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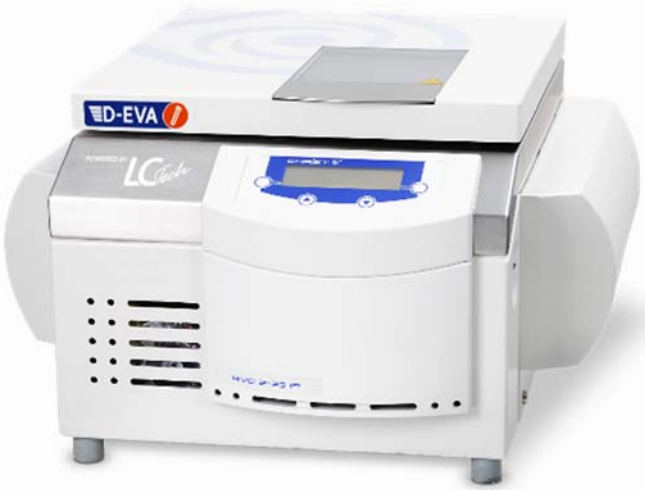

D-EVA – Automated EVAporation of PCDD/F Extracts to 100 - 200 µL PCB Extracts to 300 – 500 µL

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Content

1. Introduction

There are a lot of regulations for the analysis of polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) as well as polychlorinated biphenyls (PCBs) and all laboratories are following official standard procedures or validated house methods to fullfill these requirements. The performance of high resolution mass spectrometer (HRMS) or triple quadrupole mass spectrometer (MS/MS) is getting better and the handling is becoming easier. Nevertheless, a more effectiv clean-up is needed in order to get better quantification limits (LOQ).

One essential step during the clean-up to get very low LOQ is the evaporation of extracts to a very small volume, and the smaller the final volume the greater the risk of loosing high volatile analytes. LCTech therefore optimized a vacuum centrifuge, manufactured by Martin Christ (RVC 2-33 CDplus), to evaporate solvents to a very low final volume with no losses of analytes, the D-EVA.

A good evaporation has to fullfill following critical parameters:

- No further evaporation when the sample runs dry
- No generation of aerosols
- Only one transfer step
- No rinsing steps
- No cross-contamination

Most evaporation techniques warm up the cleaned extract and remove solvents using nitrogen or low pressure. In order to avoid losses of the volatile compound a permanent supervision is absolutely necessary. Not with the D-EVA. It stops the evaporation right in time, so that the lab technician is not bound to supervise the evaporation any more.

As a vacuum centrifuge, the D-EVA generates no aerosol thus keeping all analytes at the bottom of the centrifuge tubes, and consequently no rinsing is necesssary. The reason for cross-contamination is inhibited by the centrifugal forces as well!

The D-EVA is optimized as perfect completion for the DEXTech 16, DEXTech Plus, DEXTech Pure or DEXTech Heat, so all the glassware used in the automated clean-up can be used directly with the D-EVA. Only the final transfer into the GC-vial is necessary.

Without the need for supervision, a clean-up of the sample performed by a DEXTech system only takes 47 to 70 minutes, depending on the chosen method. The DEXTech 16 system even processes up to 15 samples in one sequence around the clock, day and night. The result of all DEXTech systems are two fractions, the PCB-Fraction (F1) and the PCDD/F-Fraction (F2) which have to be evaporated to a lower volume for subsequent analysis. Using D-EVA, a simultaneous evaporation of up to 26 samples for the PCDD/F-Fraction (F2) with a final volume of 30 to 100 µL or up to 23 samples for the PCB-Fraction (F1) with a final volume of 300 to 500 µL is possible. The centrifuge chamber is heated via infrared light using halogen lamps free from UV wavelengths and even a pressure of down to 10 mbar is possible.

2. Method Development

To quantify the efficiency of an evaporation method, possible losses have to be quantified. Therefore an isotopically labelled standard solution has to be spiked to the sample right at the beginning of the the evaporation. After evaporation a recovery standard solution has to be added to the sample and thus can be used as 100 % indication for calculating any losses.

2.1 Reagents and Materials

- Standard Solutions
  - EPA1613-LCS, ISS and CSS, Wellington Laboratories
  - EPA1613-PAR/Stock, Wellington Laboratories
  - PCB-LCS-H, ISS-H and CSS-H, Wellington Laboratories
  - PCB-Stock-A20, Wellington Laboratories
  - EDF-5526, Recovery Standard, CIL
  - EDF-5525-100x Internal Standard, CIL

- Solvents
  - n-Hexane picograde
  - Toluene picograde
  - Dichloromethane picograde

- D-EVA, Martin Christ enhanced by LCTech GmbH
  - Centrifuge vials
  - Temperature sensor

- DEXTech Pure & Heat, LCTech GmbH
  - Acidic silica gel column
  - Aluminium-oxide column
  - Carbon column
  - eVOL RT, SGE

- DFS HRMS, Thermo Fisher Scientific
  - HT8-PCB, 60m, 0.25 mm film, 25 mm ID, Trajan
  - RTX-Dioxin2, 60m, 0.25 mm film, 25 mm ID, Restek

2.2 Sample Preparation

2.2.1 Solvent Blank:

2.2.1.1 PCDD/F-Fraction (F2)

10 mL of toluene are spiked with EPA1613-LCS. After evaporation with D-EVA vacuum centrifuge the solution is spiked again with EPA1613-CSS and evaporated nearly to dryness with nitrogen. No additional rinsing is necessary! Subsequently, it is transferred with EPA1613-ISS into the GC-vial.

2.2.1.2 PCDD/F-Fraction (F2) with 2.5 to 10 pg absolut

10 mL of toluene in 15 mL centrifuge tubes are spiked with EDF5526 100x internal standard. Afterevaporation down to different volumes with D-EVA it is evaporated to nearly dryness with nitrogen directly in the centrifuge tubes where it is resolved in EDF5525 recovery standard and transferred to the GC-vial.

2.2.1.3 PCB-Fraction (F1)

24 mL of n-hexane/dichloromethane 1/1 are spiked with PCB-LCS-H. After evaporation with D-EVA vacuum centrifuge down to different final volumes the solution is spiked again with PCB-CSS-H and evaporated nearly to dryness with nitrogen. No additional rinsing is necessary! Subsequently, it is transferred with PCB-ISS-H into the GC-vial.

2.2.2 Matrix Fish Oil

3 g of fish oil are solved in 1 mL of toluene and 9 mL of n-hexane. After the solution is spiked with EPA1613-LCS and PCB-LCS-H, it is cleaned using a DEXTech system which separates PCBs from PCDD/Fs.

<u>PCDD/F in F2</u>

F2 (= 10 mL toluene) is spiked with 1613EPA-PAR/Stock. After evaporation with D-EVA vacuum centrifuge the solution is spiked with EPA1613-CSS and evaporated nearly to dryness with nitrogen. Subsequently, it is transferred with EPA1613-ISS into the GC-vial.

<u>PCB in F1</u>

F1 (= 24 mL n-hexane/dichloromethane 1/1) is spiked with PCB-stock-A20 and evaporated nearly to dryness with nitrogen. Subsequently, it is transferred with PCB-ISS-H into the GC-vial.

2.3 Instrumentation

2.3.1 Clean-up:
With a standard column set-up (acidic silica gel column, aluminium-oxide column and carbon column) the matrix is cleaned using the "Alox Pure" Method on a DEXTech Pure system.

2.3.2 Analysis:
All samples, solvent blanks as well as the fish oil are analysed with a DFS HRMS from Thermo Fisher Scientific. Fraction 1 is injected in SSL mode onto a 60 m HT8 PCB capillary column from Trajan and fraction 2 is injected in PTV mode onto a 60 m RTXDioxin2 capillary column from Resteck.

2.3.3 Evaporation:
The volume of the cleaned fractions is very important. If necessary, the samples have to be filled up to a common volume. For fraction 1 the optimal volume is 24 mL, for fraction 2, 10 mL. Because of the thermal capacity of the temperature sensor in the reference vial, the volume in that vial has to be 20 % higher than in the sample vials.

Because of the varying vapour pressure of the solvents, the cryo trap has to be emptied before changing evaporation methods. Otherwise, the evaporation is more slowly and insufficient recoveries could be achieved.

The centrifuge is running with 800 rpm only. Therefore an exact balancing with a scale is not necessary. However, to preserve the motor from scuffing, a balanced positioning of the vials in the rotor is strongly recommended.

2.3.4 Sample Transfer into GC-Vials:
In order to avoid losses of the volatile analytes, a final evaporation nearly to dryness has to be done directly in the centrifuge vials without rinsing of the glass ware. The analytes are dissolved in the injection standard, mixed by vortexing and subsequently transferred into the GC-vials via microliter pipettes.

2.3.6 D-EVA Programs:

Fraction 1, PCB:
Method: PCB-24 mL

	Start	1	2	3	4
t [min]	40	00:08	00:06	00:01	00:30
T [°C]		40	40	40	40
p [mbar]		500	150	70	70
ps [mbar]		575	250	120	100

Final Volume: 300 – 500 µL
Run Time: 30 – 35 min

Fraction 2, PCDD/F:
Method: DF-10 mL

	Start	1	2	3	4
t [min]	40	00:01	00:05	00:01	00:40
T [°C]		40	40	40	40
p [mbar]		30	20	10	10
ps [mbar]		50	35	30	30

Final Volume: 100 – 200 µL
Run Time: 40 – 45 min

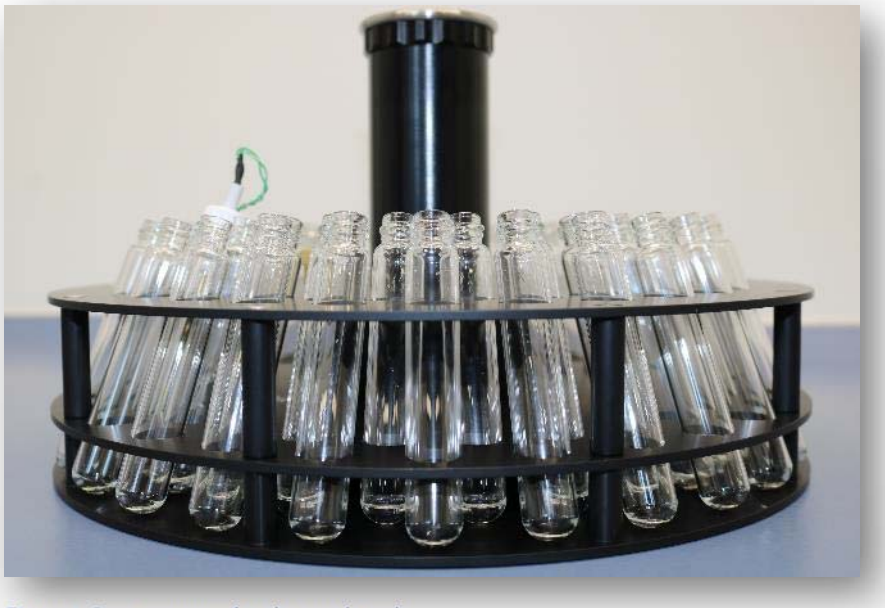


Figure 1: Rotor equipped with sample vials.

2.4 Final Volume

The final volume of evaporation with D-EVA can individually be optimized down to 30 µL for 15 mL centrifuge tubes or 100 µL for 35 mL centrifuge tubes. Just keep in mind: The lower the final volume the greater is the risk of some samples running dry.

For the safety of the samples, the standard program installed on the D-EVA will stop the evaporation at a point with a final volume of 100 – 200 µL for PCDD/F and 300 – 400 µL for PCB. Blowing down this final volume in the centrifuge tubes within a few minutes to nearly dryness is possible without any rinsing steps. Just transfer the sample with the recovery standard solution into the GC-vial and the ^{13}C-recoveries of 90-100 % are an easy challenge for the D-EVA for the PCDD/F fraction (*see Table 1*) and the PCB fraction (*see Table 2*).

3. Results

Table 1: ^{13}C-recoveries PCDD/F

- *Sample 1 - 3: spiked solvent blanks from method development "dioxin-only"-clean-up*
- *Sample 4 - 7: spiked solvent blanks, evaporation with D-EVA by LCTech, transfer in GC-vial, blowing down with nitrogen and quantification by Helmholtz Centre Munich*
- *Sample 8: complete sample preparation, including extraction, clean-up and evaporation*
- *Sample 9 – 12: spiked solvent blanks (2.5 – 10 pg abs), evaporation with D-EVA by LCTech, transfer in GC-vial, blowing down with nitrogen and quantification by LCTech*

^{13}C-Recoveries PCDD/F														
Congeners	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9	10	11	12	**Ø**	**SD**
^{13}C-2,3,7,8-TCDF	91	81	75	110	106	97	104	91	108	90	102	83	95	11
^{13}C-1,2,3,7,8-PeCDF	111	96	92	109	112	96	113	100	89	105	99	97	102	8
^{13}C-2,3,4,7,8-PeCDF	117	106	103	114	113	100	112	104	114	92	100	86	105	10
^{13}C-1,2,3,4,7,8-HxCDF	104	90	94	101	99	94	102	99	96	92	97	83	96	6
^{13}C-1,2,3,6,7,8-HxCDF	106	90	91	105	101	92	103	96	108	98	86	82	97	8
^{13}C-2,3,4,6,7,8-HxCDF	106	89	93	117	118	103	117	94	109	102	98	106	104	10
^{13}C-1,2,3,7,8,9-HxCDF	107	109	120	104	99	95	98	94	106	94	97	80	100	10
^{13}C-1,2,3,4,6,7,8-HpCDF	101	90	101	103	102	93	102	96	84	85	91	101	96	7
^{13}C-1,2,3,4,7,8,9-HpCDF	115	95	103	108	109	98	112	102	80	80	93	104	100	11
^{13}C-2,3,7,8-TCDD	93	85	84	109	105	90	96	90	92	82	84	89	92	8
^{13}C-1,2,3,7,8-PeCDD	113	101	103	115	115	103	120	95	106	104	110	95	107	8
^{13}C-1,2,3,4,7,8-HxCDD	112	87	92	106	104	94	100	94	100	105	100	94	99	7
^{13}C-1,2,3,6,7,8-HxCDD	111	86	89	101	97	93	102	91	116	100	101	104	99	9
^{13}C-1,2,3,4,6,7,8-HpCDD	111	92	101	100	102	92	100	101	109	109	103	99	102	6
^{13}C-OCDD	131	113	118	103	98	93	100	109	125	109	107	101	109	11

Table 2: ^{13}C-recoveries PCB

- *Sample 1 - 4: spiked n-hexane/dichloromethane 1:1 without clean-up*
- *Sample 5: spiked solvent blank with clean-up*
- *Sample 6: spiked fish oil with clean-up*
- *Sample 7 – 9: spiked n-hexane/dichloromethane 1:1 without clean-up*

^{13}C-Recoveries PCB											
Congeners	1	2	3	4	5	6	7	8	9	Ø	SD
#28L	80	71	81	99	79	74	91	95	96	85	10
#52L	92	77	91	97	91	97	82	96	106	92	9
#77L	100	96	102	109	104	89	95	100	94	99	6
#81L	91	82	95	98	96	88	97	99	95	93	6
#101L	87	82	83	92	78	83	92	89	92	86	5
#123L	92	90	91	104	92	92	108	103	90	96	7
#118L	99	94	94	106	106	105	111	106	92	101	7
#114L	91	91	91	103	102	87	119	114	100	100	11
#105L	87	86	91	99	91	77	103	111	92	93	10
#126L	104	112	104	125	105	93	108	106	86	105	11
#153L	115	108	113	115	110	100	115	119	112	112	5
#138L	117	119	124	128	115	101	104	110	102	113	10
#167L	97	91	100	108	88	78	100	106	92	96	9
#156L	112	108	114	122	102	95	102	104	95	106	9
#157L	108	109	115	119	101	98	103	110	92	106	8
#169L	101	104	108	113	96	88	95	106	86	100	9
#180L	102	110	107	116	95	92	105	109	112	105	8
#189L	116	109	118	119	94	85	104	102	99	105	12

4. Conclusion

D-EVA (Dioxin-Evaporation) is a brilliant solution for fast, parallel, and reproducible evaporation of your PCB and dioxin samples without any cross-contamination. The system concentrates your samples with vacuum and energy supply via light to a low volume and reliablly prevents evaporation to dryness due to a special LCTech sensor.

The sensor allows the system to stop automatically at the defined volume for PCDD/F extracts between 100 and 200 µL and for PCB at about 500 µL, as described in this application note. The subsequent transfer into the insert of a GC-vial is easy to handle.

5. General Accessories & Spare Parts

5.1 D-EVA Vacuum Concentrator

1. Rotational-Vacuum-Concentrator D-EVAporation P/N 16900

5.1.1 Rotor with 48 Positions

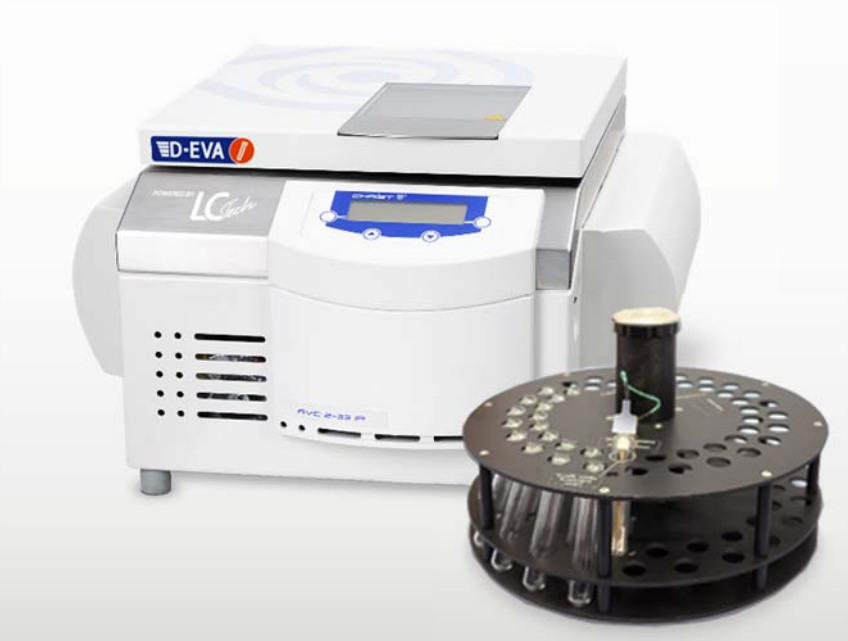
1. Angle rotor, 48 positions P/N 16742
2. Sensor for below mentioned vial P/N 16741
3. Centrifuge tube, GL14 P/N 15781
4. Screw cap GL 14 P/N V0014-SL
5. Seal for centrifuge tube GL 14 P/N V0014-D

5.1.2 Rotor with 24 Positions

1. Angle Rotor, 24 positions P/N 16802
2. Sensor for below mentioned vial P/N 16738
3. Centrifuge tube, GPI 24-400 P/N 16452
4. Screw cap GPI 24-400 P/N V0024-SL
5. Seal for centrifuge tube GPI 24 P/N V0025-D

5.1.3 Rotor with 12 Positions

1. Angle Rotor, 12 positions P/N 16929
2. Sensor for below mentioned vial P/N 16755
3. Centrifuge tube, GL 32 P/N 16725
4. Screw Cap, GL 32 P/N 16754



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