed with this treatment method for urine samples.

The test was performed according to the kit instructions and the absorbances were measured at 450 nm with the Multiskan FC microplate photometer and SkanIt for Multiskan FC PC software.

A template protocol for this assay can be downloaded from the SkanIt Protocol library. (See Further Information.)

Results

The calculations were performed according to the kit instructions with SkanIt software.

The calibration curve was fitted using the four parameter logistics-fitting formula (Figure 2).

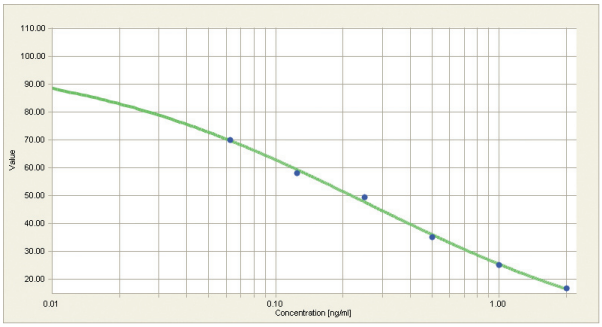

Figure 2. **Calibration curve of the assay**

The assay contained four control samples; each sample was spiked to concentration 1 ng/ml. Each control was assayed in replicates. The control results are reported in Table 1.

	Mean concentration (ng/ml)	CV%
Control 1	0.9	21.5
Control 2	1.3	22.1
Control 3	1.3	19.3
Control 4	1.1	10.3

Table 1. **Measured concentration of the controls**

The control results correspond well to the calculated concentration of 1 ng/ml.

38 bovine urine samples were assayed. The manufacturer reports the detection limit of 0.1 ppb for urine samples with liquid-liquid extraction. Eight of the samples were below this limit (Figure 3).

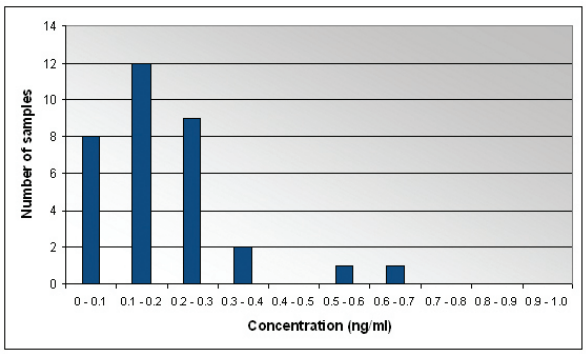

Figure 3. **Distribution of the samples over the kit assay range**

Five of the samples were above the detection range and must be diluted and re-assayed or verified using another detection method.

It is also possible to add a qualitative classification step to the results. With this step, the positive samples can be easily identified from the results, as shown in Figure 4.

Figure 4. **Example of a qualitatively classified result view**

Summary

Multiskan FC and SkanIt software provide an easy way to perform this β-agonist assay and the data management needed.

References

1. European Union (1996). – Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stock farming of certain substances having a hormonal orthyrostatic action and of β-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. Off. J. Eur. Union, L 125 of 23.5.1996, 3-9.
2. COMMISSION REGULATION (EC) No 2391/2000 of 27 October 2000 amending Annexes I, II and III to Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin L276 (2000)

Further information

For further information about the Thermo Scientific Multiskan FC photometer, please refer to: www.thermo.com/readingroom

The assay protocol for the β-agonist EIA can be downloaded from the Multiskan FC protocol library: http://www.thermoscientific.com/wps/portal/ts/news/detail?relationTypeCode=NE&contentId=50416

For further information about the reagents, please refer to: www.europroxima.com/

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