APPLICATION NOTE | AN0012

NEW: With HPLC Direct Injection Module

SOLUTIONS BY LCTech

DETERMINATION OF PAH
via GPC with On-line EVAporation and HPLC-FLD Measurement

APPLIKATIONSNOTE | www.LCTech.de
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Determination of PAH via GPC with on-line EVAporation and HPLC-FLD-measurement

Principle of the Method

The extracted samples, i.e. food, are handled automatically by the FREESTYLE GPC. They are processed via the on-line evaporation in the EVAporation module and filled into an HPLC vial ready for injection.

Procedure

The extracted, dried and filtered raw extract is adjusted to 10.0 mL and placed on the FREESTYLE system in a 16 mL vial that is closed with a cap/septum.

The sample is processed on the system using the method shown in the report on page 4.

The description of the process in brief:
5.0 mL are applied by the calibrated sample loop on the GPC column. The matrix gets separated and the main run with the PAH is already concentrated on-line in the EVAporation module, that means while the process in the GPC is still running. After the evaporation an automated solvent exchange to Acetonitrile/1% 1-Butanol takes place. Finally the EVAporation chamber is rinsed, the concentrate is precisely adjusted to 1.0 mL and filled into an HPLC vial.

The measurement of the analytes happens on Thermo 3000 Ultimate with a fluorescence detector.

Devices and Materials

1.	FREESTYLE Basic	P/N 12663
2.	FREESTYLE EVAporation	P/N 13841
3.	FREESTYLE GPC	P/N 12664
4.	FREESTYLE Direct Injection	P/N 13890
5.	GPC-Column	P/N GPC10011
6.	Frame for rack 11933	P/N 11915
7.	Rack for 16 mL vials	P/N 11933
8.	Frame for rack 11920	P/N 11915
9.	Rack for GC vials	P/N 11920
10.	16 mL- vials	P/N V0016 (100 pcs/pck)
11.	Screw cap for 16 mL vials	P/N V0016-SL (100 pcs/pck)
12.	Seals	P/N V0016-D (100 pcs/pck)
13.	GC vials	P/N V0001 (100 pcs/pck)
14.	Crimp cap for GC-vials with seal	P/N V0001-B (100 pcs/pck)
15.	Chiller	P/N 12060, 230 VAC, 50 Hz

16. Ethyl acetate/Cyclohexane for the analysis of organic trace compounds
17. Aceton p.a.
18. Acetonitrile p.a. with 1 % 1-Butanol
19. Standard laboratory glassware and -apparatus

On-line connection from GPC directly into the EVAporation chamber - possibility of precise concentration up to 0.2 mL.

Parameterisation of the Method on the FREESTYLE-System

FREE-STYLE

LCTech FreeStyle - Report on Methods: GPC -> EVA Date: 10.10.2014 Time: 09:23:02

Name: PAH_Flex.fmt

GPC - Method: PAH_Flex_GPC.gpc	Online ======= >	EVA - Method: PAH_Flex_EVA.evp

GPC:	Type:	Loop Overfill	
	Source / Input of Sample:	from vial / vials	Transfer Speed: 10 ml / min.
	Volume of Sample Loop:	5 ml	
	Flow:	5 ml/min	
	Min. Pressure:	0.3 bar	
	Max.Pressure:	12 bar	
	Forerun:	30 min.	
	Collection Time:	20 min.	
	Tailing Time:	0.2 min.	
	UV Recording:	OFF	
	Column:	D25_1.clm	
	Method of Fractioning:	GPC Pump OFF	
	Source vial list:		
		Nr.: 1 1 x 5.5 ml --> Type1@16	Sediment position: 0 mm
	Collection vial list:		
		Nr.:1 1 x 240 ml --> Type1@240	Dump: no Online: yes

EVA:	Temperature water heating 40 °C		Temperature bottom cone 50 °C
	Sample input: Online from GPC or SPE process		
	Batch volume = limit from where concentration starts: 5 ml (fix) + Waiting time: 10 min.		
	Vacuum during GPC online sample input: 200 mbar		
	Phase 1: Concentrate to level: 1 ml		
	Vacuum absolute: 180 mbar		
	Rinsing volume after phase 1: 5 ml	Rinsing steps: 1 x	Solvent from Port: 8 (Acetonitrile-1-Butanol)
	Skip phase 2		
	Time control for vacuum process: no		
	to dryness: no		
	Nitrogen blow-down: no		
	Remove Aliquot: no		
	Solvent exchange: yes		
	At reach of level: 2 ml		
	Solvent addition per exchange: 5 ml	Solvent from Port: 8 (Acetonitrile-1-Butanol)	
	Number of solvent exchanges: 1	End volume after exchange: 0.5 ml	
	Vacuum starts at level: 180 mbar abs.	Gradient of vacuum : -20 mbar/min	Vacuum end at level: 100 mbar abs.
	Rinsing, filling up, mixing and transfer into vials:		
	Rinsing volume at the end: 0.5 ml	Rinsing steps: 1 x	Solvent from Port: 7 (Acetonitrile)
	Fill up to volume:	1 ml	Way of mixing: suck up and release
	Concentrate: into vials		
	Nr.: 1 1 [each]	Type: Type1@1 ml	Volume per vial 1 ml
	Fill Quantitativ: no		
	1. Cleaning cycle		
	Rinsing volume: 5 ml	Rinsing steps: 1 x	Solvent from Port: 9 (Acetone)
	2. Cleaning cycle		
	Rinsing volume: 5 ml	Rinsing steps: 1 x	Solvent from Port: 1 (EA/CY 1/1)

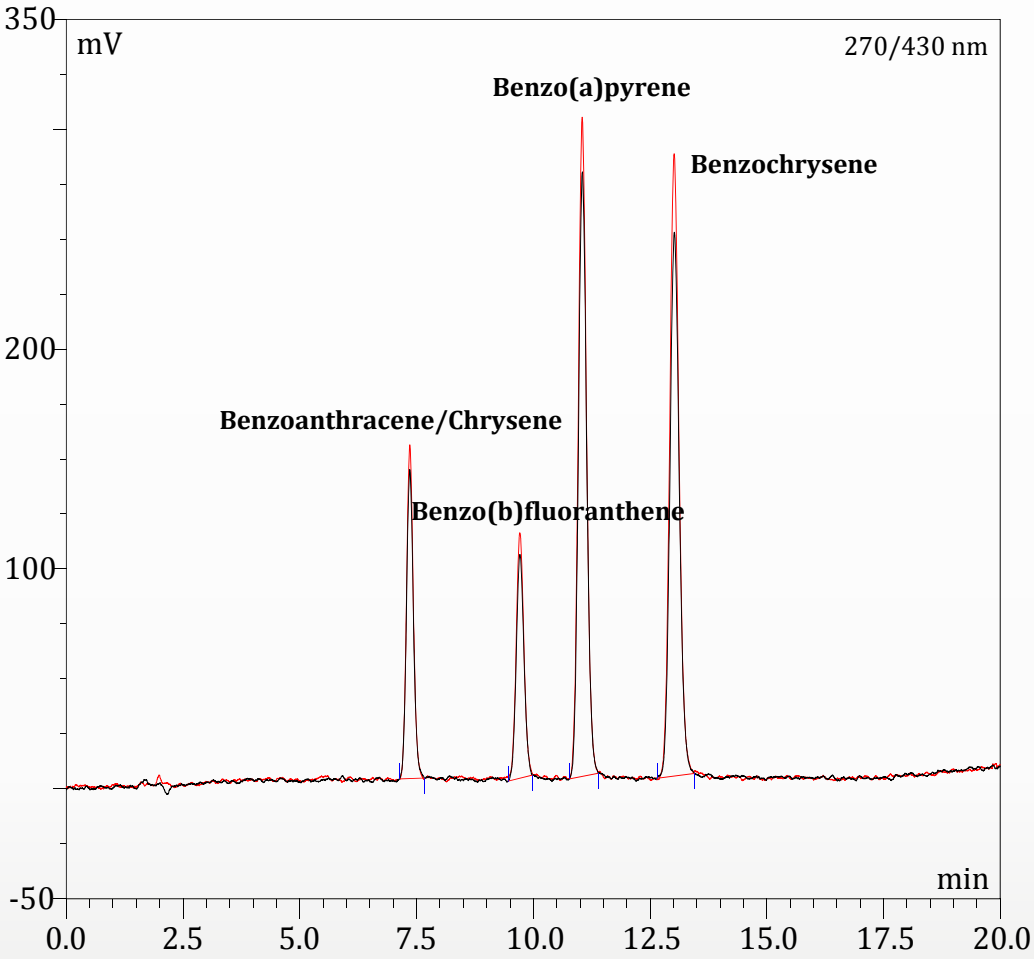
Results without HPLC Direct Injection Module

The process time of a sample including solvent exchange and transfer into a GC vial is just 1 h 35 min.

Remark: The process time takes at least 10 minutes less, if the sample is measured in a GC and a solvent exchange isn't necessary

Recovery rates of an exemplary experiment (n=3)

Description	Recovery Rate
Benzoanthracene/Chrysene	**91 ± 4 %**
Benzo(b)fluoranthene	**90 ± 4 %**
Benzo(a)pyrene	**85 ± 5 %**
Benzochrysene	**83 ± 8 %**



The figure shows the overlay of two fluorescence chromatograms: external PAH standard (red) and a finished sample (black).

Parameterisation of the Method on the FREESTYLE-System with HPLC Direct Injection Module

FREE-STYLE™

LCTech FreeStyle - Report on Methods: GPC -> EVA Date: 27.02.2015 Time: 16:12:03

Name: PAK_Flex_Direct.fmt

GPC - Method: PAK_GPC.gpc	Online =======>	EVA - Method: PAK_EVA.evp

GPC:	Type:	Loop Overfill	
	Source / Input of Sample:	from vial / vials	Transfer Speed: 10 ml / min.
	Volume of Sample Loop:	5 ml	
	Flow:	5 ml/min	
	Min. Pressure:	0.3 bar	
	Max.Pressure:	12 bar	
	Forerun:	30 min.	
	Collection Time:	20 min.	
	Tailing Time:	0.2 min.	
	UV Recording:	OFF	
	Column:	D25_1.olm	
	Method of Fractioning:	GPC Pump OFF	
	Source vial list:		
		Nr.: 1 1 x 5.5 ml --> Type1@16	Sediment position: 0 mm
	Collection vial list:		
		Nr.:1 1 x 240 ml --> Type1@240	Dump: no Online: yes

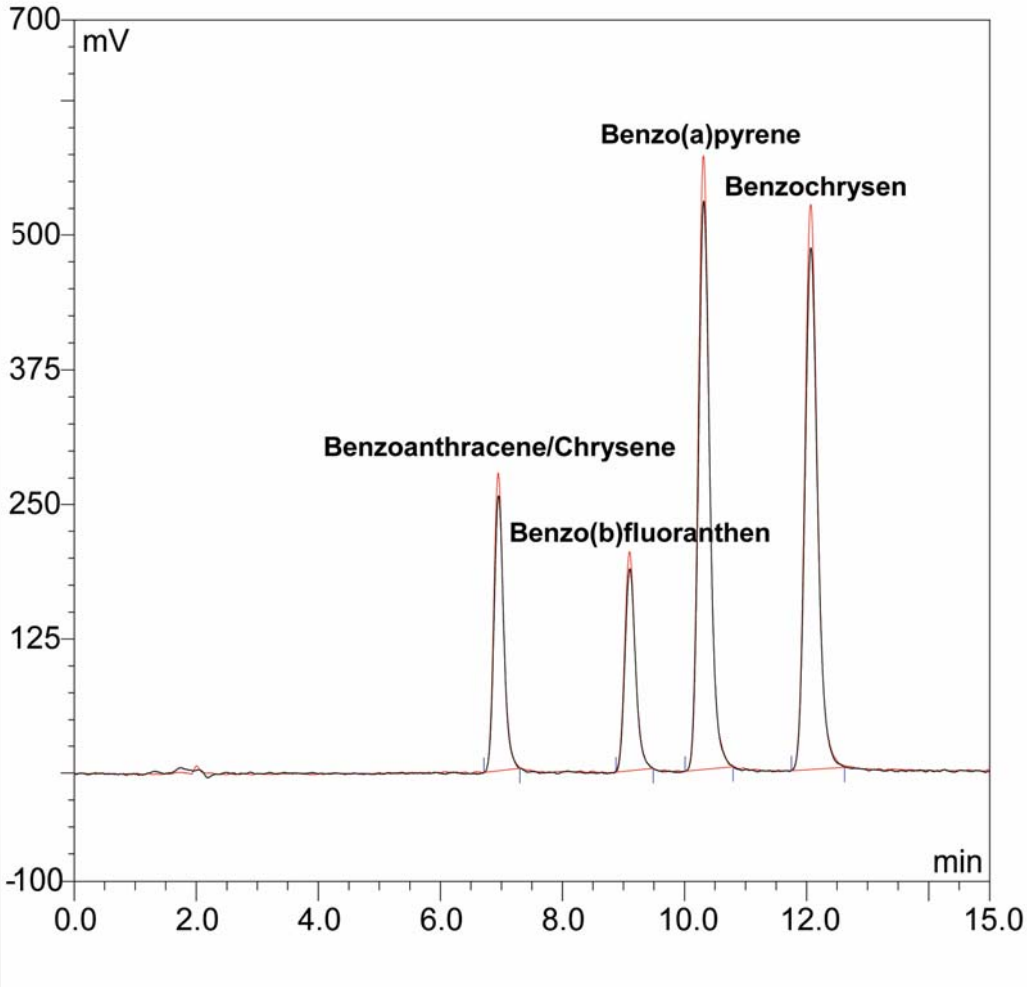
EVA:	Temperature water heating 40 °C		Temperature bottom cone 50 °C
	Sample input: Online from GPC or SPE process		
	Batch volume = limit from where concentration starts: 5 ml (fix) + Waiting time: 10 min.		
	Vacuum during GPC online sample input: 200 mbar		
	Phase 1: Concentrate to level: 1 ml		
	Vacuum absolute: 180 mbar		
	Rinsing volume after phase 1: 5 ml	Rinsing steps: 1 x	Solvent from Port: 11 (Acetonitril-Butanol)
	Skip phase 2		
	Time control for vacuum process: no		
	to dryness: no		
	Nitrogen blow-down: no		
	Remove Aliquot: no		
	Solvent exchange: yes		
	At reach of level: 2 ml		
	Solvent addition per exchange: 5 ml	Solvent from Port: 11 (Acetonitril-Butanol)	
	Number of solvent exchanges: 1	End volume after exchange: 0.5 ml	
	Vacuum starts at level: 180 mbar abs.	Gradient of vacuum : -20 mbar/min	Vacuum end at level: 100 mbar abs.
	Rinsing, filling up, mixing and transfer into vials:		
	Rinsing volume at the end: 0.5 ml	Rinsing steps: 1 x	Solvent from Port: 9 (Acetonitril)
	Fill up to volume:	1 ml	Way of mixing: suck up and release
	Concentrate: into vials		
	Nr.: 1 1 [each]	Type: HPLC ml	Volume per vial 0.5 ml
	Fill Quantitativ: no		
	1. Cleaning cycle		
	Rinsing volume: 5 ml	Rinsing steps: 2 x	Solvent from Port: 1 (EA/CH)
	2. Cleaning cycle		
	Rinsing volume: 5 ml	Rinsing steps: 1 x	Solvent from Port: 1 (EA/CH)

Results with HPLC Direct Injection Module

The process time of a sample including solvent exchange and transfer directly into the HPLC system via FREESTYLE Direct Injection module is just 1 h 45 min (process time FREESTYLE). The processing of the sample is done fully automatically from raw extract to the chromatogram without any manual working step.

Recovery rates of an exemplary experiment (n=3)

Description	Recovery Rate
Benzoanthracene/Chrysene	**94 ± 2 %**
Benzo(b)fluoranthene	**93 ± 3 %**
Benzo(a)pyrene	**93 ± 2 %**
Benzochrysene	**94 ± 2 %**



The figure shows the overlay of two fluorescence chromatograms:
external PAH standard (red) and a finished sample (black).

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