



Validation of two flow-through rapid tests for the quick and easy detection of ochratoxin A in green and roasted coffee with a cut-off value of 3 µg/kg

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Overview

- Ochratoxin A is a mycotoxin which can occur on contaminated green coffee beans.
- Because of thermostability Ochratoxin A can also occur on roasted coffee beans.
- In the European Union the maximum limit for OTA in roasted coffee is 3 µg/kg in accordance with Commission Regulation 2022/1370.
- In order to reach a detection limit of 3 µg/kg two new immune-based flow-through rapid tests were developed and validated.
- The specificity and sensitivity were 100% and 100% for both assays, respectively.

Introduction

Ochratoxin A (OTA) is a nephrotoxic and hepatocarcinogenic toxin produced by fungi. OTA can occur on contaminated (incoming) green coffee beans and due to thermostability also on the roasted coffee beans (end product). In the European Union the maximum limit for OTA in roasted coffee is 3 µg/kg in accordance with Commission Regulation 2022/1370.

In order to test incoming green coffee beans or roasted coffee beans as end product two new immune-based flow-through rapid tests were developed with a screening target concentration (STC) of 3 µg/kg in line with legislation for roasted coffee.

Method

- The membrane of the device is precoated with OTA-protein conjugate (test line) and anti-mouse IgG (control line).
- Specific antibody (mouse anti-OTA) labelled with horseradish peroxidase (HRP) is provided in a reaction vial.
- Green or roasted coffee is mixed with the extraction buffer and filtered. The green or roasted coffee extract is added to the reaction vial and incubated for 5 min.
- The mixture is transferred onto the membrane where any unbound antibodies bind to OTA-protein conjugate on the test line and bound/unbound antibodies bind to the anti-mouse IgG on the control line.
- The membrane is washed with a washing buffer.
- A substrate solution is then added. Bound HRP transforms the substrate into a blue coloured product and this appears as a line (Fig. 1).



Fig. 1. If the sample is negative for OTA (≤ 3 µg/kg) then two blue coloured lines appear (control and test). If the sample is positive for OTA (≥ 3 µg/L) then only one blue coloured line (control line) appears.

Results

- Twenty-one blank roasted/green coffee samples and 21 roasted/green coffee samples spiked at 3 µg/kg with OTA were analysed using 3 different batches of each specific OTA test kit. Additionally, in order to determine the ability of the method to distinguish between different OTA concentrations, sets of samples were also spiked at 0.75, 1.5, 2.5 and 3.5 µg/kg levels of OTA. Visual interpretation of each result (in total 243 results for roasted coffee and 198 results for green coffee) was performed by three different analysts and the results were classified as screen negative (2 lines visible) or positive (only 1 line visible) (Table 2 and 3.)
- The specificity was determined to be 100% meaning all samples containing no OTA (negative) gave correctly negative result. The overall sensitivity was 100% meaning 0% of samples gave false negative result.

Table 2. The results of testing of sets of 21 blank roasted coffee samples and multiple sets of roasted coffee samples spiked at different levels with OTA.

OTA concentration (µg/kg)	Number of positive/negative results (Batch 1)	Number of positive/negative results (Batch 2)	Number of positive/negative results (Batch 3)	Number of positive/negative results (Total)
0	0/21	0/21	0/21	0/63
0.75	0/6	0/6	0/6	0/18
1.5	1/5	1/5	1/5	3/15
2.5	3/3	3/3	3/3	9/9
3	21/0	21/0	21/0	63/0
3.5	21/0	21/0	21/0	63/0

Table 3. The results of testing of sets of 21 blank green coffee samples and multiple sets of green coffee samples spiked at different levels with OTA.

OTA concentration (µg/kg)	Number of positive/negative results (Batch 1)	Number of positive/negative results (Batch 2)	Number of positive/negative results (Batch 3)	Number of positive/negative results (Total)
0	0/21	0/21	0/21	0/63
0.75	0/6	0/6	0/6	0/18
1.5	2/4	2/4	2/4	6/12
2.5	4/2	4/2	4/2	12/6
3	21/0	21/0	21/0	63/0
3.5	6/0	6/0	6/0	18/0



Conclusions

- Two new methods for the quick and easy detection of OTA at the level 3 µg/kg in roasted and green coffee in just 30 min were developed and validated.
- The methods were found to be highly specific (100%) and sensitive (100%).
- The tests can be used by inexperienced users and does not require any additional reagents or equipment.