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Rapid ochratoxin A determination in red wine using supported liquid membrane extraction followed by fluorescence spectroscopy

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This work describes a rapid sample preparation coupled with independent fluorescence detection for ochratoxin A determination in red wine samples, which has been proven to have the advantages of low solvent consumption, high recovery efficiency and user-friendly operating processes. The validated linear detection range was from 12.5 ppb to 200 ppb with a $R^2$ coefficient of 0.9959. The limit of detection is 50.7 ppb, which needs further optimisation to reach the maximum acceptable limit regulated by European Commission (2 ppb).

### Supported Liquid Membrane Extraction (SLME)

Based on the principle of pH gradient assisted molecular transport, SLME is suitable for molecules of which the polar status can be manipulated. OTA is a weak organic acid with a pKa value of 7.1 and a log P value of 4.74, allowing the molecule to achieve protonation and deprotonation by the adjustment of pH, making it a great target for SLME.

![Diagram of SLME](image)

**Fig. 1** A diagram of a single well in the 96-well microplate extraction, where the basic principle of SLME and the OTA enrichment direction are illustrated.

### Multi-well SLME

![Diagram of Multi-well SLME](image)

**Fig. 2** The schematic view of the essential parts in the multi-well SLME: left: the assembling order of the sample plate, acceptor plate and sealing film; right: the top-down view of the acceptor plate, showing PVDF membrane. The laser comes vertically from the bottom and through the sealing tape.

### Validation of SLME recovery efficiencies

![Diagram of Validation](image)

**Fig. 3** 96-well microplate SLME acceptor phase OTA concentration versus sample OTA concentration (sample volume 250 µL, sample OTA linear range 1 - 10 µg L$^{-1}$; acceptor solution volume 50 µL; n = 3) using red wine samples with error bars representing relative standard deviations. The concentration of the extracted phase corresponds with 5 times the sample concentration, with a $R^2$ coefficient of 0.9966, therefore it can be regarded as exhaustive extraction.

### Fluorescence Spectroscopy

The fluorescence measurements were carried out with a macroscopic laboratory set-up consisting of a Ti-sapphire laser, a harmonic generating unit, a sample holder and an optical spectrum analyser. The sample was positioned on the sample holder, which contains a circular aperture. In this work, the sample plate was put upside down and the laser came from the bottom, exciting the extracted solution through the sealing tape.

![Diagram of Fluorescence Spectroscopy](image)

**Fig. 4** Fluorescence measurement setup: left: tuneable titanium-sapphire laser and harmonic generating unit; right: optical path for the excitation of the sample, after which the fluorescence spectrum is captured by the detecting fibre.

### OTA fluorescence spectra and the linearity of the calibration curve

![Diagram of OTA Fluorescence](image)

**Fig. 5** left: OTA fluorescence spectra from four different concentrations (excitation wavelength 370nm, power 30mW); right: linearity of the calibration curve using sample OTA concentrations from 12.5 to 200 ppb; error bars represent the standard deviations of 12 independent measurements.

**DID YOU KNOW**

Ochratoxin A (OTA) is a well-known mycotoxin found in several types of food. It is not only classified as possibly carcinogenic but also nephrotoxic and immunotoxic.

Wine is considered to be the second most significant source of human OTA intake.

Red wine is more likely to be contaminated due to the longer contact time between grape skins and juices during fermentation.