Ochratoxin in Wine and Beer using the Caliper Life Sciences' RapidTrace

1.0 Detection instrument: HPLC with fluorescence detector

2.0 Sample type: White wine, Red wine and Beer

3.0 Column capacity: 100 ng Ochratoxin A

4.0 Limit of detection:
0.01ng/mL (based on signal-to-noise ratio of 3:1)

5.0 Assay range listed in AOAC Official Method:

Commodity	Assay Range
White wine	0.1 – 2.0 ng/mL
Red wine	0.2 – 3.0 ng/mL
Beer	0.2 – 2.0 ng/mL

6.0 Recovery:

Commodity	Recovery
White wine	88.2 – 105.4%
Red wine	84.3 – 93.1%
Beer	87.0 – 95.0%

7.0 Material required:

7.1 OchraTest™ immunoaffinity column (Vicam cat # 13012, G1021)

7.2 4mL silanized glass vials or glass cuvettes (Vicam cat # 34000)

7.3 Whatman GF/A or Microfibre filter, 1.5 µm, 11 cm (Vicam cat # 31955)

7.4 Pump Assembly Stand (Vicam cat # 21020) or vacuum manifold

7.5 Polyethylene glycol (PEG) 8000 (Vicam cat # G1015, 250g)

7.6 Methanol, HPLC grade

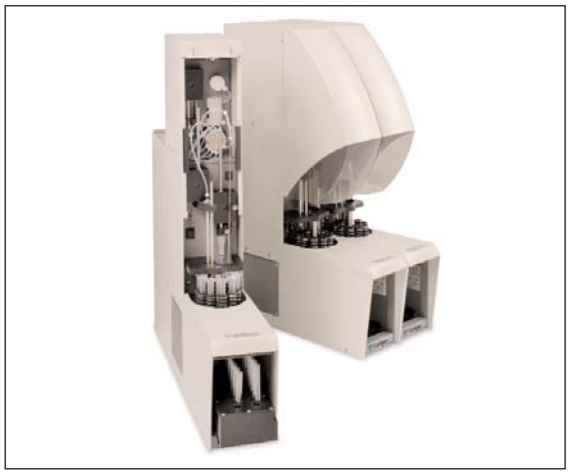
7.7 Acetonitrile, HPLC grade.

7.8 Distilled, reverse osmosis or deionized water

7.9 Glacial acetic acid, 99% purity.

7.10 Diluting Solution: 1% PEG + 5% $NaHCO_3$, pH 8.3. Dissolve 10 g PEG, and 50 g $NaHCO_3$ in approximately 950 mL water and dilute to 1 L with water.

7.11 Washing Solution: 2.5% NaCl + 0.5% $NaHCO_3$, pH 8.1. Dissolve 25 g NaCl and 5 g $NaHCO_3$ in approximately 950 mL water and dilute to 1L with water.



8.0 HPLC set up:

8.1 Column: Stainless steel (150 x 4.6 mm id) packed with 5 µm C18 reversed-phase material preceded by a reversed-phase guard column (i.e., 20 x 4.6 mm id, 5 µm particle size) or guard filter (i.e., 0.5 µm, Rheodyne). Columns of different dimensions may be used, if they adequately resolve the Ochratoxin A peak from all other peaks.

8.2 LC mobile phase: Water:acetonitrile:glacial acetic acid (99+99+2, v/v/v) pH 3.2. Mix 990 mL water with 990 mL acetonitrile and 20 mL acetic acid, filter through 0.45 µm filter, and degas.

8.3 Flow rate: 1 mL/min.

8.4 Fluorescence detector: Fitted with a flow cell and set at 333 nm (excitation) and 460 nm (emission) indicating a peak from > 0.02 ng of Ochratoxin.

8.5 Injection volume: 100 µL (equivalent to 2 mL wine or beer)

8.6 Retention times: approximately 6 minutes

9.0 Sample preparation:

9.1 Cool beer at 4 °C for 30 min to prevent fast foam formation. Degas by sonicating for 1 hour.

9.2 Pour 10 mL wine or beer into a 100 mL container.

9.3 Add 10 mL Diluting Solution (1% PEG + 5% $NaHCO_3$, pH 8.3). Mix vigorously.

9.4 Filter through glass microfiber filter (Vicam cat # 31955) if solution is cloudy or if solid residue forms after dilution.

10.0 Immunoaffinity column cleanup

RapidTrace Automated Method for Wine:

Column Vicam Ochratoxin A

Method VICAM.SPE

Step #	Step	Source	Output	Vol (ml)	Rate (ml/min)
1	Purge-Cannula	MeOH	Cannula	2	42
2	Purge-Cannula	water	Cannula	2	42
3	Load	Sample	aqueous	5	2
4	Load	Sample	aqueous	5	2
5	Rinse	vent	aqueous	6	30
6	Rinse	wash	aqueous	6	3
7	Rinse	water	aqueous	6	3
8	Rinse	vent	aqueous	6	30
9	Collect	MeOH	Fraction 1	1.5	2
10	Rinse	water	Fraction 1	1.5	2
11	Purge Cannula	water	Cannula	2	42

Manual:

10.1 Connect the OchraTest™ immunoaffinity column, to pump stand or vacuum manifold.

10.2 Add 10 mL diluted solution (equivalent to 5 mL wine/beer) to the reservoir, and pass solution through the immunoaffinity column at a flow rate of about 1 drop/second. Do not permit the immunoaffinity column to run dry.

10.3 Wash the OchraTest immunoaffinity column with 5 mL Washing Solution at a flow rate of 1-2 drops/second.

10.4 Wash the OchraTest immunoaffinity columns with 5 mL water at a flow rate of 1–2 drops/second. Dry the column by passing air through it.

10.5 Elute Ochratoxin A into the glass vial or cuvette by passing 2 mL methanol through the column at a flow rate of 1 drop/second.

10.6 Evaporate the eluate to dryness at 50 °C under nitrogen.

10.7 Redissolve eluate immediately in 250 µL HPLC mobile phase and store at 4 °C in dark until HPLC analysis.

11.0 References

Visconti, A., Pascale, M. and Centonze, G. (2001) Journal of AOAC International. Determination of Ochratoxin A in Wine and Beer by Immunoaffinity Column Cleanup and Liquid Chromatographic Analysis with Fluorometric Detection: Collaborative Study. 84 (6), 1818-1827.

Visconti, A., Pascale, M. and Centonze, G. (1999) Journal of Chromatography A. Determination of Ochratoxin A in Wine by Means of Immunoaffinity Column Clean-up and High-Performance Liquid Chromatography. 864, 89-101.

12.0 Technical assistance

For assistance please contact Vicam Technical Services by phone at 800-338-4381 (in the United States, Mexico, Canada and outside Massachusetts) or at 617-926-7045 (outside the United States and in Massachusetts) or by fax at 617-923-8055 or e-mail to techservice@vicam.com

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