**Methodology**

Sample Preparation:
Addition of diluted wash buffer (37°C) to 1 g of honey sample. After rolling and filtration the sample is ready for application onto the microtitre plate.

Competitive ELISA
In-house made capture antibody was immobilised and stabilised on % well microtitre plates. The streptomycin assay is based on a competitive reaction where any free analyte contained in the standard/sample competes for binding sites of the capture antibody with homologous peroxidase labelled conjugate. Following the incubation and washing steps, enzyme substrate is added. Measurement of the optical density (OD) from the zero calibrator and reading the OD reading at 450 nm.

Analytical parameters

**Limit of Detection (LOD)**
LOD was defined as mean concentration of negative samples + 3SD.

**Limit of Detection**
6.4ppb

**Specificity/Cross-reactivity (CR)**
The specificity, expressed as % cross-reactivity (XCR) was calculated as follows:

\[
XCR = \left( \frac{IC_{50} \text{ (Streptomycin)}}{IC_{50} \text{ (Cross-reactant)}} \right) \times 100
\]

\(XCR\) was calculated as follows:

\[
\%CR = \left( \frac{IC_{50} \text{ (Streptomycin)}}{IC_{50} \text{ (Cross-reactant)}} \right) \times 100
\]

Precision
Intra-assay precision (n=12) was determined from the mean results corresponding to six different concentration levels for three microtitre plates batches within the same and was expressed as %CV.

Recovery
Commercially available positive honey samples were examined along with a negative sample spiked for a range of concentration levels.

Recovery
100%

Recovery in honey for Streptomycin

**References:**

1. The Merck Manual of Diagnosis and Therapy, Section 13: Infectious Diseases, Chapter 153: Antibacterial Drugs, Aminoglycosides.

**Conclusions:**

- In veterinary medicine streptomycin is one of the first line antibiotics used mainly to treat infections caused by aerobic gram-negative bacteria although they also act synergistically against some gram-positive bacteria.\(^{10}\) Streptomycin and dihydrostreptomycin are aminoglycosides with related structures. Aminoglycosides have also been used as growth promoters in food producing animals and there has been controversy surrounding this due to the potential for the development of aminoglycoside resistant pathogens.\(^{10}\) Honey is generally accepted as being a natural food product by the general public and its consumption has increased due to its perceived health benefits. However, over the last few years traces of streptomycin have been found in a growing number of honey samples worldwide. Contamination of honey may also occur through antimicrobial treatment of plants.\(^{10}\) Regarding the European legislation, no maximum residue limits are fixed for antibiotics in honey, therefore their use is not accepted.

- The development of simple and sensitive methods for the detection of streptomycin and dihydrostreptomycin in honey are of value for monitoring and regulatory purposes. We report the development of a sensitive competitive ELISA for the screening of these compounds in honey after simple sample preparation.

**Chemical Structures**

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