Application Note

067/2011

SpeedExtractor E-916

Extraction of Soil using the SpeedExtractor E-916 for the Determination of Pesticides

SHORT NOTE

Extraction of Soil using the SpeedExtractor E-916 for the Determination of Pesticides

This application note describes a fast and reliable way to extract organochlorine pesticides (OCP) from soil with the SpeedExtractor E-916. After reducing the volume with the Syncore Analyst the extracts were cleaned by using Florisil. The cleaned extracts were concentrated again and quantified by GC-ECD.

Introduction

A pesticide is any substance or mixture of substances intended for, preventing, destroying, repelling, or mitigating any pest. There are three main types of pesticides: organochlorine, organophosphate and carbamates.

OCP can cause severe health problems, are persistent and tend to bio-accumulate. Therefore the use of some OCP is banned by the Stockholm convention

Experimental

Instrumentation: SpeedExtractor E-916 with 20 mL cells, Syncore Analyst, Thermo Trace GC Ultra

Samples: CRM 847-050 and CRM 804-050 by R.T. Corporation.

Depending on the expected values of the OCP in the sam¬ples 1 to 5 g of sample were weighed and mixed with sand. The mixture was transferred to the cell and two surrogates were added prior to the extraction. The cells were extracted using the parameters in Table 1. 150 mL Syncore® vessels with appendix were used as collection vials.

After the extraction, 1 mL of Internal Standard (IS) was added to the extracts. After concentration on Syncore® Analyst a clean-up with Florisil was performed. The volume of the cleaned solution was reduced again and quantification of 16 different pesticides was performed by GC-ECD.

A fourfold extraction of the samples was done. Two blanks were extracted in parallel on two positions not used for samples. In addition post-extractions of the samples were to show the performance of the method.

Table 1: Method SpeedExtractor E-916

Temperature	100 °C
Pressure	100 bar
Solvent	Cyclohexane 50%, Ethyl acetate 50%
Cells	20 mL
Vials	150 mL Syncore with 1 mL appendix
Cycles	3
Heat-up	1 min
Hold	10 min
Discharge	2 min
Flush with solvent	3 min
Flush with gas	2 min

Results

The measurements be within the prediction interval (P.I.) at least 19 out of 20 times. Out of the 22 quantified values only one value was above the P.I. All pesticide values in the blanks were below the limit of quantification.

Figure 1 and 2 show the results of a few selected pesticides. Error bars of CRM represent the prediction intervall; error bars of SpeedExtractor represent the rsd (n = 4). For detailed results see Application Note 067/2011

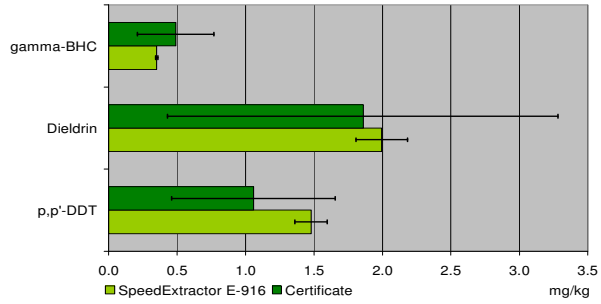

Figure 1: CRM 804-050

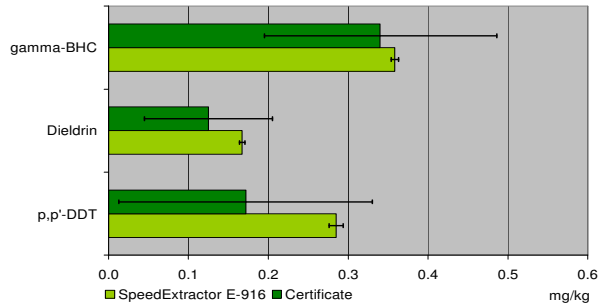

Figure 2: CRM 847-050

Conclusion

The method presented in this application note demonstrates a fast and reliable way to extract pesticides from soil using the SpeedExtractor E-916.

Acknowledgement

We sincerely thank Bachema AG, Schlieren, Switzerland for the analytical work.

References (Short Überschrift)

SpeedExtractor E-916 operation manual

For more details see Application Note 067/2011

1 Introduction

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests.

Highly hazardous pesticides may have acute and/or chronic toxic effects, and pose particular risks to children. Their widespread use has caused health problems and fatalities in many parts of the world, often as a result of occupational exposure and accidental or intentional poisoning. Environmental contamination can also result in human exposure through consumption of residues of pesticides in food and possibly drinking water [1].

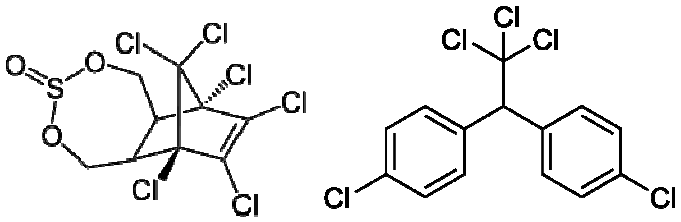

Figure 1: Endosulfan and p,p-DDT

There are three main types of pesticides: organochlorines, organophosphates and carbamates. Organochlorine pesticides (OCP) are persistent and tend to bio-accumulate. Several persistent organic pollutants (POP) on the list of Stockholm convention are OCPs. Therefore most organochlorine pesticides have been phased out or are subjected to strict regulations. Most OCP have been substituted by organo-phosphates and carbamates.

Organochlorine pesticides are lipophylic and extremely stable. There are four main types of organochlorine pesticides: cyclodiens, chlorinated benzenes, dichlorodi-phenylethanes and cyclohexanes.

This application note describes the extraction and quantification of several typical organochlorine pesticides from soil samples using the SpeedExtractor E-916.

The concentration, clean-up and quantification of the extracts were done by Bachema AG, Schlieren, Switzerland. Bachema is an ISO 17025 accredited labora-tory.

2 Equipment

- SpeedExtractor E-916 with 20 mL cells, 4-port mixer
- Analytical balance
- Halogen moisture analyzer HR 73, Mettler Toledo
- Syncore® Analyst with Flush Back Module, BUCHI
- Syncore® Analyst 150 mL Vials with 1 mL Appendix and Screw Cap, BUCHI (11056498)
- Screw Cap with Septa for Syncore® Analyst vials, BUCHI (Screw Cap 11056528, Septa 53677)
- Thermo Trace GC Ultra with double column mode, Thermo Scientific
- Glass syringes, 100 µL
- Positive-displacement pipette, Pos D, 100-1000 µL, Rainin
- 4 mL glass vial
- Weighing dishes

3 Chemicals and Materials

- Cyclohexane, Pesti-S, Biosolve (63132602)
- Ethyl acetate, Pestanal, Fluka (31063)
- Acetone, for GC ultra-trace analysis, Scharlau (AC0309.025S)
- Certified Reference Material, CRM 847-050, R.T. Corporation
- Certified Reference Material, CRM 804-050, R.T. Corporation
- Tetrachlor-m-xylol (TMX) 10 ng/µL in Cyclohexane, Dr. Ehrenstorfer GmbH, L 17382500CY
- PCB-209 10 ng/µL in Cyclohexane, Dr. Ehrenstorfer GmbH, L 20020900CY
- 1-Bromododecane, purum, Fluka (18580), alternatively Aldrich (245038)
- Quartz Sand, BUCHI (37689)
- Florisil SPE column, Isolute FL SPE, 1 g / 6 mL, Biotage (712-0100-C)
- Standard solutions containing 10 ug/mL of each pesticide in cyclohexane, customized standard, LGC Standards GmbH

4 Samples

4.1 Certified Reference Material, CRM 847-050

The CRM is a soil fortified with pesticide compounds. Being a natural matrix waste sample the analysis is challenged by the same preparation problems, analytical interferences, etc. as it is typical for similar matrices. The CRM has a texture classification as clay loam soil.

The CRM 847-050 represents a medium level contaminated sample.

4.2 Certified Reference Material, CRM 804-050

The CRM is a natural soil containing incurred pesticide compounds, from an agricul¬tural region of the Western United States. Being a real-world waste sample the analysis is challenged by the same preparation problems, analytical interferences, etc. as is typical for similar matrices. The CRM has a texture classification as sandy loam soil.

The CRM 804-050 represents a high level contaminated sample.

5 Procedure

5.1 Procedure

The extraction and analysis of pesticides in soil includes the following steps:

- Preparation of the samples
- Preparation of the cells
- Addition of surrogate standards
- Extraction with SpeedExtractor E-916
- Addition of internal standard to the extracts
- Concentration with Syncore® Analyst, 150 mL vessels with 1 mL appendix
- Nitrogen blow down to 1 mL
- Clean-up on Florisil SPE column
- Concentration with Syncore® Analyst to 1-2 mL
- Nitrogen blow down to 1 mL
- Quantification by GC-ECD

For quality measures the two surrogate standards and an internal standard are used during the procedure. For details, see 5.4 and 5.5.

The blanks are prepared in the same way, using sand only.

In addition a daily surrogate solution prepared. For details, see 5.4.

5.2 Extraction scheme

Parallel blanks and post-extractions of the samples are performed to show the performance of the extraction.

Table 1: Extraction scheme

	Pos. 1	Pos. 2	Pos. 3	Pos. 4	Pos. 5	Pos. 6
1	CRM847-050 Medium level	CRM847-050 Medium level	CRM847-050 Medium level	CRM847-050 Medium level	Parallel-Blank	Parallel-Blank
2	Post-extraction	Post-extraction	Post-extraction	Post-extraction	Blank	Blank
3	CRM804-050 High level	CRM804-050 High level	CRM804-050 High level	CRM804-050 High level	Parallel-Blank	Parallel-Blank
4	Post-extraction	Post-extraction	Post-extraction	Post-extraction	Blank	Blank

5.3 Determination of dry matter

The dry matter of the sample was determined by using a halogen moisture analyzer. The dry weight was used to calculate the values of the pesticides in the sample on a dry weight base.

5.4 Surrogates and daily surrogate solution

Tetrachlor-m-xylol (TMX) and PCB-209 are used as surrogates and added to each sample before extraction.

A daily surrogate solution is prepared in parallel to the extraction. The daily surrogate solution will be handled like an extract after clean-up.

The values found for TMX and PCB in the daily surrogate solution and in the extracts are an indicator for the performance of the extraction and clean-up procedure. The values found for TMX and PCB in the daily surrogate solution to 100%. Con¬centrations of pesticides in the extracts are recalculated by using the surrogate concentrations in the extracts compared to the values found in the daily surrogate. This procedure compensates any loss of analyte during extraction and clean-up

If the value of the two surrogates in the extracts is less than 60% of the value found in the daily surrogate solution the whole analysis will be repeated.

Preparation of daily surrogate solution:

- Give 1 mL Cyclohexane into a 4 mL Vial
- Add 30 µL TMX 10 µg/L
- Add 50 µl PCB-209 10 µg/L
- Add 1 mL internal standard solution

5.5 Internal Standard (ISTD) Solution

The internal standard solution is added to every extracts, the daily surrogate solution and the standard solutions. It is used to compensate differences between extracts,

standards and blanks caused by differences in volumes after concentration, variations of injection volumes etc.

Preparation of ISTD:

- Give 50 µL of 1-Bromododecane into a 500 mL volumetric flask
- Fill up with Cyclohexane

5.6 Preparation of the samples

- Samples are stored at 4° C. Allow samples to reach room temperature before opening them.
- Due to potential settling and stratification in storage, shipping and handling the whole sample must be thoroughly mixed before application.
- Weigh in approx. 5 g of CRM847-050 or 1 g of CRM 804-050 in a weighing dish.
- Add about 9 g of sand and mix well with a spatula.

5.7 Preparation of the cells containing sample

- Prepare the cell using a bottom glass fibre filter
- Weigh in about 9 g of Sand
- Fill in the prepared sample into the 20 mL cell using a funnel
- Fill up any void volume with sand
- Add 30 µL of TMX with glass syringe
- Add 50 µL of PCB-209 with glass syringe
- Close the cell with a cellulose filter
- Wait for about 10 min before extraction

5.8 Preparation of the cells containing blanks

- Prepare the cells using a bottom glass fibre filter
- Fill up the cell with sand
- Add 30 µL of TMX with glass syringe
- Add 50 µl of PCB-209 with glass syringe
- Close the cell with a cellulose filter
- Wait for about 10 min before extraction

5.9 Preparation of the cells for post-extraction

The cells used for the extraction filled with sample and sand are extracted again with the same method. No additional surrogates are added.

5.10 Extraction with SpeedExtractor E-916

Table 2: Extraction method for SpeedExtractor E-916

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	Cyclohexane 50% Ethyl acetate 50%
Cells	20 mL
Vials	150 mL
Cycles	3
Heat-up	1 min
Hold	10 min
Discharge	2 min
Flush with solvent	3 min
Flush with gas	2 min
Total extraction time	61 min

5.11 Concentration after extraction

- Add 1 mL of ISTD to every collection vessel
- Transfer the vessels to a Syncore® Analyst
- Reduce the volume of the extracts to < 1 mL by using the following parameters: 50 °C, 150 mbar, 220 rpm, ca 25 min
- Transfer the extracts to a 4 mL vial
- Rinse vessel with 1 mL of cyclohexane and combine rinsing and extract solution
- Blow down the volume to 1 mL with nitrogen

5.12 Clean-up

- Condition the Biotage Isolute SPE column with 10 mL cyclohexane/acetone 99:1 applying a vacuum of max. 0.5 bar
- Load 0.5 mL of extract onto the column
- Wait for about 2-3 min
- Elute without vacuum with 50 mL cyclohexane/acetone 99:1

5.13 Concentration after clean-up

- Reduce the volume on Syncore® Analyst to 1 mL
- Transfer the extract to a 4 mL vial
- Rinse vessel with 1 ml of cyclohexane and combine rinsing and extract solution
- Blow down the volume to 1 mL with nitrogen

5.14 Quantification with GC-ECD

The samples are quantified by GC-ECD using calibration solutions containing the pesticides of interest and the ISTD.

5.14.1 Calibration solution

10 levels in the range of 2.5 ng/mL to 1000 ng/mL.

5.14.2 GC parameters

Table 3: GC parameters

GC-ECD	Thermo Trace GC Ultra				
Mode	Double column mode: 1 injector, 2 columns, 2 ECD				
Columns	RTX-CL Pesticides and RTX-CL Pesticides 2 (Restek)				
Carrier gas	Hydrogen, made by a gas generator type Schmidlin NMH2-1000				
Auto sampler	CTC GC-PAL				
Injector parameters	- 200 °C - Constant flow: 3.2 mL / min - Split 40 after 0.6 min				
Injection volume	2 µL				
Oven program		Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]
	1	120		1	1
	2	320	9		23
	3	320		3	26

6 Results

The found results were compared to the reference values and the prediction intervals (P.I.) of the certified reference materials (CRMs). The laboratories participating in the round robin for the certification of the CRMs used soxhlet extraction or sonication for the extraction. According to the certificate of the CRM the results should fall within the prediction interval range 19 of 20 times.

Due to the high analyte concentrations of the pesticides in the extracts of the samples, the CRM extracts had been diluted prior to quantification resulting in differences of the LOQs between CRMs and blanks.

For CRM 847-050 the values for alpha-Endosulfan and Heptachlor were below the limit of quantification. Thirteen values were in the prediction interval and the value for p,p-DDD was above the prediction interval. For CRM 804-050 the value for Aldrin was below the limit of quantification. The other 8 values were within the prediction interval. Of the 22 pesticide values quantified in the two CRMs, 17 values were in the upper half of the prediction interval and one value was above the prediction interval demonstrating the high efficiency of the extraction compared to the average extraction efficiency achieved by the laboratories participating in the round robin for the certification of the CRMs.

All relative standard deviations are below 10 %.

The results of CRM 804-050 were also compared to the values found by PLE and Soxhlet by Concha-Graña et al. [2]

Table 4: Results for CRM-847-050. Values for p,p'-DDD is above prediction interval.

Pesticide	Mean Value n = 4	rsd	Certified value Prediction Interval (95%)	Parallel Blank n = 2	Post extraction n = 4
	mg/kg	%	mg/kg	mg/kg	mg/kg
Aldrin	0.139	2.5	0.049-0.181	<0.001	0.001
p,p'-DDD	0.371	2.7	0.129-0.327	<0.0005	0.001
p,p'-DDE	0.280	3.3	0.125-0.311	<0.0005	0.001
p,p'-DDT	0.285	3.2	0.014-0.331	<0.0005	0.001
Dieldrin	0.167	2.1	0.045-0.205	<0.001	0.001
alpha-Endosulfan	<0.1		0.073-0.248	<0.01	<0.01
beta-Endosulfan	0.163	3.1	0.11-0.357	<0.01	<0.01
Endrin	0.492	3.8	0.106-0.647	<0.001	0.001
Endrinaldehyd	0.065	6.0	0.015-0.083	<0.005	<0.005
alpha-BHC	0.227	2.4	0.121-0.328	<0.0005	0.001
beta-BHC	0.107	2.6	0.056-0.129	<0.0005	<0.0005
gamma-BHC	0.358	1.3	0.194-0.485	<0.0005	0.001
delta-BHC	0.081	3.4	0.033-0.102	<0.0005	<0.0005
Heptachlor	<0.1		0.028-0.189	<0.01	<0.01
cis-Heptachlorepoxid	0.110	0.0	0.043-0.154	<0.01	<0.01
Methoxychlor	0.311	4.9	0-0.345	<0.01	<0.01

Table 5: Results for CRM 804-050. All results are within the prediction interval.

Pesticide	Mean value n = 4	rsd	Certified value Prediction Interval (95%)	Parallel Blank n = 2	Post extraction n = 4
	mg/kg	%	mg/kg	mg/kg	mg/kg
Aldrin	<0.05		0-0.0384	<0.01	<0.01
p,p'-DDD	1.951	6.2	0.499-2.560	<0.005	0.03
p,p'-DDE	1.939	6.5	0.633-2.410	<0.005	0.03
p,p'-DDT	1.474	8.0	0.465-1.660	<0.005	0.02
Dieldrin	1.994	9.6	0.437-3.290	<0.01	0.03
alpha-Endosulfan	0.452	4.3	0.386-0.596	<0.1	<0.1
beta-Endosulfan	0.648	7.9	0.247-2.010	<0.1	<0.1
Endrin	0.069	8.0	0.042-0.082	<0.01	<0.01
gamma-BHC	0.352	2.6	0.212-0.717	<0.005	0.06

Table 6: Values for CRM 804-050 by Concha et al. [2]

Pesticide	Concha et al. PLE	rsd	Concha et al. Soxhlet	rsd
	mg/kg	%	mg/kg	%
Aldrin	0.014	27.7	0.025	2.0
p,p'-DDD	1.384	6.9	1.655	3.8
p,p'-DDE	1.406	7.8	1.66	4.2
p,p'-DDT	1.101	2.4	1.317	18.0
Dieldrin	1.582	8.0	1.907	4.8
alpha-Endosulfan	0.456	8.6	0.554	6.1
beta-Endosulfan	0.771	9.5	0.872	5.7
Endrin	0.062	7.0	0.133	7.5
gamma-BHC	0.299	10.7	0.498	11.0

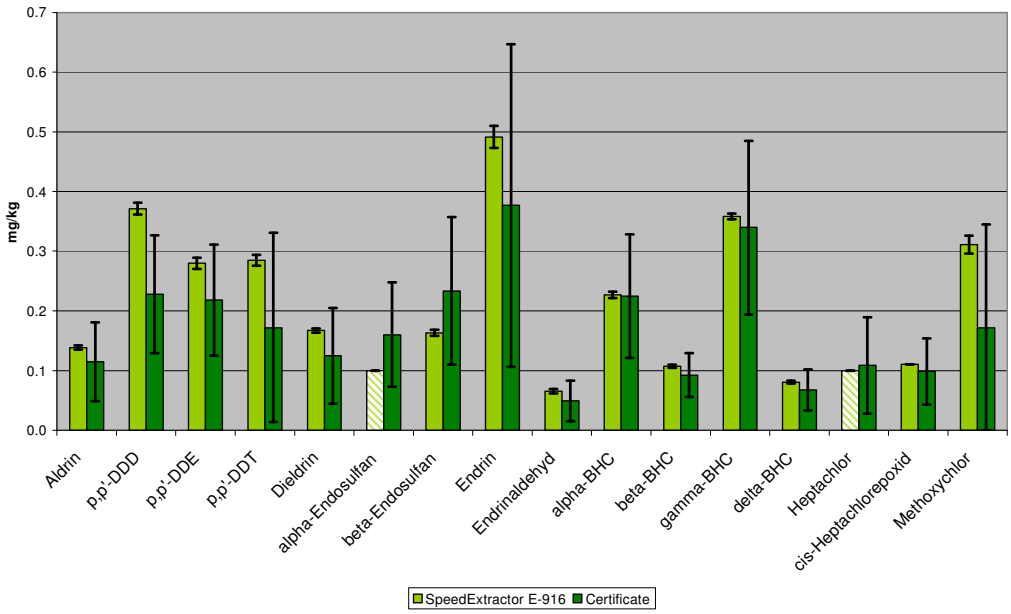

Figure 2: CRM 847-050. Results for alpha-Endosulfan and Heptachlor are below limit of quantification (LOQ). Showed value represents LOQ. The error bars for the CRM represent the prediction interval and the error bars for SpeedExtractor represent the relative standard deviation.

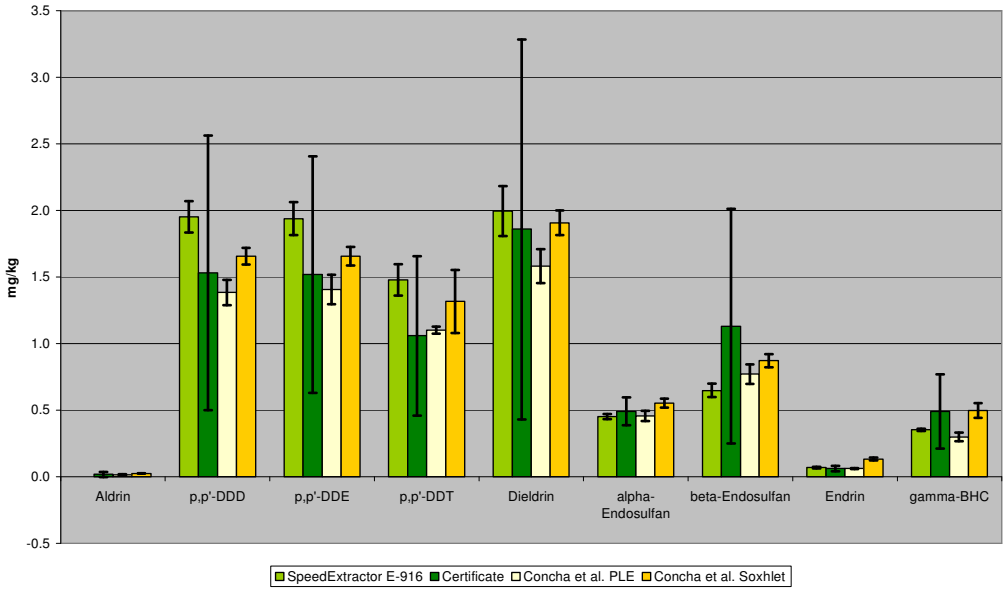

Figure 3: CRM 804-050. The error bars for the CRM represent the prediction interval, the error bars for SpeedExtractor and Concha et al.[2] represent the relative standard deviation.

7 Conclusion

The method presented in this application note demonstrates a fast and reliable way to extract pesticides from soil using the SpeedExtractor E-916. In reference to the 22 pesticide levels quantified only one was above the P.I. Most values found for the extraction with the SpeedExtractor E-916 are in the upper half of the prediction interval, demonstrating the high extraction efficiency.

8 Acknowledgements

We greatly acknowledge Mr. Urs Maier and his team of Bachema AG, Schlieren, Switzerland for their measurements and support in developing this application note.

9 References

[1] WHO, Preventing disease through healthy environments - Exposure to highly hazardous pesticides: a major public health concern (2010)

[2] Development of pressurized liquid extraction and cleanup procedures for determination of organochlorine pesticides in soil - E. Concha-Graña, M.I. Turnes-Carou et al., Journal of Chromatography A, 1047 (2004) 147-155

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