



COMUNICACIONES
A CONGRESOS

2004



ÁREA DE CALIDAD Y SEGURIDAD ALIMENTARIA

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Título: Selectivity improvement in the fluorimetric detection for the HPLC determination of Benzo(a)Pyrene in refined olive oil
Congreso: 11th Symposium on Sample Handling for Environmental and biological Analysis
Lugar de celebración: Bayona, España
Fecha: Abril 2004



SELECTIVITY IMPROVEMENT IN THE FLUORIMETRIC DETECTION FOR THE HPLC DETERMINATION OF BENZO(a)PYRENE IN REFINED OLIVE OIL

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ABSTRACT

A fast and high sensitive HPLC method for the determination of benzo(a)pyrene in refined olive oil is presented. The method is based on the use of a reversed-phase column and a gradient elution program. The detection is performed by fluorescence. The method is simple, sensitive and selective. The detection limit is 0.1 ng mL⁻¹.

Keywords: benzo(a)pyrene, refined olive oil, HPLC, fluorescence, detection limit.

The determination of benzo(a)pyrene in refined olive oil is a complex task due to the presence of many other compounds in the sample. The use of a reversed-phase column and a gradient elution program allows the separation of benzo(a)pyrene from the other compounds. The detection is performed by fluorescence, which is a very sensitive and selective method. The detection limit is 0.1 ng mL⁻¹.



INTRODUCTION

Benzo(a)pyrene is a polycyclic aromatic hydrocarbon (PAH) that is found in refined olive oil. It is a known carcinogen and its presence in food is a concern. The determination of benzo(a)pyrene in refined olive oil is a complex task due to the presence of many other compounds in the sample.

Materials and a fast and sensitive HPLC method for the determination of benzo(a)pyrene in refined olive oil is presented.

MATERIALS AND METHOD

The HPLC method consisted of a reversed-phase column and a gradient elution program. The detection is performed by fluorescence.

RESULTS

The method is simple, sensitive and selective. The detection limit is 0.1 ng mL⁻¹.

CONCLUSIONS

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HPLC-FLD ANALYSIS CONDITIONS

Flow: 1.0 mL/min
 Injection volume: 10 µL
 Column: Reversed phase, Chromspher PAH (Chrompack) 3 µm, 150 x 4.6 mm ID
 Column oven: 25°C
 Programming of solvents is given in table 1

GRADIENT ELUTION PROGRAM		
Time (minutes)	Water	Acetonitrile
0	90 %	10 %
3	90 %	10 %
30	0 %	100 %
32.5	0 %	100 %
35	90 %	10 %

DETECTION PARAMETERS	
Wavelength	... exc = 360 nm
	... em = 430 nm
Range of wavelength	... exc = (25-30) nm
	... em = 300-500 nm



Fig. 1. Chromatogram of olive oil sample, λ_{exc} = 430 nm and λ_{em} = 250 nm



Fig. 2. Chromatogram of olive oil sample, λ_{exc} = 430 nm and λ_{em} = 300 nm



Fig. 3. Excimer spectrum, Range 300-500 nm.



Fig. 4. Excimer spectrum, Range 250-500 nm.



- **Autores:** Sobrado C, Blanco L., Quintela MC, Cabaleiro S., González JC, Vieites JM.
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Congreso: Dioxin 2004
Lugar de celebración: Berlín (Alemania)
Fecha; Septiembre 2004



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Título: Dioxin and furan levels in feedinstuffs used in Spanish turbot aquaculture
Congreso: Dioxin 2004
Lugar de celebración: Berlín (Alemania)
Fecha; Septiembre 2004



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Congreso: 11th Symposium on Sample Handling for Environmental and biological Analysis
Lugar de celebración: Bayona, España
Fecha: Abril 2004
- **Autores:** Francisco Santaclara, Jorge Lago, Ana G Cabado, Juan M. Vieites
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Fecha: Diciembre 2004