

SOLUTIONS BY



DETERMINATION OF THE HYDROCARBON-INDEX DIN EN ISO 9377-2:2000

Keywords: DIN EN ISO 9377-2:2000, DEV H53; LAGA-Richtlinie KW/04 – 2009, Hydrocarbon Oil Index, Elufix, TPH, Total Petroleum Hydrocarbons, EPA Methods 418.1 and 1664A

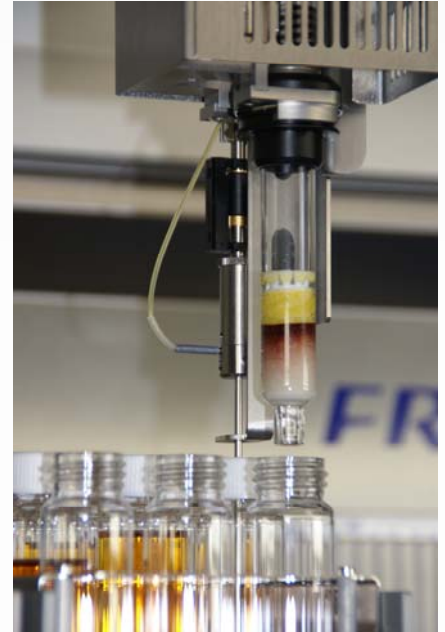
Determination of the Hydrocarbon Oil Index EN ISO 9377-2:2000 (H53-Method)

The European method EN ISO 9377-2:2000 for the determination of the hydrocarbon index in water replaces the German CFC-based (R113; 1,1,2-Trichlorotrifluorethane) method DIN DEV Method 38409-H18. It is suitable for drinking, surface, ground, waste water and water from sewage treatment plants with a hydrocarbon oil index of higher than 0.1 mg/L.

The method can be separated into several main steps; two of these steps, the clean-up via Elufix Florisil ready-to-use columns and the concentration of the obtained extracts can be performed automated via the FREESTYLE SPE and/or EVAporation module.

This application note describes the automated handling of the SPE and EVAporation step, as well as both steps in combination together.

The Elufix columns of course can also be used manually via gravitation or via the vacuum manifold (EluVac; P/N 11146).



Elufix – Ready-to-use Columns of LCTech

Automated Handling with FREESTYLE SPE

Materials and Chemicals

- | | |
|---|----------------------------|
| 1. FREESTYLE BASIC | P/N 12663 |
| 2. FREESTYLE SPE | P/N 12668 |
| 3. Frame for Rack 12399 | P/N 12103 |
| 4. Rack for 60 mL vials
(Purpose-built for the method:
Sample reservoirs and storage vessel
in one rack, for up to 12 samples) | P/N 12399 |
| 5. Frame for rack 13416 | P/N 12103 |
| 6. Rack for SPE columns
(Rack for Elufix columns, 18 pos.) | P/N 13416 |
| 7. Column adapter (reusable) | P/N 12337 (10 pcs/pck) |
| 8. Elufix columns (Florisil/Na 2g/2g) | P/N 9370 (50 pcs/pck) |
| 9. 60 mL vials | P/N F060 (100 pcs/pck) |
| 10. Screw cap for 60 mL vials | P/N V0024-SL (100 pcs/pck) |
| 11. Seals | P/N V0024-D (100 pcs/pck) |
| 12. Petroleum ether with boiling range
between 40 and 60 °C p.a. | |
| 13. MHC standard (e.g. BAM K010e) | |
| 14. Standard laboratory glassware and -apparatus | |



Standard Application Elufix Columns

The standard application is used for loading extracted samples method compliant and fully automated onto the Elufix columns, **without any further EVaporation step in the system.**

In this procedure the sample is aspirated by a needle and a valve, respectively, without any motion of the robotics, transferred onto the Elufix column and subsequently into a 60 mL vial. The column is hold by the second z-axis and an appropriate adapter ring. For this procedure a special rack is needed. The sample reservoir as well as the column can be rinsed fully automated to optimise the result.

The rinsing solution from the sample reservoir is passed over the column as well. The processed sample is taken out of the system and evaporated externally.

Procedure

Make sure that the operating state of the FREESTYLE SPE system is as described in the user manual.

Hook the frame and the racks for 60 mL vials and Elufix columns into the robotic system.

The Elufix columns can be used immediately without any testing. Each lot is tested according to point 9.6 of the method (determination of the retention capacity of stearyl stearate and recovery of the MHC standard).

Put in racks with Elufix columns and 60 mL vials (sample reservoir/vial for collection) if not already done before.

Parameterise the software for sample handling as described in attachment 1 (supplied with the system, method is saved as default method in the software).

Create a corresponding sample list and start the sequence.

After the sequence has finished, take out the used columns and the samples. Please dispose of the columns professionally. The samples are evaporated.

Recovery Test without EVaporation

For the determination of recovery rates of Elufix columns, which are handled in the FREESTYLE SPE system without EVaporation step, a method is used that correlates with point 9.6 (suitability determination of Florisil) of DIN EN ISO 9377-2:2000. Here a 10 mL portion of a 2.0 mg/mL standard (e.g. BAM K010e) is applied onto an Elufix column.

In this way it can be determined, whether the FREESTYLE SPE system is operating correctly and the recovery rates of the MHC standard are as specified in the lot certificate. A basic requirement for this test is that the columns are taken out of a new Elufix package. In this test the determined recovery rates should be between 90 and 100 %.

Procedure

The procedure is the same as with the standard application of Elufix columns, but with parameterisation described as in attachment 2.

In the last step the eluate (approx. 20 mL) is quantitatively transferred into a 25 mL volumetric flask, filled up with petroleum ether and compared with a correlating standard (0.8 mg/mL), the corresponding recovery rate is determined.

Result

A recovery rate of 96 % (specified 98,8 %) for lot 2233 was determined in a typical experiment.

Determination of Retention Capacity of Stearyl Stearate

For the determination of the retention capacity of Elufix columns, which are handled in the FREESTYLE SPE system without EVAporation step, a method is used that correlates with point 9.6 (suitability determination of Florisil) of DIN EN ISO 9377-2:2000. 10 mL of a stearyl stearate solution (200 mg in 100 mL) is applied onto the Elufix column and compared with a correlating standard.

In this way it can be determined, whether the FREESTYLE SPE system is operating correctly and whether the retention capacity of stearyl stearate is as specified in the certificate. A basic requirement for this test is that the columns are taken out of a new Elufix package. The peak area ratio of the stearyl stearate/Florisil sample measured against the standard should be less than 1.

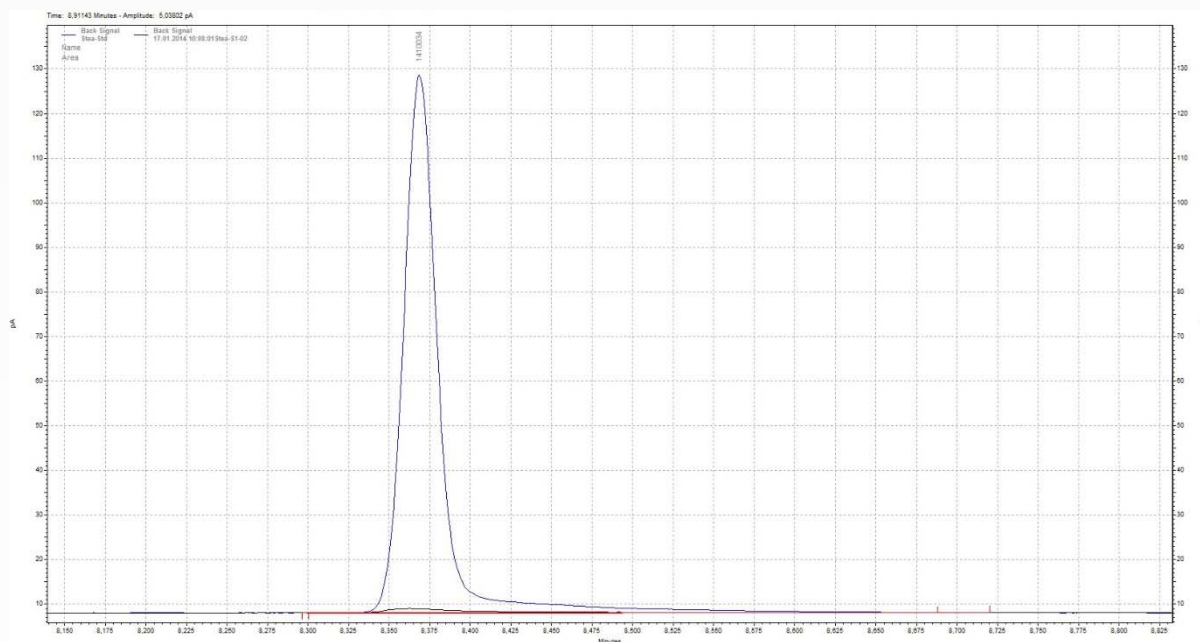
Procedure

The procedure is the same as with the standard application of Elufix columns, but with parameterisation described as in attachment 2.

In the last step the eluate (approx. 20 mL) is quantitatively transferred into a 25 mL volumetric flask, filled up with petroleum ether and compared with a correlating standard (0.5 mg/mL diluted to 25 mL) via the peak area ratios.

Result

In a typical experiment a value of 0.01 (certificate 0.01) was determined for lot 2236 (see chromatogram below).



Stearyl stearate standard (blue)
Sample cleaned-up with Florisil column (black)

FREESTYLE **EVAporation**

Materials und Chemicals

- | | |
|---|----------------------------|
| 1. FREESTYLE BASIC | P/N 12663 |
| 2. FREESTYLE EVAporation | P/N 13841 |
| 3. Frame for Rack 12399 | P/N 12103 |
| 4. Rack for 60 mL vials
(Purpose-built for the method:
Sample reservoirs and storage vessel
in one rack, for up to 12 samples) | P/N 12399 |
| 5. Frame for rack 11920 | P/N 11915 |
| 6. Rack for GC vials
(60 samples/rack; quantity according
to number of samples) | P/N 11920 |
| 7. 60 mL vials | P/N F060 (100 Pcs/pck) |
| 8. Screw caps for 60 mL vials | P/N V0024-SL (100 Pcs/pck) |
| 9. Seals | P/N V0024-D (100 Pcs/pck) |
| 10. GC vials | P/N V0001 (100 Pcs/pck) |
| 11. Crimp-caps for GC vials
with screw cap | P/N V0001-B (100 Pcs/pck) |
| 12. Chiller | P/N 12060, 230 V AC, 50 Hz |
| 13. Petroleum ether with boiling range
between 40 and 60 °C p.a. | |
| 14. Acetone p.a. | |
| 15. MHC standard (e.g. BAM K010e) | |
| 16. Standard laboratory glassware and -apparatus | |

EVAporation to a Defined Final Volume

Eluates of approximately 50 to 60 mL petroleum ether are evaporated to a precise final volume of 1 mL in the EVAporation module. As needed the final volume can be set between 0.2 mL and 5.0 mL in 0.1 mL steps easily via the software.

The EVAporation chamber is washed after the vacuum phases to transfer all MHC residues from the EVAporation chamber wall into the sample. After filling up to 1 mL the solution is transferred into a GC vial and subsequently can be put into the GC-FID autosampler. The EVAporation chamber is rinsed with acetone first, then with petroleum ether.

Procedure

Make sure that the operating state of the FREESTYLE EVAporation system is as described in the user manual.

Hook the frame and the racks for 60 mL vials and 1 mL GC vials into the robotic system.

Put in the racks with vials (sample reservoir/GC) if not already done before.

Parameterise the software for sample handling as described in attachment 3.

Create a corresponding sample list and start the sequence.

After the sequence has finished take out the used sample reservoirs and put the GC vials into the GC-FID autosampler.

Results

In a typical experiment the BAM K010e standard was used with different concentrations (0.2; 0.5; 1.0 and 2.0 mg/mL) with the parameters denoted in the attachment. The recovery rates were determined with external standards.

The recovery rates of all measured concentrations of the BAM K010e standard were > 90 %.

FREESTYLE SPE **with On-line EVAporation**

Materials and Chemicals

- | | |
|--|----------------------------|
| 1. FREESTYLE BASIC | P/N 12663 |
| 2. FREESTYLE SPE | P/N 12668 |
| 3. FREESTYLE EVAporation | P/N 13841 |
| 4. Frame for rack 12399 | P/N 12103 |
| 5. Rack for 60 mL vials
(Purpose-built for this method:
Sample reservoir and collect vials
in one rack, for up to 12 samples) | P/N 12399 |
| 6. Frame for rack 13416 | P/N 12103 |
| 7. Rack for SPE columns
(Rack for Elufix columns, 18 pos.) | P/N 13416 |
| 8. Column adapter (reusable) | P/N 12337 (10 pcs/pck) |
| 9. Elufix columns (Florisil/Na 2g/2g) | P/N 9370 (50 pcs/pck) |
| 10. Outlet for Elufix columns
for direct elution into EVAporation
chamber | P/N 13460 (10 pcs/pck) |
| 11. 60 mL vials | P/N F060 (100 pcs/pck) |
| 12. Screw cap for 60 mL vials | P/N V0024-SL (100 pcs/pck) |
| 13. Seals | P/N V0024-D (100 pcs/pck) |
| 14. Frame for rack 11920 | P/N 11915 |
| 15. Rack for GC vials
(60 samples/Rack; quantity according
to number of samples) | P/N 11920 |
| 16. GC vials | P/N V0001 (100 pcs/pck) |
| 17. Crimp-caps for GC vials
with screw cap | P/N V0001-B (100 pcs/pck) |
| 18. Chiller | P/N 12060, 230 V AC, 50 Hz |
| 19. MHC standard (e. g. BAM-K010e) | |
| 20. Petroleum ether with boiling range
between 40 – 60 °C p.a. | |
| 21. Acetone p.a. | |
| 22. Standard laboratory glassware and -apparatus | |

SPE with On-line EVaporation

The method of SPE with on-line EVaporation is operating a single Elufix column sequentially; the resulting eluate is transferred out of the special rack directly into the EVaporation chamber. The eluate is evaporated to a defined final volume between 0.2 mL and 0.5 mL, usually down to 1.0 mL.

The whole process runs **fully automated without any user intervention**. This method combines the two individual procedures (SPE and EVaporation) described above in a so called "FLEX method".

Thus the user only puts the crude sample extracted from the water into the system and takes out a concentrated and precisely adjusted extract filled into a crimped GC vial ready for measurement on a GC-FID system.

Procedure

The procedure follows the above-mentioned procedures with the difference that the rack combination is different and both individual processes (SPE and EVaporation) need to be combined to a method in the software.

The SPE and EVaporation steps are listed in the table below.

SPE steps	Fully automated
Loading	53 mL sample, 10 mL/min.
Drying	5 sec., nitrogen
Washing	2 mL petroleum ether, 15 mL/min.
Drying	10 sec., nitrogen

Evaporation parameters	Fully automated
Temperature	Water heater 40 °C Bottom cone 38 °C
Vacuum 1	Volume defined to 1 mL, 300 mbar
Rinsing volume	4 mL petroleum ether
Vacuum 2	Volume defined to 1 mL, 300 mbar
Backfill to final volume	1 mL

Parameterise the software for sample handling as described in attachment 4.

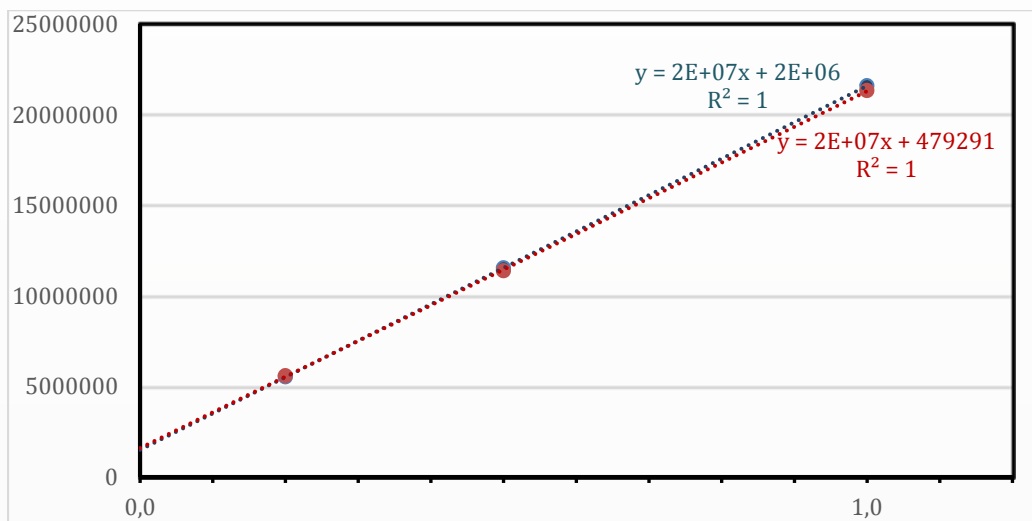
Results

In a typical experiment, the BAM K010e standard was processed with concentrations of 0.2, 0.5, and 1.0 mg/mL in 50 mL petroleum ether. The resulting concentrated extracts were measured against corresponding external standards with a GC-FID and the recovery rates were determined. For evaluation of the GC data solvent blanks and concentrated solvent blanks were determined and the GC data corrected accordingly.

The recovery rates of the processed samples were > 90 %; for data of a typical experiment refer to the table shown below.

Concentration [mg/mL]	Mean Value (n = 3)	Standard Deviation [%]
0,2	101	3
0,5	99	4
1,0	99	4

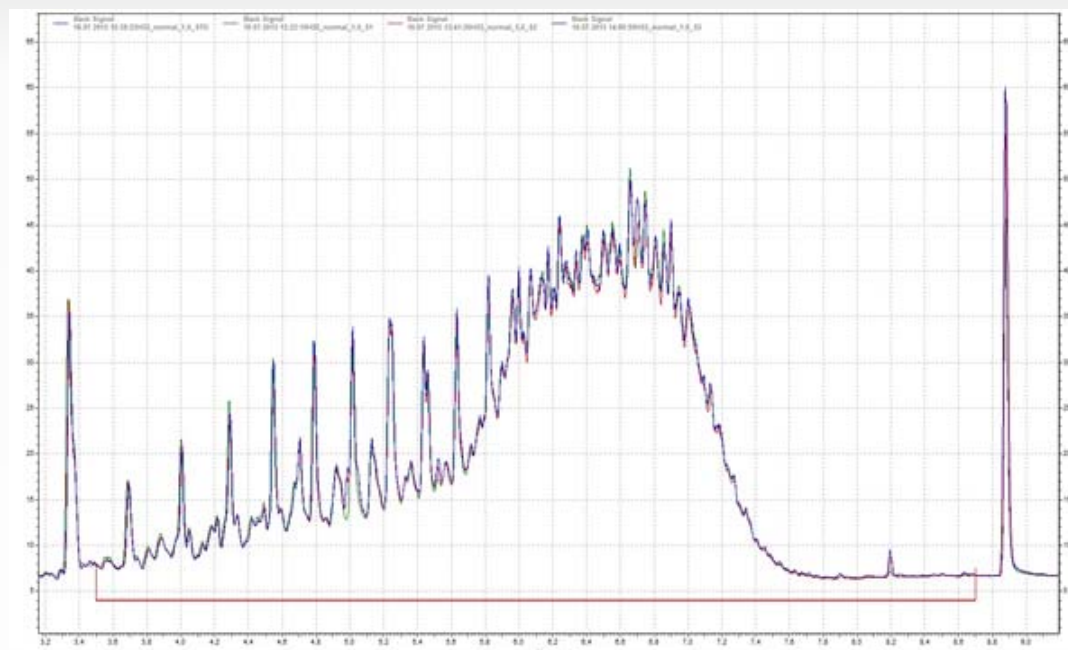
Notable is the low standard deviation in the fully automated process. It includes the handling with SPE, the subsequent evaporation as well as the measuring error of the GC-FID.



The figure above shows the linear equation of the mean values of the external standards (blue) and the MHC samples processed in the system at three different concentrations (0.2; 0.5, and 1.0 mg/mL; reddish brown) and their coefficient of determination.

APPLICATION NOTE | AN0002

The processing reproducibility of an identical MHC standard (1.0 mg/mL in 50 mL petroleum ether; n = 3) is exemplarily shown in the overlaid GC-FID chromatograms below.



Important Information

For characterising of hydrocarbons in waste please refer to DIN EN 14039:2005-01 *Characterization of waste - Determination of hydrocarbon content in the range of C10 to C40 by gas chromatography; German version EN 14039:2004*

Due to the comparatively high boiling point of n-hexane at 69 °C and the neurotoxicity, it is recommended to use petroleum ether with a boiling point of 40 to 60 °C in the system. Suitable petroleum ether qualities are available on the market; nevertheless, each lot should be checked for suitability!

Never use petroleum ether of inferior quality as blank value may increase significantly!

For a trouble-free performance of the Florisil clean-up, take care of reducing residual water from the extraction step to a minimum! Otherwise the sodium sulphate becomes encrusted and a higher pressure has to be applied. Furthermore neither an emulsion nor particulate matter should be applied onto the column!

Please make sure to use a cooler, which is dimensioned sufficiently in order to condense all solvent! In any other case, the system performance may be affected adversely and emission of gaseous solvent into the environment is increased.

Make sure to use an appropriate GC injection, GC column, and data integration technique in order to get reliable results!

Although toxicity of most of the chemicals used is low, take care of your personal safety measures!

Please dispose of the used materials and chemicals professionally!

Attachments No. 1-4

Attachment 1: SPE-Method

Date: 19.07.2017 Time: 10:38:43



LCTech FreeStyle - Report on Methods: SPE Date: 19.07.2017 Time: 10:38:43

Name: H53_SPE_2014.spe	SPE Column: LCTech_H53.col
Extension cannula:	no
Processing speed selection:	Standard (organic solvents)
Rinsing intensity:	Standard rinsing cycle wo. Extra Cleaning after Load
Use pressure limitation function during loading and washing:	no

Step: Load	Basic type: Load - Quantitative transfer in parallel mode	Step: - ID: 622
Volume: 53 ml Vial Type: Type_H53@60	Suction Speed: 15 ml/min Waiting Time after Dosage: 0 sec.	Dispensing Speed: 10 ml/min Waiting Time after Step: 150 sec.
rinsing cycle included	Rinsing volume: 3 ml Suction Speed: 20 ml/min Port: 1 Petroleum ether	Dispense: into vials Number of vials: 1 Vial Type: Type_H53@60 Rinse repetitions: 1 x Dispensing Speed: 40 ml/min Tube rinse volume: 0.1 ml

Step: Drying	Basic type: Drying - Nitrogen drying by defined time	Step: - ID: 624
Drying time with nitrogen 5 sec.		Dispense: stay on actual position

Step: Washing	Basic type: Washing	Step: - ID: 625
Volume: 2 ml Suction Speed: 20 ml/min Repetitions: 0 Waiting Time after Dosage: 0 sec.	Dispensing Speed: 15 ml/min Waiting Time after Step: 0 sec. Dispense: stay on actual position	Port: 1 Petroleum ether

Step: Drying	Basic type: Drying - Nitrogen drying by defined time	Step: - ID: 626
Drying time with nitrogen 10 sec.		Dispense: stay on actual position

Time required for this example: 21 minutes

Attachement 2: SPE-QS-Method

Date: 19.07.2017 Time: 11:32:53



LCTech FreeStyle - Report on Methods: SPE Date: 19.07.2017 Time: 11:32:53

Name: H53_SPE_QS.spe	SPE Column: LCTech_H53.col
Extension cannula:	no
Processing speed selection:	Standard (organic solvents)
Rinsing intensity:	Standard rinsing cycle
Use pressure limitation function during loading and washing:	no
	wo. Extra Cleaning after Load

Step: Load Basic type: Load - Quantitative transfer in parallel mode Step: - ID: 622

Volume: 10 ml	Suction Speed: 15 ml/min	Dispensing Speed: 10 ml/min	
Vial Type: Type_H53@60	Waiting Time after Dosage: 0 sec.	Waiting Time after Step: 150 sec.	
		Dispense: into vials	Number of vials: 1
			Vial Type: Type_H53@60
rinsing cycle included	Rinsing volume: 3 ml	Rinse repetitions: 2 x	
	Suction Speed: 20 ml/min	Dispensing Speed: 40 ml/min	Tube rinse volume: 0.1 ml
	Port: 1 Petroleum ether		

Step: Drying Basic type: Drying - Nitrogen drying by defined time Step: - ID: 624

Drying time with nitrogen 5 sec.	Dispense: stay on actual position
----------------------------------	-----------------------------------

Step: Washing Basic type: Washing Step: - ID: 625

Volume: 2 ml	Suction Speed: 20 ml/min	Dispensing Speed: 15 ml/min	Port: 1 Petroleum ether
	Repetitions: 0	Waiting Time after Step: 0 sec.	
	Waiting Time after Dosage: 0 sec.	Dispense: stay on actual position	

Step: Drying Basic type: Drying - Nitrogen drying by defined time Step: - ID: 626

Drying time with nitrogen 10 sec.	Dispense: stay on actual position
-----------------------------------	-----------------------------------

Time required for this example: 21 minutes

Attachment 3: EVaporation-Method

Date: 19.07.2017 Time: 10:39:15



LC Tech FreeStyle - Report on Methods: EVA Date: 19.07.2017 Time: 10:39:15

Name: H53_EVA_2014.evp		
Temperature water heating 40 °C	Temperature bottom cone 38 °C	
Sample input: suck from vial / vials into chamber over sample probe and tubing, option with rinsing cycle		
Number of vials: 1 x Type_H53@60 rinsing cycle included	Vacuum at suction: 300 mbar	Maximum time vacuum suction: 60 min.
Rinsing volume: 5 ml	Rinsing steps: 2 x	Solvent from Port: 1 Petroleum ether
Phase 1: Concentrate to level: 1 ml Vacuum absolute: 300 mbar		
Rinsing volume after phase 1: 4 ml	Rinsing steps: 1 x	Solvent from Port: 1 Petroleum ether
Phase 2: Concentrate to level: 1 ml Vacuum absolute: 300 mbar		
Rinsing volume after phase 2: 0 ml	Rinsing steps: 0 x	Solvent from Port: 1 Petroleum ether
Time control for vacuum process: no		
to dryness: no		
Nitrogen blow-down: no		
Remove Aliquot: no		
Solvent exchange: no		
Rinsing, filling up, mixing and transfer into vials: Rinsing volume at the end: no		
Fill up to volume: of Port: 1 Petroleum ether	1 ml	Way of mixing: with gas / air, Volume = 0 ml
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: 7 Acetone
2. Cleaning cycle		
Rinsing volume: 5 ml	Rinsing steps: 1 x	Solvent from Port: 1 Petroleum ether

Time required for this example: 35 minutes

Attachment 4: Flex-Method

Date: 19.07.2017 Time: 10:44:02



LCTech FreeStyle - Report on Methods: SPE_H53 -> EVA Date: 19.07.2017 Time: 10:44:02

Name: H53_SPE_EVA_2014.fsh		
SPE - Method: H53_SPE_2014.spe	Online =====>	EVA - Method: H53_EVA_2014.evp

SPE:	
SPE Column: LCTech_H53.col	
Extension cannula: no	
Processing speed selection: Standard (organic solvents)	
Rinsing intensity: Standard rinsing cycle	wo. Extra Cleaning after Load
Use pressure limitation function during loading and washing: no	

Step: Load		Basic type: Load - Quantitative transfer in parallel mode		Step: - ID: 622	
Volume: 53 ml	Suction Speed: 15 ml/min	Dispensing Speed: 10 ml/min			
Vial Type: Type_H53@60	Waiting Time after Dosage: 0 sec.	Waiting Time after Step: 150 sec.	Dispense: into vials	Number of vials: 1	
rinsing cycle included	Rinsing volume: 3 ml	Rinse repetitions: 1 x	Vial Type: Type_H53@60		
	Suction Speed: 20 ml/min	Dispensing Speed: 40 ml/min	Tube rinse volume: 0.1 ml		
	Port: 1 Petroleum ether				

Step: Drying		Basic type: Drying - Nitrogen drying by defined time		Step: - ID: 624	
Drying time with nitrogen 5 sec.			Dispense: stay on actual position		

Step: Washing		Basic type: Washing		Step: - ID: 625	
Volume: 2 ml	Suction Speed: 20 ml/min	Dispensing Speed: 15 ml/min	Port: 1 Petroleum ether		
	Repetitions: 0	Waiting Time after Step: 0 sec.	Dispense: stay on actual position		
	Waiting Time after Dosage: 0 sec.				

Step: Drying		Basic type: Drying - Nitrogen drying by defined time		Step: - ID: 626	
Drying time with nitrogen 10 sec.			Dispense: stay on actual position		

EVA:	Temperature water heating 40 °C	Temperature bottom cone 38 °C
Sample input: suck from vial / vials into chamber over sample probe and tubing, option with rinsing cycle		
Number of vials: 1 x Type_H53@60	Vacuum at suction: 300 mbar	Maximum time vacuum suction: 60 min.
rinsing cycle included		
Rinsing volume: 5 ml	Rinsing steps: 2 x	Solvent from Port: 1 Petroleum ether
Phase 1: Concentrate to level: 1 ml		
Vacuum absolute: 300 mbar		
Rinsing volume after phase 1: 4 ml	Rinsing steps: 1 x	Solvent from Port: 1 Petroleum ether
Phase 2: Concentrate to level: 1 ml		
Vacuum absolute: 300 mbar		
Rinsing volume after phase 2: 0 ml	Rinsing steps: 0 x	Solvent from Port: 1 Petroleum ether
Time control for vacuum process: no		
to dryness: no		
Nitrogen blow-down: no		
Remove Aliquot: no		
Solvent exchange: no		
Rinsing, filling up, mixing and transfer into vials:		
Rinsing volume at the end: no		
Fill up to volume: of Port: 1 Petroleum ether	1 ml	Way of mixing: with gas / air, Volume = 0 ml
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: 7 Acetone
2. Cleaning cycle		
Rinsing volume: 5 ml	Rinsing steps: 1 x	Solvent from Port: 1 Petroleum ether

Time required for this example: 55 minutes

Contact

LCTech GmbH
Daimlerstraße 4
84419 Obertaufkirchen
Germany

Tel.: +49 8082 2717-0
Fax: +49 8082 2717-100
E-Mail: info@LCTech.de

www.LCTech.de
www.LCTech-online.com

SOLUTIONS BY

