



# Rapid Clean-Up and Analysis of Aflatoxins in Virgin Olive Oil

## Introduction

Virgin olive oil is traditionally the major source of fat in the mediterranean and has also become more important in western diet due to its pleasant flavour and proven beneficial properties on consumers health. Nevertheless during the production process the olive oil can be contaminated by aflatoxins that are naturally produced by moulds of the genus *Aspergillus*. Aflatoxins are known to be highly toxic and potent carcinogenic metabolites that can lead to serious health problems. The major four Aflatoxins AFB<sub>1</sub>/AFB<sub>2</sub> and AFG<sub>1</sub>/AFG<sub>2</sub> are named after their blue or green fluorescence upon exposure in UV-light.

We developed a rapid, simple and sensitive HPLC method to determine Aflatoxins in olive oil samples. Silica (SiOH) based solid phase extraction is used for a rapid and direct clean-up of the toxins and an HPLC-post-column photochemical derivatization is used to increase the detection sensitivity of AFB<sub>1</sub> and AFG<sub>1</sub>.

## Procedure

### Aflatoxin Standards

The standard stock solution of the Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (REA-MIXAFL-1ml) was obtained from AFFINISEP Co.

### Extraction and Clean-Up of Aflatoxins

2g of olive oil sample was dissolved in 6 ml of n-Hexane and applied to a 200 mg, 6 ml silica solid phase extraction cartridge, previously conditioned with 3 ml n-hexane. The cartridge was washed with 3 ml n-hexane and 4 x 3ml n-hexane:2-propanol (9:1, v/v) and subsequently dried under vacuum for 3 minutes. Aflatoxins

were eluted directly into a 1.5 ml autosampler vial with 1 ml Methanol.

## HPLC Analysis

### Equipment:

SunChrom HPLC 2000 System consisting of:

- SunFlow 2040 HPLC Organizer
- SunFlow 2010 HPLC Pump
- SunTherm 2070 Column Thermostat
- UV-Reactor
- Fluorescence Detector
- Optimas Autosampler



Set-Up of the SunFlow HPLC System

### Analytical Conditions:

<b>Analytical Column</b>	YMC-Aqua, 5 $\mu$ m, 150 x 4,6 mm (Yamamura Chemicals Co.)
<b>Mobile Phase</b>	10 mM Sodium-Phosphate buffer (pH 7.2): Methanol: Acetonitrile (72:23:5, v/v/v)
<b>Flowrate</b>	1.2 ml/min
<b>Injection Volume</b>	20 $\mu$ l
<b>Fluorescence Detection</b>	Excitation ( $\lambda$ ): 365 nm Emission ( $\lambda$ ): 430 nm



## Results

### Calibration Curve

A six-point calibration curve was generated in the range of 2.5 - 10 ng/ml for AFB1 and AFG1 and 0.625 - 2.5 ng/ml for AFB2 and AFG2. The  $R^2$  value exceeded 0.999.

### Recoveries

Table I: Recovery results for olive oil samples (Directly injected Aflatoxin standard represents 100%)

Mycotoxin	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
Aflatoxin-Standard	100	100	100	100
Spike Level ( $\mu\text{g}/\text{kg}$ )	5	2.5	5	2.5
Recovery (%)	103	108	109	103

### Chromatogram

The elution profiles of the samples show that the olive oil was not naturally contaminated with aflatoxins and there were no interfering matrix effects present after the SPE clean-up.

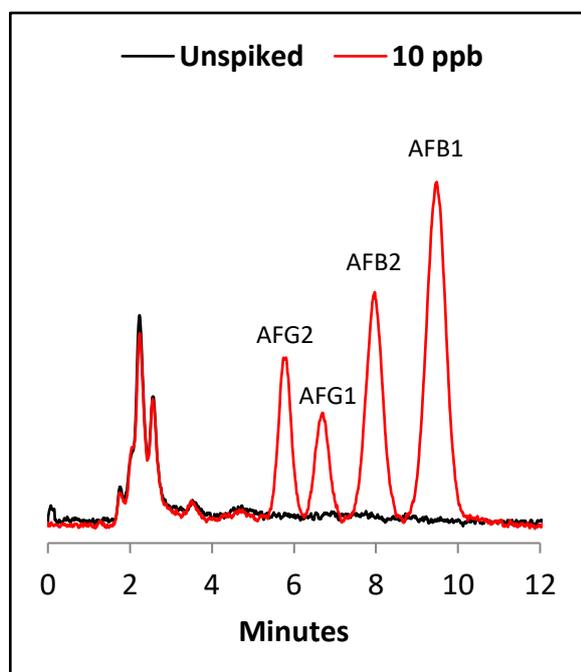


Figure 1: Elution profile of olive oil sample unspiked (black line) and sample spiked with 10 ppb AFB1/AFG1 and 2.5 ppb AFB2/AFG2 (red line) respectively.

### Conclusion

The described procedure allows a fast and simple clean-up of aflatoxins from oil samples in less than 45 minutes with a limit of quantification  $< 1$  ppb AFB1. Removal of matrix interferences and post-column photochemical derivatization highly enhances the sensitivity of aflatoxin analyses in olive oil. If desired, the methodology also allows the quantification of much lower concentrations by using more oil sample, concentrating the SPE eluates and using an increased injection volume for HPLC analysis.

The methodology can in principle be applied to all kind of natural oils like argan, canola, sunflower or pumpkin seed oil and more.

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