SOLUTIONS BY LCTech

D-EVA

automated EVAporation in dioxin analysis

PCDD/F: 10 mL toluene to 30 – 150 µL
PCB: 24mL n-hexane to 300 - 500 µL

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Content

1. Introduction

For the analysis of polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) as well as polychlorinated biphenyls (PCB), laboratories apply a wide variety of standard procedures or in-house methods to meet the requirements of the numerous regulations. The measurement technology, be it GC-HRMS or GC-MS/MS is getting better and its handling is getting easier, but it still needs powerful extraction and clean-up methods to achieve clean chromatograms with good signal-to-noise ratios around the extremely low limits of quantification. Thanks to automated systems such as the XTRACTION® for extraction and the DEXTech family (DEXTech 16, DEXTech Plus, DEXTech Pure or DEXTech Heat) for clean-up, these tasks, which are very time-consuming and demanding in the manual process, can be performed fully automatically, quickly and efficiently. All extractions or clean-ups, whether manual or automated, have one critical step in the clean-up process in common:

The evaporation of sample extracts to a very small volume

An extraction with the X-TRACTION® requires 1-3 cycles depending on the matrix, each only 15 min with 30-90 mL final volume that has to be reduced to approx. 1 mL for further sample clean-up. Sample clean-up by a DEXTech system takes only 47 to 70 minutes, depending on the program; the DEXTech 16 system even operates up to 15 samples in a sequence day and night. The result of all DEXTech clean-up systems is two fractions per sample, the PCB fraction (F1) with 24 mL n-hexane/dichloromethane and the PCDD/F fraction (F2) with 10 mL toluene, which must be evaporated to the lowest possible volume for subsequent analysis.

Small final volumes are necessary to meet the low limit of quantification (LOQ), but the lower the final volume should be, the higher the risk of losing the highly volatile analytes with conventional evaporation methods. In most evaporation techniques, the sample is heated in water baths or thermal blocks and the solvent is removed using negative pressure or blowing off with nitrogen. Conventional processes require permanent monitoring of the process to ensure that the process is stopped when the target volume is reached, in order to prevent losses of the volatile compounds due to further heat input.

LCTech has therefore optimized the vacuum centrifuge RVC 2-33 CDplus (Martin Christ) to automate this monitoring and allow evaporation without loss of analytes and to a very low final volume!

The D-EVA

Criterias for a good performed evaporation:

- **Stop evaporation in time when final volume is reached**
  If a sample is evaporated beyond the target volume, there is a risk of losing higher volatile analytes. The D-EVA stops the active evaporation in time, even a subsequent drying of the samples due to time is loss-free, so that a monitoring of the evaporation by laboratory personnel is no longer necessary.

- **No cross contamination**
  Aerosol formation leads to analyte losses and, in the worst case, even cross-contamination. The D-EVA programs are optimized for the perfect balance between pressure and temperature control to avoid aerosols despite the fastest possible evaporation.

- **No rinsing steps during sample transfer**
  Aerosol formation, capillary forces and turbulence of the solvent make additional rinsing steps necessary during quantitative sample transfer. As a vacuum centrifuge, the D-EVA keeps all analytes in solution and prevents aerosol formation and capillary action on the glass walls of the centrifuge tubes. No rinsing steps are required for quantitative sample transfer!

- **No loss of analytes due to UV light**
  Heat is required for evaporation. Infrared light using halogen lamps is used to heat the centrifuge chamber. This means that even photosensitive analytes can be evaporated with the D-EVA without loss.

- **Only one transfer step**
  The D-EVA is optimized as a perfect complement for the DEXTech 16, DEXTech Plus, DEXTech Pure, or DEXTech Heat, so that all glass vials used in automated clean-up can be used directly with the D-EVA. Only the final transfer into the GC vial is necessary. The centrifuge tubes for 90 mL initial volume can also be used with the X-TRACTION.

- **Only one transfer step**
  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.

- **Maximum Speed**
  - 39 min. are required for up to 26 samples for the PCDD/F fraction (F2).
  - 31 min. are required for up to 23 samples for the PCB fraction (F1).

- **Smallest final volumes**
  - Only 30 - 150µL for the PCDD/F fraction (F2)

- Only 300 - 500 µL for the PCB fraction (F1)

2. Principle of D-EVA

By lowering the pressure, the boiling point of the solvents to be evaporated is reduced. The energy required for evaporation is drawn from the immediate environment and causes a drop in the temperature of the solvent (enthalpy of evaporation). The infrared lamps of the D-EVA compensate for this energy requirement and thus accelerate the process. Once the solvent has evaporated, the temperature in the sample vessels rises rapidly. The D-EVA regulates the evaporation on the basis of this temperature curve. For this purpose, LCTech GmbH has designed a sensor that precisely monitors the course of evaporation in a reference glass. As soon as the sensor detects the rapid rise in temperature, the active heat supply is stopped immediately and the vacuum chamber of the centrifuge is ventilated to raise the boiling point. This terminates the active evaporation. The subsequent passive evaporation of the solvent under normal conditions does not cause any loss of analytes.

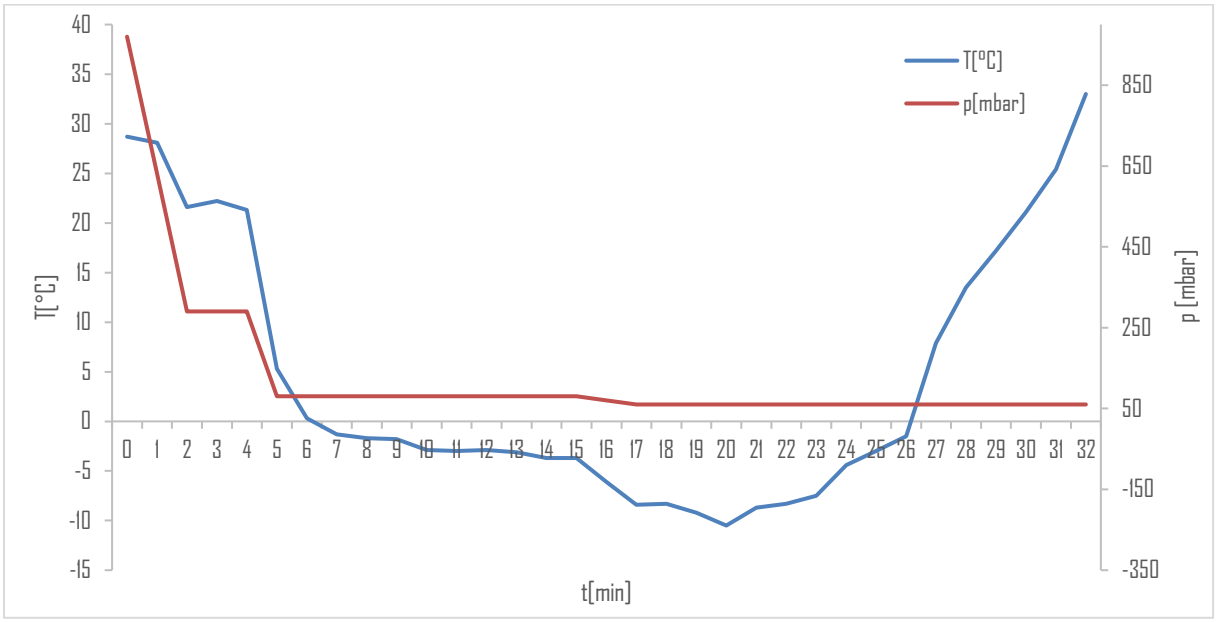

Figure 1: T/p diagram of the n-hexane method with 24 mL initial volume.

Figure 1 shows the T/p diagram of the evaporation of an n-hexane-dichloromethane mixture with the D-EVA. A gentle pressure ramp lowers the pressure to 290 mBar at where dichloromethane begins to boil and at this point the temperature of the solvent drops rapidly due to the enthalpy of evaporation. The infrared lamp of the D-EVA is switched on to heat the samples and accelerate the process, while the centrifugal forces prevent the formation of aerosols. Then the pressure is lowered to 80 mBar, at this pressure n-hexane starts to boil, and again the evaporation is accelerated by the infrared lamps.

3. Development of the method

To capture the efficiency of an evaporation method, potential losses has to be quantified. For this purpose, a standard solution containing isotopically labeled analytes is added to all samples for quantification prior to evaporation. After evaporation, another isotopically labeled standard solution will be added to the samples as a recovery standard, which will be used as a 100% indicator for calculating potential losses.

3.1 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-5526-100x, Recovery Standard, CIL
  - EDF-5525-100x Internal Standard, CIL

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

- D-EVA, Martin Christ extended 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

3.2 Sample Preparation

So-called solvent blank values are used as test samples to assess the cleanliness and efficiency of the process, and samples are prepared to record possible influences by matrix fish oil. To determine cross-contamination, a solvent mixture is mixed with a highly concentrated standard.

3.2.1 Solvent – blank value (blow off nitrogen effect)

- **PCDD/F-Fraction (F2)**
  10 mL toluene is spiked with EPA1613-LCS (labeled compound solution). After evaporation with the D-EVA vacuum centrifuge, the solution is again spiked with EPA1613-CSS (cleanup standard solution) and evaporated with nitrogen almost to dryness. No additional rinsing is required! It is then redissolved with EPA1613-ISS (internal standard solution) and transferred to the GC vial.

3.2.2 Solvent - blank value (efficiency)

- **PCDD/F fraction (F2) with 2.5 to 10 pg absolute.**
  10 mL toluene in 15 mL centrifuge tube are spiked with the internal standard EDF5526-100x. After evaporation to different volumes with the D-EVA, the samples are transferred to GC vials and spiked and quantified by the Helmholtz Center with nitrogen blown down with ISS.

- **PCB-fraction (F1)**
  24 mL of n-hexane/dichloromethane 50/50 v/v is doped with PCB-LCS-H. After evaporation with the D-EVA vacuum centrifuge to different final volumes, PCB-CSS-H is added again to the solution and evaporated with nitrogen almost to complete dryness. No additional rinsing is required! It is then transferred to the GC vessel with PCB-ISS-H.

3.2.3 Matrix Fish Oil

- Dissolve 3 g fish oil in 1 mL toluene and make up to 10 mL with n-hexane. After the solution is doped with EPA1613-LCS and PCB-LCS-H, it is cleaned up with a DEXTech system, separating PCBs from PCDD/Fs.

- **PCDD/F fraction (F2)**
  F2 (= 10 mL toluene) is doped with 1613EPA-PAR/stock. After evaporation with the D-EVA vacuum centrifuge, the solution is spiked with EPA1613-CSS and evaporated with nitrogen almost to dryness. It is then transferred to the GC vessel with EPA1613-ISS.

- PCB fraction (F1)
  F1 (= 24 mL n-hexane/dichloromethane 50/50 v/v) is doped with PCB stock-A20 and evaporated with nitrogen almost to dryness. It is then transferred to the GC vessel with PCB-ISS-H.

3.3 Implementation

3.3.1 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.

3.3.2 Analysis

- All samples, solvent blanks as well as the fish oil will be analyzed using a Thermo Fisher Scientific DFS HRMS. 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.

3.3.3 Evaporation

- The volume of the cleaned-up fractions is important. The lower the desired final volume is to be, the more precisely all samples must be brought to the same volume. The accuracy of the DEXTech systems is sufficient.
  Filling Reverence centrifuge tube must be done with the same solvent mixture corresponding to the sample extract.

- Due to the different vapor pressure of the solvents, the cryotrap must be emptied before changing the evaporation method. Otherwise, evaporation will be slower and insufficient recoveries may 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.

3.3.4 Sample Transfer into GC-Vials

- The PCDD/F samples are mixed with the respective ISS solution after evaporation and can be transferred directly into the 300µL inserts of the GC vials without further rinsing steps and, if necessary, reduced to 20µL with nitrogen in 2-3 min. The PCB fraction must be mixed with ISS in the centrifuge tube and brought to the final volume with nitrogen in 2-3 min and then transferred to GC vials without rinsing steps.

3.3.5 D-EVA Programs

n-hexane	Start	1	2	3	4	5	6
t [min]	40	00:02	00:02	00:01	00:10	00:01	01:00
T [°C]		45	45	45	45	45	45
p [mbar]		290	290	80	80	60	60
ps [mbar]		300	300	85	85	65	65
Rotor [rpm]		800	800	800	800	800	800

Program stopping temperature: 30°C

Fraction 1, PCB (24 mL):

Final volume: 300 – 500 µL (optional 100-200µL, see chapter 3.4)
Run time: ~31 min

Extraction, n-Hexane (90 mL):

Final volume: 1 to 1.5 mL
Run time: ~48 min

toluene	Start	1	2	3
t [min]	40	00:03	00:10	01:30
T [°C]		40	40	40
p [mbar]		30	10	10
ps [mbar]		200	80	50
Rotor [rpm]		800	800	800

Program stopping temperature: 30°C

Fraction 2, PCDD/F (10 mL):

Final volume: 30 – 150 µL
Run time: ~39 min

Extraction, n-Hexane (90 mL):

Finales volume: 1 bis 1,5 mL
Run time: ~58 min

3.4 Final Volume (optional note)

- Chapter 3.3.4 recommends a final blowdown with nitrogen for evaporation to near dryness. An optional alternative is possible if the recovery standard solution can be added to the sample immediately before evaporation. The final volume can be optimized for individual needs by varying the initial volume of solvent in the sensor vial.

- Because the initial volume varies slightly, the risk of running samples dry increases as the final volume is reduced. The lower the desired final volume, the greater the likelihood that more volatile analytes can be lost. Example PCB:
  PCB#28 loses 20-30% recovery and PCB#52 loses 10-20% recovery with a residual volume of 100-200µL. This is not relevant for the native quantification.

- If the recovery standard solution can be added immediately before evaporation, these losses due to evaporation would no longer be detected by 13C recovery, and the signals from these analytes are still detectable in chromatography with a very good signal-to-noise ratio.

- If losses for the PCB#28 of 20% are acceptable, or the recovery standard can be added before evaporation, final volumes of 100-200 µL are also achievable for the PCB fraction without problems

Figure 1: Rotor equipped with sample vials.

4. Results

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

- **Sample 1 - 3**: spiked solvent blanks from the method development "dioxin-only" clean-up.
- **Sample 4 - 7:** spiked solvent blanks, evaporation with D-EVA from LCTech, transfer to GC vessel, blowdown with nitrogen and quantification by Helmholtz Zentrum München.
- **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 from LCTech, transfer to GC vessel, blowdown with nitrogen and quantification with LCTech.

^{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

Table 2: ^{13}C-recoveries PCB

- **Sample 1 - 4**: doped n-hexane/dichloromethane 50:50 (v/v) without clean-up
- **Sample 5:** doped solvent blank with clean-up
- **Sample 6:** spiked fish oil with clean-up
- **Sample 7 - 9:** doped n-hexane/dichloromethane 50:50 (v/v) without clean-up

5. 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 automatically stops the system at the defined volume of 30 and 150 µL for the PCDD/F fraction and 300 - 500 µL (optional 100-200µL, see chapter 3.4) for the PCB fraction, as described in this application note. The subsequent transfer into the insert of a GC-vial is easy to handle.

6. General Accessories & Spare Parts

6.1 D-EVA Vacuum Concentrator

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

6.1.1 Rotor with 54 Positions

- Angle rotor, 54 positions P/N 16742
- Sensor for below mentioned vial P/N 16741
- Centrifuge tube, GL14 P/N 15781
- Screw cap GL 14 P/N V0014-SL
- Seal for centrifuge tube GL 14 P/N V0014-D

6.1.2 Rotor with 24 Positions

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

6.1.3 Rotor with 12 Positions

- Angle Rotor, 12 positions P/N 16929
- Sensor for below mentioned vial P/N 16755
- -Centrifuge tube, GL 32 P/N 16725
- Screw Cap, GL 32 P/N 16754

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