Product: OtaCLEAN; 3 mL widebore

According to the matrix please select the appropriate method.

1 For non fatty matrices recommended e.g. wheat, malt:

- Extract 20 g of sample with 100 mL (80 % methanol : 20 % water) in a blender jar at high speed for three minutes.
- Pass the extract through a plaited filter.
- Add 12 mL of the purified extract to 48 mL PBS buffer (pH 7.2).
- Continue with 7.

(Note: If there is a strong precipitation by mixing with PBS buffer, a further filter or centrifuge step is highly recommended before passing through the IAC column).

2 For fatty matrices recommended e.g. nuts, some fatty spices, and pistachio paste:

- Add 2.0 g NaCl to the sample.
- Extract with 100 mL of methanol: water (8:2) and 50 mL of n-hexane in a blender jar at high speed for three minutes.
- Pass the extract through a plaited filter

(Note: If there is a separation of phases to be found, the lower liquid phase is used for the following steps). To accelerate phase separation the extract could be centrifuged at 1000xg for 5 min.

- Add 12 mL of the purified extract to 48 mL PBS buffer (pH 7.2).
- Continue with 7.

3 For spices recommended e.g. black pepper, coriander, cumin, turmeric, and ginger:

- Add 2.0 g NaCl to the sample.
- Extract with 100 mL of methanol: water (8:2) and 50 mL of n-hexane in a blender jar at high speed for three minutes.
- Pass the extract through a plaited filter

(Note: If there is a separation of phases to be found, the lower liquid phase is used for the following steps). To accelerate phase separation the extract could be centrifuged at 1000xg for 5 min.

- Add 5 mL of the purified extract to 30 mL PBS buffer (pH 7.2) containing 8% Tween20.
- Continue with 7.

4 For coffee matrices recommended e.g. coffee beans, instant coffee:

- 5,0 g of the sample were homogenized and solved in 40 mL (50 % methanol / 50% 3 % $NaHCO_3$). 10 mL of this solution are mixed thoroughly with 10 mL of dichloromethane for 5 min.

  The upper liquid phase was mixed with 10 mL dichloromethane for5 min. *(Note: If there is a separation of phases to be found, the upper liquid phase is used for the following steps). To accelerate phase separation*
- *the extract could be centrifuged at 1000xg for 5 min.*

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- Add 2.4 mL of the purified extract to 57,6 mL PBS buffer (pH 7.2).
- Continue with 7.

5 For wine matrices recommended e.g. red wine, white wine:

- 10 mL of the sample was mixed with 10 mL (1 % PEG, 5 % NaHCO3) for 3 min.
- The sample is passed through a plaited filter to remove precipitations.
- 12 mL of the sample was diluted with 48 mL PBS (pH 7.2).
- Continue with 7.

6 For beer matrices recommended:

- 20 mL of the sample were degassed by sonication.
- 8 mL of 3 % NaHCO3 solution was added and mixed well.
- The sample is passed through a plaited filter to remove precipitations and suspended particles.
- 12 mL of the sample was diluted with 48 mL PBS (pH 7.2).
- Continue with 7.

7 Immunoaffinity chromatography procedure:

- The diluted extract is passed through a 0.2 µ syringe filter.
- 5-50 mL (depending on the sensitivity of the detection) (for spices, e.g. black pepper, cumin, turmeric, ginger, coriander, a maximum of 14 mL) is applied on the OtaCLEAN column. A gentle vacuum or overpressure may be used in all steps passing liquid through the column; nevertheless, it is indispensable to maintain a maximum flow rate of 2 mL/min. (50 mL diluted extract (using protocol 1 or 2) represent 2 g matrix. 14 mL diluted extract (using protocol 3) represent 0.4 g matrix. 50 mL diluted extract (using protocol 4) represent 0.25 g matrix. 50 mL diluted extract (using protocol 5) represent 5 g matrix. 50 mL diluted extract (using protocol 6) represent 7.142855 g matrix.)
- To wash the column, pass 10 mL of distilled water through the column, which could be used to wash residual sample material from the reservoir.
- Carefully remove the residual water in the column.
- Elute with at least 2 times 1 mL of methanol; let the first addition of methanol act on the gel for 5 minutes.
- Dilute or concentrate eluate to your requirements and measure directly by HPLC with fluorescence detection. The use of a post-column derivatization is recommended!

If you have any questions, please contact: mycotoxins@LCTech.de

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