

Validation of screening test kit OCHRACARD for the determination of Ochratoxins in wine



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Introduction

Ochratoxin A (OTA) is produced by various moulds of the genera *Aspergillus* and *Penicillium* and is a known carcinogen and nephrotoxin of animals and humans. Ochratoxin A can be found in a variety of foodstuffs including cereals, dried vine fruits, coffee and wine. In January 2005, the European Commission fixed a limit of 2.0 mg/l (ppb) for OTA in wine, therefore it is important to validate qualitative screening methods, at detection levels equal to and around the European legislative limits. The following study aims to assess the performance of **OCHRACARD**, a qualitative screening test for the determination of Ochratoxin A on 30 naturally contaminated Italian red wine samples.

Methodology

Test Procedure for Ochratoxin Analysis

Sample Preparation

Take 20ml of red wine and adjust the pH of the wine to pH 7.8 using 4N sodium hydroxide. Remove 6ml of the wine and dilute with 6ml of PBS and mix well.

Pass 12ml of the diluted sample through the Ochracard immunoaffinity column at a flow rate of approximately one drop per second or by gravity.

Wash the column with 2 X 10ml of 2PEG in PBS.

Place a sample collection tube directly under the column and slowly pass 1ml of methanol through the column by gravity to elute the ochratoxin A from the column. During the elution process backflushing or reversing the direction of flow of the methanol two or three times is recommended to ensure complete elution of the ochratoxin A from the column. Push air through the column using a syringe to collect the last few drops of eluate in the collection tube.

Pipette 2ml of sample diluent buffer provided in the **OCHRACARD** kit into the glass barrel, pass this through the column and collect in the same tube. Mix by inversion.

OCHRACARD Assay Procedure

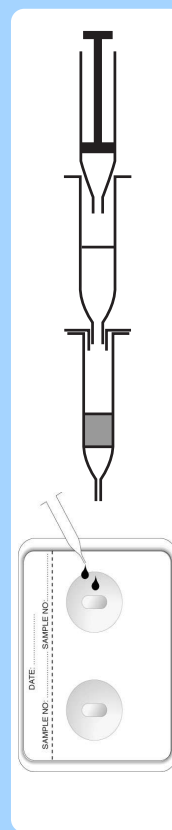
Check that the two ports on the card to be used each exhibit two light blue spots
Pipette 500µl of the sample into the corresponding port on the card and allow it to pass completely through the membrane

Pipette 100µl of the ready to use conjugate into the ports and allow this to pass completely through the membrane.

Apply 100µl of wash buffer (green label). Allow the wash buffer to pass through the membrane and wipe around the port thoroughly with a paper tissue.

Apply 100µl substrate (blue label) to the membrane, and allow the colour to develop for 5 minutes at room temperature (start the timer when the substrate is added)

Apply 100µl of stop solution (yellow label) and read results immediately after the stop solution has passed through the membrane.



Results

OCHRACARD Interpretation of results

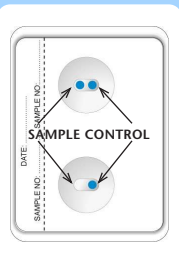
The control spot must develop a clearly visible **purple** colour in order to have a valid test result. The colour of the sample spot and the control spot does **not** need to be of the same intensity.

NEGATIVE Result

The sample should be considered to be **negative** (less than 2ppb) when the sample and the control spot both have a clearly visible colour development.

POSTIVE Result

The sample should be considered to be **positive** (more than 2ppb) when the sample spot fails to develop a readily detectable colour.



OCHRACARD TEST RESULTS FOR RED WINE COMPARED TO HPLC:

Sample	OCHRACARD RESULT (ppb)	HPLC RESULT (ppb)	Sample	OCHRACARD RESULT (ppb)	HPLC RESULT (ppb)
1	less than 2	0.52	17	more than 2	2.20
2	more than 2	3.79	18	more than 2	2.50
3	less than 2	0.71	19	more than 2	2.59
4	less than 2	0.98	20	more than 2	2.04
5	less than 2	0.09	21	more than 2	6.20
6	more than 2	4.18	22	more than 2	8.80
7	less than 2	0.18	23	less than 2	1.98
8	less than 2	0.89	24	more than 2	2.09
9	more than 2	2.89	25	less than 2	1.65
10	less than 2	1.13	26	less than 2	0.04
11	more than 2	3.80	27	less than 2	1.22
12	more than 2	4.50	28	less than 2	1.35
13	more than 2	2.96	29	more than 2	2.02
14	more than 2	1.26	30	less than 2	1.42
15	less than 2	0.20	31	less than 2	1.37 (1.5 ± 0.15 certified value)
16	more than 2	2.28	32	more than 2	2.89 (3.0 ± 0.3 certified value)

Conclusion

With the exception of one red wine sample (N° 14) the results obtained using **OCHRACARD** were in agreement with HPLC.

As part of the validation study, two wine reference materials (N° 31-32) were also analysed using **OCHRACARD** and using **OCHRAPREP** with HPLC and both methods confirmed the certified values.

OCHRACARD was found to be a suitable screening test for analysis of ochratoxin A in wine at the legal limit of 2.0 mg/l (ppb).