

SIMPLE AND RAPID SCREENING AND CONFIRMATION OF TETRACYCLINES IN HONEY AND EGG

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Aim

The aim of this study was to find a rapid and simple way for screening and confirmation of tetracyclines in honey and egg. For that purpose a newly introduced receptor based dipstick-test was evaluated, Tetrasensor® (Unisensor, Belgium). The extracts from the screening procedure were used without further purification in the LC-MS/MS confirmation. The use of antibiotics, such as tetracyclines, is illegal in honey production but allowed for treatment of egg laying hens, with an MRL of 200 µg/kg.

Samples

Honey:

Thirty different batches of Swedish honey were analysed blank and spiked with oxytetracycline (OTC, n=20), chlortetracycline (CTC, n=5) and tetracycline (TC, n=5) at a concentration of 25 µg/kg. Prior to screening, blank and spiked samples were randomised and analysed blind, ten samples at a time. In addition to the randomised samples, each run contained two known samples, one blank and one spiked at 25 µg/kg, serving as negative and positive control.

Egg:

Thirty different batches of Swedish eggs, were analysed blank and spiked with OTC (n=20), CTC (n=5) and TC (n=5) at a concentration of 200 µg/kg. Prior to screening, blank and spiked samples were randomised and analysed blind. In addition to the randomised samples, each run contained two known samples, one blank and one spiked at 200 µg/kg, serving as negative and positive control. In addition, 6 samples spiked with all three tetracyclines at 200 µg/kg, were analysed only with the confirmation method.

Screening with Tetrasensor® (Unisensor, Belgium)

Procedure

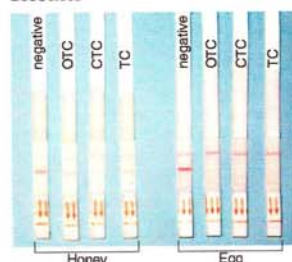
Honey:

- The samples were placed in a water bath (about 50°C)
- About 600 mg was pipetted into the buffer tubes supplied by the kit.
- The tubes were mixed vigorously on a Vortex.
- 200 µl was transferred to reagent vials and incubated for 15 min.
- The dipsticks were placed in the vials.
- After another 15 minutes the result was judged by two technicians independently.

Egg:

- 3.0 g egg was weighed.
- 9 ml tissue buffer (Tetrasensor) was added.
- The samples were shaken for 10 min and then centrifuged for 1 min at 10 000 rpm.
- 200 µl sample was added to a vial containing labelled receptor.
- After the receptor was dissolved a dipstick was placed in the vial and incubated for 10 min.
- The result was judged by two technicians independently.

Results



Tetrasensor analysis of OTC, CTC and TC in honey (spiked samples 25 µg/kg) and egg (spiked samples 200 µg/kg).

Type of sample	Total number (n)	Positives (n)	Negatives (n)
Honey, blank	30	1	29
Honey, 25 µg/kg OTC	20	20	0
Honey, 25 µg/kg CTC	5	5	0
Honey, 25 µg/kg TC	5	5	0
Egg, blank	30	0	30
Egg, 200 µg/kg OTC	20	20	0
Egg, 200 µg/kg CTC	5	5	0
Egg, 200 µg/kg TC	5	5	0

No false negative results were found, demonstrating that the Tetrasensor kit fulfils the most important criteria for screening tests. In the analysis of tetracyclines in honey, one positive result, which could not be confirmed, was found. The sample was judged as positive by both technicians, but the LC-MS/MS analysis was negative for TC, OTC and CTC. This result may be a false positive sample, which would be acceptable since 5% of false positive results are acceptable for a simple and fast screening procedure (about forty samples may be analysed in one day). In egg no false positive result was found.

Confirmation with LC-MS/MS

Instruments

HPLC: Alliance 2695, Waters
Column: Genesis C₁₈, 50 X 2.1 mm, 4 µm, protected by a guard column

Gradient table

Time (min)	% A	% B	Flow (ml/min)
0	10	90	0.3
1.0	75	25	0.3
2.5	75	25	0.3
2.6	10	90	0.3

Run time 7 minutes
A: Methanol
B: 0.2% formic acid containing 0.1 mM oxalic acid

LC-MS/MS: Quattro Ultima, Micromass
Electrospray ionisation, positive mode
Desolvation temp. 400°C
Source block temp. 135°C
Cone voltage: 30 V
Hexapole 1: 10 V

MRM table

Compound	Parent (m/z)	Collision Energy (V)	Daughter 1		Daughter 2	
			m/z	Energy (V)	m/z	Energy (V)
OTC	460.95	18	426.25	72	127.05	
CTC	479.00	26	153.98	21	444.15	
TC	445.05	26	154.06	18	410.01	

Sample preparation

Honey:

- 100 µl of the buffer phase was diluted with 900 µl of 10% methanol in water
- 10 µl was injected into the LC-MS/MS

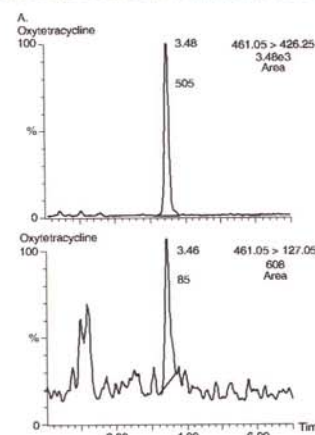
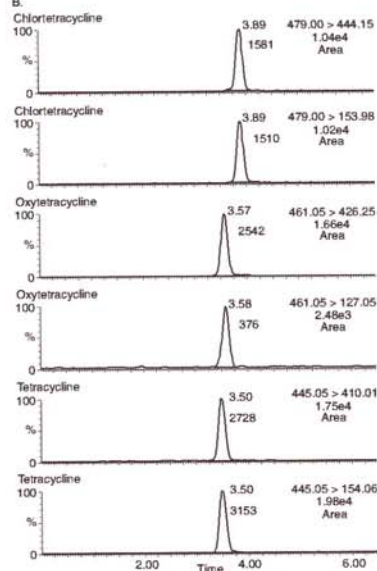
Egg:

- the buffer phase was filtered and 100 µl was diluted with 300 µl water
- 5 µl was injected into the LC-MS/MS

Results

LC-MS/MS confirmation:

- A. A honey sample spiked with OTC at 25 µg/kg.
B. An egg sample spiked with CTC, OTC and TC at 200 µg/kg.



The results from the confirmation of tetracyclines in egg, 200 µg/kg

Compound	Number	Recovery* (%)	CV (%)	Accuracy, bias (%)	CV (%)
OTC	6	67.5	4.6	-6.9	3.8
OTC	5	-	-	-8.2	4.1
OTC	5	-	-	-3.0	9.4
CTC	6	60.4	4.7	-0.9	4.9
CTC	5	-	-	-5.0	4.7
TC	6	71.4	3.8	-3.8	3.8

* Recovery was determined by comparing peak areas for samples spiked before and after sample preparation.

Conclusion

As a conclusion, the investigated test kits are suitable for screening of tetracyclines in honey and egg. The tests are rapid, simple and cheap. The possibility to use the diluted samples from the screening for LC-MS/MS confirmation without further purification makes the complete procedure, screening and confirmation, extremely convenient to use.