SpeedExtractor
Application Booklet

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Table of Contents

This document describes all aspects which are important for the development of new extraction methods using the SpeedExtractor and provides a collection of reference applications. The latter are referred to as Short Notes since they summarize the thorough and detailed documentation of the corresponding Application Note. Please contact your local Büchi representative for additional information on a particular application.

Please note: This document is continually updated with the latest reference applications and additional findings related to method development.

1 Technical Notes 3

1.1 General guidelines for pressurized solvent extraction 3

1.2 Procedure for optimizing a method 12

1.3 Comparability between the Dionex ASE® and Büchi's SpeedExtractor 14

1.4 Work-up procedures: in-cell clean-up 15

1.5 Work-up procedures: an optimized SPE approach 18

1.6 Work-up procedures: pre-extraction 20

1.7 Evaporation of the extracts 21

2 Collection of Short Notes 26

2.1 Environmental analysis 27

2.2 Trace analysis in foodstuff 36

2.3 Fat determination in foodstuff 37

2.4 Plastics and their additives 41

2.5 Pharmaceuticals and natural products 43

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1 Technical Notes

Technical Notes provide useful information which is generally applicable to pressurized solvent extraction methods. They are particularly helpful for the development of new, or the optimization of existing, methods. It is, however, important to note that the following Technical Notes are to be considered guidelines, not rules. Since the efficiency of an extraction depends on a variety of parameters, careful optimization for each type of sample is important. The following should provide a good starting point to shorten the optimization process.

1.1 General guidelines for pressurized solvent extraction

Pressurized solvent extraction is an efficient way to extract analytes from various solid or semi-solid matrices at elevated temperatures and pressure. The range of applications is comparable with classical Soxhlet or automated Soxhlet extractions. Many principles inherent to that technique are also applicable to pressurized solvent extraction. It is therefore advisable to transfer the findings for proper sample preparation and operational parameters from Soxhlet to pressurized solvent extraction.

The goals of any optimization process are to reduce time and solvent consumption, ensure high recoveries, avoid carry-over of traces from the current run to a subsequent one, and, for parallel extraction procedures, the elimination of cross-contamination between adjacent samples. All these aspects depend on the way the sample is prepared and the correct choice of extraction parameters.

The most common problems encountered are clogged cells, low recoveries, or artifacts such as co-extraction or side reactions. The first part of this Technical Note explains the influence of each parameter on the extraction efficiency and presents a guideline on how to optimize a method and solve the most common problems.

1.1.1 Sample preparation

As with Soxhlet, homogeneous, dry, and free-flowing samples work best. Therefore, if a successful sample pre-treatment procedure from Soxhlet is known, the same procedure can be followed for the SpeedExtractor. Wet samples reduce extraction efficiency and may cause blowback due to restricted flow through the sample bed. In addition, high water contents may result in interferences due to co-extractions. Generally, the same sample preparation performed for Soxhlet or sonication should also be done prior to extraction with the SpeedExtractor.

Sample weight

For most applications, the amount of sample used, is determined by the subsequent analytical method. If it is not limited by the analytics, it can be helpful to decrease the sample weight for samples that tend to clog.

In most cases it is impossible to weigh the sample directly in the extraction cell. The use of weighing boats with a sample transfer is therefore inevitable. However, this drawback can be avoided using paper thimble (see p. 7).

Homogenization

As for any analytical procedure, it is important to have a representative part of the sample. In addition, the efficiency of the extraction is proportional to the surface area in contact with the solvent. The smaller the particles, the larger the relative surface area, and thus the more efficient the extraction. It is therefore imperative to grind and/or sieve samples that are coarse, lumpy, or rocky. For samples that are efficiently extracted, sieves with pore sizes of 1 – 2 mm generally provide good results. For more critical samples, 500 µm sieves are recommended. Most food samples can be homogenized easily and safely by using the Büchi Mixer B-400.[1] Alternatively, centrifugal mills also provide satisfactory particles.[2]

Figure 1: Plant material (Genépi) before and after homogenization (ultra centrifugal mill, 1 mm sieve)

However, very fine-meshed samples may form tightly compressed beds which restrict solvent penetration and impede solvent discharge. This can be avoided when the sample is mixed with a suitable drying or dispersing agent before loading the extraction cell (see Drying and Dispersing). It is also often recommended to place a sand or Celite bed into the extraction cell before the sample is loaded. Extraction thimbles may also help to avoid cell clogging (see Section 1.1.2 Packing the cell).

Drying

Extractions with dry samples are most efficiently performed. There is no further treatment, except mixing with a suitable dispersing agent necessary (see Dispersing).

High water contents may cause clogging due to restricted flow through the sample bed. In combination with nonpolar solvents, there is a negative impact on the extraction efficiency due to shielding of the sample from contact with the extraction solvent, reduction of the surface area (particles stick together), or interferences due to co-extraction with water. The latter is more pronounced in pressurized solvent extractions (PSE) than with other extraction techniques due to the applied elevated temperatures and pressures. Therefore, polar solvent mixtures like hexane/acetone or dichloromethane/acetone often provide better results than only nonpolar solvents. For some applications, co-extracted

[1] Application Note B-400 Mixer 4000020EN Homogenizing of samples with high water, fat and fibre content

[2] Excellent results were achieved using Retsch's centrifugal mill ZM 200 with a 1 mm or 2 mm sieve.

water can easily be removed by addition of sodium sulfate in the collection vial. The mixture is then filtered and the filtrate is washed thoroughly. However, for applications where the extract has to be dried to a constant weight (e.g., fat), co-extracted water makes accurate measurements nearly impossible.

Drying the sample prior to extraction is, therefore, highly recommended and often provides better results. Mixing the wet sample thoroughly with a suitable drying agent is a common technique used to avoid loss of volatile compounds. Magnesium sulfate should not be used, because it can melt at higher temperatures. Sodium sulfate should not be used in combination with polar solvents, because it can be solubilized in the extraction and deposits in the outlet lines and valve. The drying agent of choice is diatomaceous earth (DE, P/N 053201). Generally, DE dries samples more efficiently and hence provides a cleaner transfer of the mixture to the cell.

For very wet samples, placing the sample onto metal weighing boats and oven drying them is a suitable alternative provided that no volatile compounds are analyzed. In order to avoid the loss of volatile compounds or degradation of temperature sensitive compounds, freeze-drying or spray drying are also appropriate options.

Dispersing

Dispersion of the sample with inert materials is necessary to avoid aggregation of sample particles and is recommended for almost all applications. The most commonly used dispersing agents are sand and diatomaceous earth. Sea sand entails the risk of clogging the outlet lines due to the small particle size. For trace analysis, inert materials must be extracted periodically under the same conditions as those used for the sample to verify the absence of contaminants. It is advisable to extract the dispersing agent right before every extraction.

For dry samples, sand is generally a good choice. It provides good results, is inexpensive, and there are no safety/health issues. Ottawa sand and Fontainebleau sand are also suitable for PSE. Fontainebleau sand and, particularly, Celite are recommended for very fine samples as the particle size of the sample and the inert material are similar. If Celite is used, it is necessary to make a sand bed in the cell to prevent blockage of the metal frit (see 1.1.2 Packing the cell).

For samples with residual moisture, mixing with diatomaceous earth provides best results (see Drying).

The dispersing agent can have a major impact on the extraction performance. Optimization procedures with different dispersing agents are required. Even the products of different suppliers of diatomaceous earth can have an influence on the extraction. Büchi has had good experience with its distributed product (P/N 053201).

For most samples, a ratio of approx. 1:1 (w/w) with DE, or approx. 1:5 (w/w) with sand is suitable. It is important to mix all the replicates with the same amount of dispersing agent to get reproducible results.

Dispersing and drying agents	
DE, P/N 053201	Wet samples
Extraction sand, P/N 037689	Dry samples
Ottawa sand	Dry samples
Fontainebleau sand	Fine samples
Celite	Very fine samples

Mixing ratios (w/w)	
Sample:DE	1:1
Sample:Sand	1:5
Sample:Celite	1:2

1.1.2 Packing the cell

There are various techniques to pack an extraction cell. Some are used to reduce solvent consumption, to avoid clogging of the cell, or to simplify the cleaning process after extraction. By adding suitable adsorbents such as aluminium oxide, florisil or silica gel, it is even possible to eliminate post-extraction clean-up steps. Due to the importance of the so-called in-cell clean-up procedures, more detailed information is provided in Section 1.4. In the following, we focus on commonly used techniques with different drying and dispersing agents, expansion elements, glass wool, and extraction thimbles. **Figure 2** shows different ways of filling an extraction cell.

1. **Standard filling**: sample mixed with a drying/dispersing agent
2. **Sand bed**: mixed sample with sand bed and filled void volume on top
3. **Expansion element**: voluminous fluffy sample with expansion element
4. **Paper thimble**: sample filled in paper thimble with glass wool on top

Figure 2: Examples of different filling techniques

Standard filling

Samples with a medium particle size, no tendency to clog or to contaminate the extraction cell, and sufficient volume, do not require a supplemental filling technique. The sample is simply mixed with a drying/dispersing agent and filled directly into the cells (see Drying on p. 4).

Avoid clogging: sand or Celite bed

For samples with a very fine particle size or samples that have been mixed with Celite, it is recommended to prepare a sand bed at the bottom of the extraction cell. Otherwise, the fine particles could block the filter disk and/or the metal frit. A sand bed acts as an additional filter. Quartz, Ottawa, or Fontainebleau sand can be used for the sand bed. Fontainebleau is finer grained, than the other sands. Filling the cell to up to 1/4 of its height with sand is usually sufficient.

Example: Dried sewage sludge, particle size < 200 µm. The sample tended to clog when only mixed with Celite. Hence, 40 ml cells were used with a 20 g Fontainebleau sand bed. Approximately 2 g of sample was mixed with 5 g of Celite. The sample was carefully transferred, and the void volume was filled up with Fontainebleau sand. Note: It is recommended to first add the dispersing agent in the weighing boat and then add the sample on top of it (see **Figure 3**). Any remaining grains

sticking to the weighing boat can be removed by adding another portion of dispersing agent and transferring it onto the sample in the extraction cell.

Figure 3: Dried sewage sludge with Celite (left) and sample mixed Celite (right)

Reduce solvent consumption: expansion element or sand bed

Replicates and cells that are extracted in the same run should have approximately the same void volume. It is therefore important to weigh or measure the drying/dispersing agent by volume. In addition, it is recommended to fill up any void volume of the cell with sand or diatomaceous earth.[3] This ensures a uniform extraction process and also reduces the solvent consumption per position. Expansion elements are an alternative for voluminous but low density samples (e.g., paper). For very small sample amounts, 2 ml expansion elements (P/N 053708), which are designed for the 10 ml extraction cells (P/N 051237), are available. These cylindrical elements can be placed on top of the sample instead of sand to reduce the void.

Figure 4: Ottawa sand bed, with the sample and another sand bed on top

The use of paper thimbles

The use of paper or glass fiber thimbles has several advantages:

First, it allows one to weigh the sample in the thimble directly. Thus, the risk of sample loss upon transfer is considerably reduced since direct measurement of the heavy extraction cells on an analytical balance is no option. This applies particularly to gravimetric analytical methods where the extraction efficiency is determined by measuring the weight of the sample before and after the extraction. Typical applications are the determination of the non-branched fraction of polyethylene samples.

Second, like sample beds, extraction thimbles can act as additional filters to avoid clogging of the cells.

Finally, for samples which tend to melt at elevated temperatures and thereby contaminate the extraction cell, the use of thimbles substantially facilitates the cleaning process. This applies particularly to plastic samples such as granulates or films.

For trace and ultra-trace analysis, it is important to pre-extract the thimbles in order to avoid interferences with contaminants. During the

[3] It is important not to fill the cell completely but to retain a void approx. 1 cm in height between the sample bed and the upper filter. This prevents the sample from clogging in case of swelling and therefore ensures uniform flow. See Operation Manual, Section 6.3.3.

extraction, some compounds of the thimble are co-extracted, which can cause problems in gravimetrical analysis on small output weight.

To avoid sample material being protruded from the thimble when releasing the pressure, the thimbles have to be closed by adding glass wool on top. Note: Glass wool should not get into contact with the sealing surface of the cells or the seals as this could cause leaks and damage to the seals.

1.1.3 Extraction parameters

An extraction cycle involves three steps (see **Figure 5**), one of which, the HEAT UP step, is an optimized instrument parameter and hence cannot be used in process optimization. However, it is vital to keep the whole process in mind when optimizing a procedure as every single step has an impact on the overall outcome of the process.

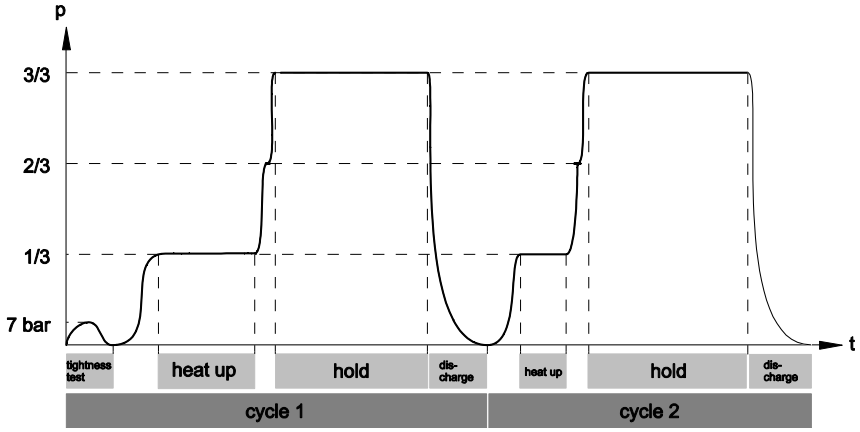


Figure 5: Pressure/time diagram of a two cycle extraction process. Only the first cycle involves a TIGHTNESS TEST during which the presence of the extraction cells and/or severe leaks is checked. During the HEAT UP step the pressure inside the extraction cells is slowly increased to the set parameters of the extraction method. During the HOLD period, these parameters remain constant, followed by the DISCHARGE of the extract via pressure compensation and collection in the vials. For more information please refer to the operation manual.

Temperature

Temperature has the highest impact on the speed and recovery of the extraction. The main advantage of PSE lays in the fact that higher temperatures can be applied based on higher pressures. As a rule of thumb, a temperature increase of 10 °C doubles the extraction speed.[4]

However, there are limitations: heat sensitive compounds degrade at elevated temperatures and side reactions may occur.[5] Generally, it is preferable to work above the boiling point of the solvent. An absolute temperature of 100 °C, or 20 °C above the boiling point of the solvent at ambient pressure, is a good starting point for method development (see also Section 1.2). Usually the temperatures for plant extractions are low

Temperature settings	
Starting point	bp_{rt} + 20 °C
Environmental samples	typ. 100 °C
Natural products	~ 50 °C
Fat determination	≤ 100 °C

[4] The temperature dependence of a chemical reaction is described by the *Arrhenius* equation ($k = A \exp(-E_a / RT)$). Doubling the speed of a kinetic process by a temperature increase of 10 °C is a useful simplification of this law.

[5] Sugar, for example, turns into caramel at higher temperatures. Fat may be decomposed, and, as a result, the fat content determined may be higher than it really is (see e.g. H.-D. Belitz, W. Grosch, P. Schieberle, Lehrbuch der Lebensmittelchemie, Springer).

(50 °C), while environmental analysis (except dioxins) and fat extraction are performed at 100 °C. If a sample contains large amounts of fat, the set temperature should not exceed 100 °C as the decomposed fat can cause cell clogging and impede accurate determination of the recovery.

Plastic samples, in particular, have a tendency to melt inside the extraction cell. In such cases, cellulose Soxhlet thimbles (P/N 11055334, 11055358) facilitate the removal of the cell content after the extraction (see also Section 1.1.2).

Pressure

Elevated pressures increase the penetration of the matrix as a consequence of decreased viscosity of organic solvents and the ability to overcome strong solvent-matrix interactions. They also keep the solvent in a liquid state and ensure fast filling and re-filling of the cell. The influence of the pressure on the recoveries is of vital interest in the literature. *Richter* found that increased pressures (i.e. up to 172 bar) provide significantly better results in PAH analysis of wet samples (water content up to 60%).[6] Similar results were achieved by *Zhu* in the extraction of herbicides from wet soil samples.[7] Higher recoveries were achieved at high pressures (i.e. 152 bar) when sodium sulphate was used as drying agent for PAH, PCB, and chlorinated pesticides in soils.[8] Pressure-assisted chelating extraction of digesting metal in solid matrices also work best at pressures of up to 210 bar.[9] As a consequence, it is important to have the ability to change the pressure, in particular for wet samples or samples with a complex matrix, such as natural products where the cell structure has to be destroyed.

However, it has been shown that 100 bar is a good starting point for a very broad range of applications.

Pressure settings	
Default	100 bar
Wet samples	150 bar

Solvent

The solvent has a major impact on the extraction result. The polarity should be similar to that of the analyte. In many cases, the same solvent as that used for the classic Soxhlet extraction can be used. However, the behavior of the solvent at elevated temperatures and pressures can differ. Mixtures of solvents with different polarities often provide better recoveries.[10] Since there are no general guidelines on how to choose the ideal solvent, the Application Notes and standard methods (such as EPA, AFNOR, etc.) should be considered.

The use of strong basic or acidic solutions such as HCl, HNO_3 or H_2SO_4 is not recommended. However, the use of weak acids such as EtOAc or H_3PO_4 in a ratio of 1-10% (v/v) with aqueous or polar solvents is fine. Hydrolyzed food samples for fat determination can be used

Non-compatible solvents
Diethyl ether
1,4-Dioxane
Tetrahydrofurane (THF)
Carbon disulfide
Strong mineral or organic acids and bases

The autoignition point of these solvents are below 200 °C or the corrosive properties may harm the instrument.

[6] B. E. Richter, *Anal. Chem.* 68 (1996) 1033 – 1039.

[7] Y. Zhu, *J. Agric. Food Chem.* 48 (2000) 4097 – 4102.

[8] M. Schantz, *Anal. Chem.* 69 20 (1997) 4210 – 4219.

[9] A. Wanekaya, *Analyst* 127 (2002) 1272 – 1276.

[10] Polar additives such as acetone decrease the shielding effect of water when wet samples are extracted with nonpolar solvents (see Drying on p. 5).

without any problems since the filtrate is washed to a neutral pH prior to extraction.[11]

Cell size

It is preferable to choose the smallest cell size that fits the samples. Not only is less solvent consumed, but the extraction is also faster.[12] The amount of dispensing agent required to complete the volume is also smaller, lowering the costs per sample. For very small samples amounts, an expansion element may be used to fill up the void volume of partially filled extraction cells (P/N 053708, 2 ml). For delicate samples (e.g., samples with high fat content), it can be advantageous to choose a larger cell in order to have less saturated and viscous extract.

Recommended cells for dry and ground samples	
1 – 1.5 g	10 ml
2 – 3 g	20 ml
4 – 6 g	40 ml
8 – 12 g	80 ml
12 – 24 g	120 ml

The values are based on the recommended mixing ratios shown on p. 5, i.e., 1:1 for DE and 1:5 for sand.

Vial size

The vials should be large enough to accommodate the entire extract. If this is not possible, utilize the “vial change” option and combine the extracts after extraction for further evaporation and/or clean-up (see Section 6.4.3 of the operation manual). In order to keep the time during which the sample is exposed to elevated temperatures as short as possible, make sure that the vials are exchanged quickly to continue the extraction immediately.

Number of cycles

The extraction efficiency may be limited by the saturation of the solvent with analyte or by the time required for the solvent to penetrate the matrix and to dissolve the analyte. In the first case, additional cycles must be added to introduce fresh solvent. In the latter, it is more efficient to increase the hold time. This is usually the case in trace or ultra-trace analysis. It is important to note though, that an additional cycle results in higher total solvent consumption. Since the ultimate goal usually is to reduce solvent and time consumption while obtaining the highest recoveries, both aspects have to be considered for optimization.

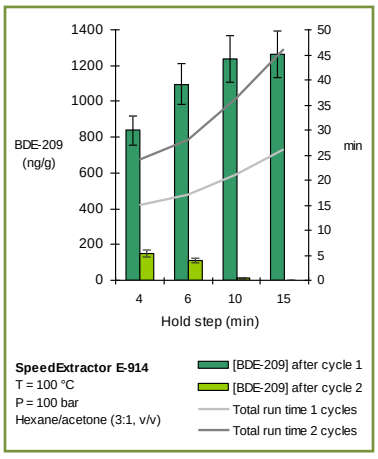


Figure 6: Example of an optimization process with highest recoveries achieved with just one cycle with a hold time of 10 min. For more information please refer to the best@buchi no. 55.

Hold time

For samples where the contact time between analyte and solvent is crucial for the extraction efficiency, the hold time should be sufficiently long. For samples with a high concentration of analyte (e.g., food samples for fat determination), it can be helpful to have a short hold time in the first cycle in order to prevent clogging and to speed up the extraction, and longer hold times for the subsequent cycles.[13] For environmental samples, in particular, optimizations with just one cycle

[11] Application Note 006/2009: Fat determination in food and feed products using SpeedExtractor E-914 after acid hydrolysis.

[12] Generally, shorter HEAT UP, DISCHARGE and FLUSH times are required for smaller cells.

[13] In fat determination with high fat contents, the hold time of the first cycle is often set to 0 min to avoid saturation of the solvent which can result in clogged cells.

but with a long hold time provided the best results in terms of recoveries, time, and solvent consumption.[14]

Discharge time

After each cycle, the outlet valve is opened and by means of pressure compensation and gravity the extracts are discharged into the collection vials. The time required to complete the discharge process, depends on the temperature, solvent, cell size, packing of the cells, the dispersing agent, and on sample properties. If the selected discharge time is too short, the solvent is not completely exchanged for the next cycle, leading to a less efficient extraction. For optimization purposes, it is advisable to observe the discharge phase to determine the time needed until no more drops are collected in the vial and to let the pressure drop to 0-1 bar.[15]

For highly concentrated samples, in particular, the discharge time for the first cycle is considerably longer then for the subsequent ones. This is due to the fact that the discharged extract of the first cycle is highly concentrated and hence more viscous.

Discharge time	
Default	2 min
Typical values	2 – 5 min
Concentrated samples	Long first cycle, following shorter

Flush with solvent

Flushing with solvent ensures that any remaining analytes in the outlet lines are transferred into the collection vials by thoroughly flushing the whole system with fresh solvent. This step is crucial to avoid carry-over of extract residues from the last cycle to a subsequent run (see also Section **Fehler! Verweisquelle konnte nicht gefunden werden.**). As a rule of thumb, the flushing time should be sufficient to exchange the entire volume of the system once. For example: 6 x 20 ml cells are approximately half filled with sample and half with solvent, resulting in 6 x 10 ml solvent for the cells. The total dead volume of the system is less than 10 ml, yielding 70 ml in total. The flowrate of the pump in the flush step is 50 ml/min. Hence, 2 min flush with solvent is sufficient.

Flush with solvent times	
Default	1 min
6 x 10 ml	1 min
6 x 20 ml	2 min
6 x 40 ml	3 min
4 x 40 ml	2 min
4 x 80 ml	4 min
4 x 120 ml	5 min

Time required to exchange the total volume when the cells are half filled with solvent.

Flush with gas

Flushing with gas at the end of the extraction process ensures that no liquids remain in the cells or lines. Remaining liquids can cause blowback of sample. The instrument therefore always automatically checks whether there is any remaining pressure before opening the lift (see also Section 8.1.3 of the operation manual).

In general, the longer the flush with gas, the better. If the temperature of the selected method lies below the boiling point of the solvent, a longer flush is needed. Water and ethanol, in particular, are more critical than solvents with a lower vapor enthalpy such as hexane. Larger cell volumes also require longer flush times. It is recommended to determine the time needed to flush with gas by observing the

Flush with gas time	
Default	3 min
Typical values	2-5 min

[14] G. Lastenet, C. Roscioli, L. Guzella, Determination of PBDEs using Pressurized Solvent Extraction and Automated Soxhlet Extraction, best@buchi no. 55, in preparation.

[15] Exception: If water is used as solvent, the pressure usually does not drop to 0 bar due to the high vapor pressure of water.

droplets collected. One minute after the last droplet is formed is a safe value. When the cell lift opens, no sound of discharging gas should be heard. Usually 2-5 min are sufficient.

1.2 Procedure for optimizing a method

If no reference applications and no good guideline for a starting point are available, a standardized preliminary test followed by iterative optimizations is recommended.

1.2.1 Preliminary tests

The adjacent table indicates the recommended parameters for a standardized preliminary test. For temperature sensitive compounds, corresponding changes in the temperature are required.

The sample weight is usually determined by the homogeneity of the sample or by the detection limits of the subsequent analysis. The cell size and the best way to prepare the sample should be determined in advance, taking Section 1.1.1 into account. In some cases, a little trial and error needed.

In order to determine the completeness of the method, it is recommended to run a subsequent extraction using the same parameters but just running one cycle. This approach is preferred over the VIAL CHANGE option as the latter may induce residual analyte concentration in the subsequent cycle based on remains in the outlet lines.[16]

Preliminary test	
Temperature	100 °C
Pressure	100 bar
Solvent	See p. 9
Cells	See p. 10
Vials	See p. 10
Cycles	2
Hold	5 min
Discharge	4-10 min (p. 11)
Flush with solvent	1-5 min (p. 11)
Flush with gas	5 min

1.2.2 Evaluation of the preliminary test and troubleshooting

If the extraction runs smoothly and with satisfying recoveries, it can be further optimized in terms of time and solvent consumption. As pointed out on p. 10, less extraction cycles with longer hold times reduce time and solvent consumption provided that the diffusion of the analyte from the matrix to the solvent is the time-determining step. The extraction time can also be reduced by shortening discharge and/or flush time (see pp. 11).

In case of unexpected problems, please refer to the troubleshooting guidelines below.

[16] It is important to note that when VIAL CHANGE is activated, there is no flushing with solvent or nitrogen between the corresponding two cycles. Hence, remains of the analyte of the first cycle may not be collected in the vials until the lines are flushed with solvent during the subsequent cycle. This is in contrast to the approach suggested above, where the lines are thoroughly flushed before the after-extraction starts.

Problem	Action	Reference
Low recoveries	1. Prolong discharge times	See p. 11
	2. Increase the temperature in steps of 20 °C	See p. 8
	3. Increase the number of cycles	See p. 10
	4. Prolong hold times	See p. 10
	5. Change the sample preparation procedure	See p. 3
	6. Change the solvent or solvent mixture	See p. 9
	7. Increase the pressure	See p. 9
Excessive recoveries	1. Artifacts could have been formed due to too high temperatures. Sometimes the color of the extract is unusual or darker or has dark spots. Decrease the temperature in 20°C steps.	See p. 8
	2. Undesired compounds were co-extracted. Change the polarity of the solvent so that it is similar to the polarity of the analyte. If further clean-up steps are required, consider the in-cell clean-up options.	See p. 15
	3. Carry-over from a preceding run. Make sure that all accessories and materials, in particular the dispersing agent, cells, and metal frits, are pre-extracted. In some cases, it may be useful to perform a “cleaning extraction” using the same extraction parameters as for the actual extraction but just running one cycle.	
Clogging	For very viscous extracts:	
	1. Use glass fiber filters instead of cellulose filters.	
	2. Decrease the sample amount if possible	See p. 11
	3. Prolong discharge times, particularly of the first cycle	See p. 10
	4. Use larger cells	
	5. Shorten the hold times, particularly of the first cycle[17]	See p. 10
	For all other samples:	
	1. Prepare cells with a (larger) sand bed	See p. 6
	2. Change the drying/dispersing agent	See p. 4

[17] For very viscous samples it is recommended to set the hold time of the first cycle to zero.

1.3 Comparability between the Dionex ASE® and Büchi's SpeedExtractor

The efficacy and reliability of pressurized solvent extraction (PSE) using the SpeedExtractor was compared to Dionex Accelerated Solvent Extraction (ASE®) for several analytes and matrices such as dioxins/furans (PCDDs/PCDFs) in soils, polybrominated diphenyl ethers (PBDEs) in lake sediments, perfluorinated compounds (PFCs) in soil and sewage sludge, and polychlorinated biphenyls (PCBs) and phthalates in plastics.[18,19] It was shown that Dionex applications are very similar to those of the SpeedExtractor. The following section describes the transfer of the corresponding parameters from one system to the other.

Equipment and materials

Dionex ASE	Büchi SpeedExtractor E-916/914
Cell size ASE 200: 11 ml, 22 ml, 33 ml ASE 350: 1 – 100 ml	E-916: 10, 20, 40 ml E-914: 40, 80, 120 ml 11 ml and 22 ml ASE cells can be replaced by 10 and 20 ml cells, respectively. For other cell sizes, when there is a question, it is better to use the next larger SpeedExtractor cell and to fill up the void with sand or an expansion element.
Hydromatrix or ASE Prep DE	Diatomaceous earth (P/N 053201)
Ottawa sand	Ottawa sand or Büchi Quartz sand (P/N 037689)

Extraction method parameters

Dionex ASE	Büchi SpeedExtractor E-916/914
Extraction solvent	The same solvents can be used
Temperature Range: ambient to 200 °C	Range: 30 to 200 °C The geometry and the principle of the Dionex heater are different from the SpeedExtractor. In the Dionex system, only half of the cell is heated by a mantle, whereas the SpeedExtractor has a compact heating block. Hence, the actual temperature of the ASE instrument is approx. 20% lower than the set temperature.

ASE	SpeedExtractor
150 °C	120 °C
120 °C	100 °C
80 °C	65 °C
50 °C	40 °C

[18] S. Cleres, L. Gruber, G. Lastennet, M. Schlummer, G. Wolz, Determination of polychlorinated biphenyls and phthalates in waste polymer samples intended for mechanical recycling, Dioxin congress in Beijing, 2009.

[19] S. Cleres, L. Gruber, G. Lastennet, M. Schlummer, G. Wolz, Parallel pressurized solvent extraction of PCDD/PCDF, PBDE and PFC from soil, sludge and sediment samples, Dioxin congress in Beijing, 2009.

Pressure Constant[20]: 1500 psi	Range: 50 – 150 bar 1 psi ≈ 0.7 bar, 1 MPa = 10 bar. Since the pressure has a less significant impact on the extraction efficiency, round up to the nearest number during unit conversion. ASE 1500 psi — SpeedExtractor 100 bar
(Oven) Heat-up time: Time required to heat up the sample and solvent. The operator has to set the time in function of the extraction temperature and is optional.	The heat-up time of the SpeedExtractor is pre-defined and optimized for different extraction cells and temperatures. Hence, the time required to heat up the solvent and sample cannot be changed by the operator in order to ensure a reproducible extraction process.
Static time	Corresponds to “hold time”. The total of the heat-up and hold times should equal the total of Dionex’ heat-up and static times.
Flush volume, given as % of the volume of the cell size. When more than one cycle is specified, the flush volume is divided by the number of cycles for each rinsing step.	Flush with solvent, given in min. Flush only occurs at the end of the extraction method, but not after each cycle. The flush time should be chosen independently of the ASE parameter. To optimize this parameter, see p. 11.
Purge time: Time required for nitrogen flush	Flush with gas, given in min. The nitrogen flush time should be chosen independently of the ASE parameter. To optimize this parameter, see p. 11.
Static cycles	Corresponds to “cycles”

1.4 Work-up procedures: in-cell clean-up

No extraction technique is absolutely selective for the desired analyte. Co-extracted substances may interfere with analyte analysis, making a reliable determination less efficient, impossible, or misleading. The extracts from a pressurized solvent extraction therefore often undergo time-consuming purification procedures such as chromatography (GPC or glass columns), solid phase extraction (SPE), or liquid-liquid extraction (LLE).

The following describes three different approaches for additional extract work-up:

- A suitable adsorbent can be mixed with the sample, and/or a layer of absorbent material can be placed at the bottom of the SpeedExtractor’s extraction cells. This technique is commonly referred to as in-cell clean-up (see Section 1.4.1).
- A more flexible, but also more time-consuming approach, combines pressurized solvent extraction (PSE) with a subsequent solid phase extraction (SPE) of the extracts. Section 1.5 presents an optimized

[20] For the new ASE 350, the pressure can no longer be changed but is kept constant at 1500 psi.

workflow based on synergies between the extraction and evaporation equipment.

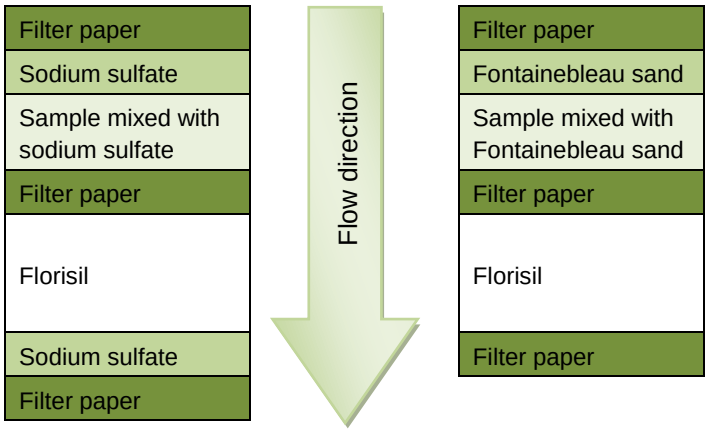
- As an alternative to chromatographic work-up procedures, the interfering compound can be pre-extracted with a suitable solvent, followed by another extraction with a different solvent to extract the desired analyte (see Section 1.6).

1.4.1 Addition of sorbent to the extraction cell

The fact that extract filtration is an inherent step of PSE while the liquid passes through a frit from the extraction cell to the collection vial, entails the possibility to trap matrix constituents by adding suitable absorbents directly to the extraction cell. This one-step extraction and clean-up process is an ideal way to optimize tedious clean-up procedures.

The ideal purification procedure, however, depends on many parameters such as the sample type, the quantity and type of co-extracted substances, the analyte, the subsequent analytics, and extraction parameters like solvent and temperature. It is therefore impossible to propose a universally valid procedure. This technical note presents general techniques and describes some examples of successful applications.

The following figure schematically represents two commonly used packing techniques for the different layers of an extraction cell. The flow direction of the extraction solvent is indicated by an arrow.



1.4.2 Frequently used adsorbents

The most commonly used adsorbents are listed in **Table 1**.

Table 1: Adsorbents and their properties[21]

Adsorbent	Properties and application field
Florisil (magnesium silicate with basic properties)	Adsorbs lipids and fats, but also other organic compounds of the matrix.
Aluminum oxide, basic, acidic or neutral	Adsorbs lipids
Silica gel	Binds polar and unsaturated compounds

[21] Schwedt, G. (2008) Analytische Chemie, Wiley-VCH, Weinheim, 2nd edition.

1.4.3 Examples from literature

A variety of different in-cell clean-up techniques have been published in literature, each of them using different adsorbents for different applications. **Table 2** provides a useful selection of different application fields.

Table 2: Selection of in-cell clean-up procedures published in the literature

Adsorbents	Uses	Applications
Florisil	Cleaning of cocoa and coffee extracts.[22]	Determination of acrylaimide
Florisil, silica/sulfuric acid	Several different "fat retainers" have been tested for their ability to hold fat in the cells while extracting fatty food samples. Co-extracted fat negatively influences the GC-MS measurements. Florisil and silica/sulfuric acid have been identified as the most effective sorbents, which lead to extracts with less than 0.3 mg fat. The best results were found with a ratio of 1:40 fat to adsorbent. The fat retaining capacity also depends on the extraction temperature (better retention at lower temperatures).[23,24] Florisil was less convenient to apply, but with the silica/sulfuric acid material which is very aggressive, the stainless steel parts of the instrument were damaged. In addition, some organic solvents react with sulphuric acids, such as acetone, which is commonly used.[25] The authors Gomez-Ariza et al. also achieved good results with Florisil for the determination of PCBs in oysters and eggs of the common spoonbill bird.[26]	Determination of PCB in a fatty matrix
Florisil, pre-extraction of non-polar interfering compounds	Paraben and triclosan are widely used bactericides in cosmetics, textiles, and cleaning agents. There have been some controversies due to potential allergic reactions, harms to the skin microflora, and even possible carcinogenicy. The compounds are ubiquitous in indoor dust.[27]	Determination of paraben and triclosan
Silicic acid and cyanopropyl sorbent	In-cell cleanup has been used to fractionate polar phospholipids from non-polar lipids in biological samples. Layers of silicic acid or cyanopropyl sorbent were placed into the extraction cells.[28]	Fractionation of polar phospholipids

[22] Delatour, T. et al. (2004) Improved sample preparation to determine acrylamide in difficult matrixes such as chocolate powder, cocoa and coffee by liquid chromatography tandem mass spectroscopy. J. Agric. Food Chem. 52, 4625-4631.

[23] Björklund, E.; Muller, A. von Holst, C. (2001) Comparison of fat retainers in accelerated solvent extraction for the selective extraction of PCBs from fat-containing samples.

[24] Sporring, S.; Björklung, E. (2004) Selective pressurized liquid extraction of polychlorinated biphenyls from fat-containing food and feed samples, influence of cell dimension, solvent type, temperature and flush volume. J. Chromat. A., 1040, 155-161.

[25] Focant, J.-F.; Pirard, C.; De Pauw, E. (2004) Automated sample preparation-fractionation for the measurement of dioxins and related compounds in biological matrices: a review. Talanta, 63, 1101-1113.

[26] Gomez-Ariza, J. L.; Bujalance, M.; Giraldez, I.; Velasco, A.; Morales, E. (2002) Determination of polychlorinated biphenals in biota samples using simultaneous pressurized liquid extraction and purification.) J. Chromat. A. 946, 209-219

[27] Canosa, P.; Perez-Palacios, D. Garrido-Lopez, A.; Tena, M. T.; Rodriguez, I.; Rubi, E.; Cela, R. (2007) Pressurized liquid extraction with in-cell clean-up followed by gas chromatography-tandem mass spectrometry for the selective determination of parapens and triclosan in indoor dust. J. Chromat. A. 1161, 1-2, 105-112.

[28] Poerschmann, J.; Carlson, R. (2006) New fractionation scheme for lipid classes based on „in-cell fractionation" using pressurized liquid extraction. J. Chromatography A. 1127, 18-25.

1.5 Work-up procedures: an optimized SPE approach

For complex matrices, a two-step extraction work-up procedure is often required. Solid phase extraction (SPE) is the method of choice, particularly in trace analysis. The following Section presents an improved parallel SPE concentration process where all essential work-up steps, including evaporation of the eluates, are achieved without any sample handling between the individual steps.

General considerations

In contrast to an ordinary separation on column chromatography, in SPE the analytes are usually not separated on the column but are retained as a sharp band on the solid phase and are then eluted by liquid organic solvent. Successful SPE therefore has two major requirements: first, a high, reproducible percentage of the sample analytes must be taken up by the sorbent; second, the solutes must then be easily and completely eluted from the solid particles.

For normal-phase SPE silica gel, activated aluminum and, in particular, Florisil are extensively used for example in trace analysis of herbicides or pesticides in vegetable matter. To do so, an aqueous slurry of the sample is prepared in a blender. Pesticides are extracted along with portions of the vegetable matter by a mixture of organic solvents that are not miscible with water. The organic extract is then passed through a Florisil column and eluted with one or more organic solvents. Since pesticides are usually more hydrophilic than vegetable matter, they are eluted while the vegetable matter remains tightly held by the column.[29]

Steps of a typical SPE procedure

Conditioning: Pretreatment of the sorbent usually involves washing with the solvent (used for elution step) in order to exclude contamination by the sorbent or cartridge. Second, a mediating solvent which promotes better surface contact between the phases is required.

Adsorption: The liquid sample to be extracted is passed through a packed column by suction (or applied pressure). The flow should be at a reasonable and constant rate. The flow rate, however, is strongly dependant on the particle size, porosity and matrix. Very small particles (~10 µm) are more efficient and thus permit a faster flow rate.

Washing: Co-adsorbed matrix materials which influence the analytics of the analyte need to be removed from the column. The proper choice of a wash solution is highly dependent on the solid sorbent, the sample matrix, and the analyte. Example: Inorganic ions and organic components from the matrix are to be removed from a reverse-phase column with C18 bonded-phase silica particles. Water will easily remove the ions but may not be sufficient to remove the sample matrix components. Addition of organic solvents (~10%) may

[29] A comprehensive list of commercially available sorbents is published online: http://www.biotage.com/DynPage.aspx?id=22311.

help, but the percentage of organic solvent must not be high enough to partially elute the sample analytes.

Elution: The elution step removes (desorbes) the adsorbed sample analytes from the sorbent and returns them to a liquid phase suitable for analysis. This is mostly done by an organic liquid or sometimes thermally assisted by a gas stream. In order to achieve high recovery rates, the eluting liquid must remove the analyte completely from the solid phase using small solvent volumes. In addition, the solvent must be compatible with the respective analytical procedure (e.g. no impurities that disturb chromatographic peaks).

Classical equipment

Most solid phase extractions are performed with the aid of suction, although pressure alternatives are available. A typical vacuum manifold permits simultaneous filtration of 12 or 24 samples (see **Figure 7**). [30]

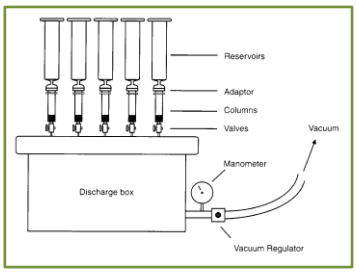


Figure 7: Example of a classic manual SPE vacuum manifold with SPE columns, valves, discharge box, manometer and vacuum regulator.

The vacuum needed for filtration can be obtained by a simple water jet or vacuum pump with a suitable controller. Individual flow control for each position is achieved either by a PTFE stopcock or a rotating screw-type valve built into the cover. This ensures that the packing of some tubes will not go dry while others are still draining.

This setup entails a number of time consuming and tedious manipulations. The liquids passed through the tubes are collected in individual test tubes or a lager waste vessel. At a minimum, aeration and an exchange of the collection vessels are required between the washing and elution step. In addition, the subsequent evaporation or concentration step involves another aeration of the manifold and sometimes even sample transfer into suitable vessels for evaporation.

The Syncore® approach

To reduce manual sample handling during sample preparation, an SPE-module for Büchi's Syncore® Analyst was developed in collaboration with the Cantonal Department for Food Control, St. Gallen, Switzerland.

The system requirements were as follows: The operational steps for the SPE concentration (conditioning, adsorption, washing, elution) should be carried out without interruption and sample handling, cross-contamination must be avoided, and the outlet of each SPE-cartridge must be individually adjustable to the positions *"stop"*, *"waste"*, and *"elute"*.

An SPE-module with 6 or 12 cartridge ports consistent with the Analyst racks with 6 or 12 positions was developed. The key feature of this setup is a three-way stopcock which allows for liquid separation in either a waste vessel or a collection vessel after passing through a SPE cartridge. This makes it possible to first transfer the liquids of the condition, adsorption and washing step into the vessel, and then to elute directly into the evaporation vessel. There is no exchange of glassware or aeration of the vacuum manifold required whatsoever.

[30] Special setups for 96 port microtitre plates are common in microbiology.

Moreover, by turning the stopcock in the stop position, the elute can directly be evaporated to either dryness or to a pre-defined residual volume. The latter is achieved by means of a locally cooled appendix at the bottom of each evaporation vessel (see **Figure 8**).

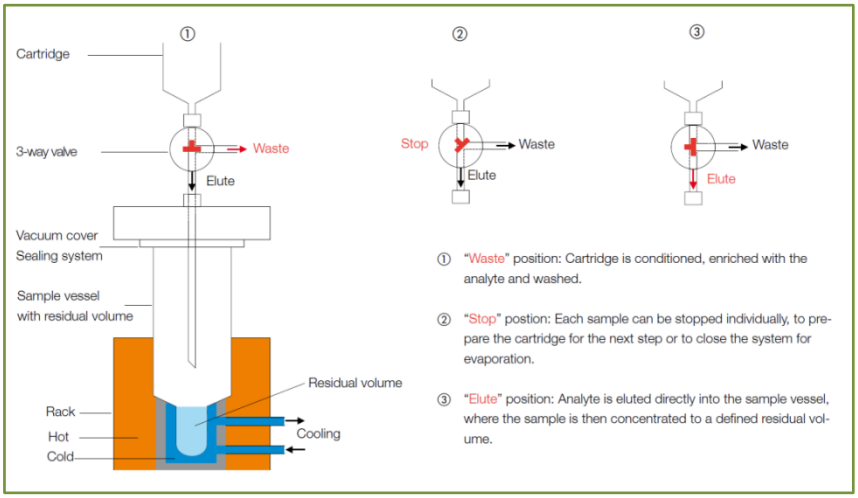


Figure 8: Operation principle of the three-way stopcock of the advanced Syncore® SPE module. Step 1 also shows the locally cooled appendix to evaporate the desorbed extract to a pre-defined residual volume.

A three times higher sample throughput was achieved using the SPE module instead of the Analyst for the determination of arbutin as a measure of the pear content in apple juice samples, compared with the classical SPE vacuum manifold followed by rotary evaporation.[31]

1.6 Work-up procedures: pre-extraction

The PSE instrument setup enables the use of different solvents with the same sample without changing the setup. It is therefore possible to use up to four different solvents with different polarities with the SpeedExtractor's integrated 4-port solvent mixer. By changing the polarity of the solvent, different matrix constituents are extracted from the solid sample. This approach can either be used for the fractional extraction of as many analytes as possible from one sample, or to pre-extract inferring matrix constituents prior to the actual extraction of the target analyte. The first approach is often used in R&D in natural product extraction when a variety of different ingredients are to be determined and quantified. An example of the latter is given in the next section.

1.6.1 Determination of hypericin in St. John's Wort

Hypericin is the main active compound in St. John's Wort. According to the European Pharmacopoeia, hypericin is determined photometrically. Co-extracted chlorophyll, however, interferes with the analysis due to its absorbance which is close to the wavelength of hypericin.

[31] P. Kölbener, A. Wernli, R. Hartmann, Determination of the pear content in apple juice, best@buchi no. 43/2006.

The ground sample was therefore pre-extracted with dichloromethane to remove chlorophyll. The hypericin is extracted in a second step using methanol. For further information please refer to the corresponding short note.[32]

1.6.2 Examples from literature

Corticosteroids were extracted selectively from bovine liver. The sample was pre-extracted with *n*-hexane to remove the lipids. The analyte was then extracted with a mixture of *n*-hexane/ethyl acetate.[33]

1.7 Evaporation of the extracts

Büchi Labortechnik AG offers two systems which are designed for parallel extract evaporation, the Syncore® Analyst R-12 and the Multivapor™ P-6. Both are compatible with the glassware used to collect the extracts of the SpeedExtractor, making the time-consuming sample transfer step obsolete. For evaporation to dryness, the Multivapor™ is the instrument of choice, whereas for concentration to pre-defined residual volumes, the Syncore® Analyst is preferred.

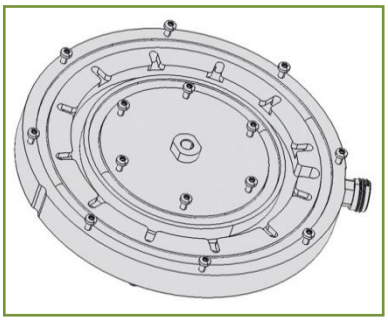


Figure 9: Vacuum lid of the Multivapor P-12 showing the grooved channels.

1.7.1 Multivapor™: Evaporation to dryness

The Multivapor™ is designed to simultaneously evaporate 6 or 12 samples to dryness. This parallel vortex evaporator is characterized by a unique sealing system which allows for accommodation of a variety of different sample vessels, all of them individually sealed. In order to prevent cross-contamination, the lid has grooved channels (see **Figure 9**), and the vapor duct of each position can optionally be equipped with a porous frit.

Figure 10: Sample vessels compatible with the Multivapor: ① P/N 052672, ② P/N 053208, ③ P/N 049535.

Compatible glassware

The Multivapor™ P-6 is the best fit for the SpeedExtractor since it is compatible with all of the SpeedExtractor's collection vials (except those with an appendix). Hence, whether the samples are collected in 240 ml narrow-necked flat bottom bottles ①, 220 ml wide-necked round bottom vessels ②, or 60 ml vials ③ (see **Figure 10**), all sample vessels can be placed directly into the Multivapor's evaporation rack, making sample transfer from one sample vessel into another obsolete.

1.7.2 Syncore®: Concentration to pre-defined residual volumes

In trace or ultra-trace analysis concentration of the extract is required in most cases to lower the detection limit of the analyte. The Syncore® Analyst R-12 is designed to concentrate up to 12 samples

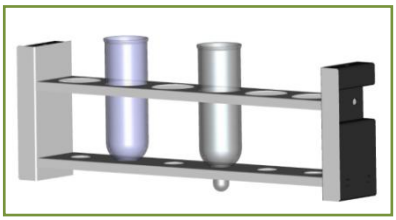


Figure 11: Analyst collection unit (P/N 11055574) with sample vessels with appendixes.

[32] Application Note 015/2009, in preparation.

[33] Draisci, R.; Marchiafava, C.; Palleschi, L.; Camarata, P.; Cavalli, S. (2001) Accelerated solvent extraction and liquid chromatography – tandem mass spectrometry quantification of corticosteroid residues in bovine liver. J. Chromatograph. B. Biomed Sci Appl. 753, 217-223.

with working volumes of 10 – 120 ml down to pre-defined residual volumes of 0.3 or 1.0 ml by means of gentle heating under vacuum. A SpeedExtractor collection unit is available (P/N 11055574) which is tailored to the evaporation vessels of the Syncore. This allows extract collection directly into the evaporation vessels (see **Figure 11**).

Cooled appendix

The key feature of the Syncore® Analyst is an integrated cooling zone which stores the sample in a cooled environment (see **Figure 12**). Thus, a sudden increase in temperature at the end of the evaporation process can be avoided (see p. 23). The cover is constructed from inert materials to avoid the leaching of contaminants. The risk of cross-contamination is eliminated by an individual sample sealing system and separate vacuum channels.

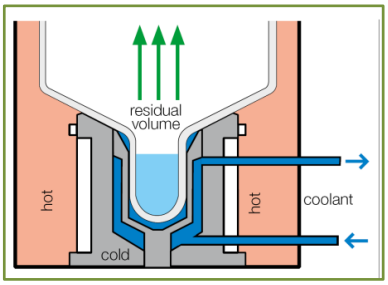


Figure 12: Analyst rack cools the appendix of the sample vessel avoiding evaporation to dryness.

The Flushback effect

The Analyst can optionally be equipped with a so-called Flushback Module. With this unique feature, the top of each vessel is cooled, which partially condenses the vaporized solvent as it leaves the sample vessel (see **Figure 13**). In turn, a gentle continuous rinse of the glass wall during the entire evaporation process occurs. This ensures that the dissolved sample remains in the cooled appendix and does not stick on the glass wall. It has been demonstrated that the Flushback Module has a significant impact on the recoveries, in particular for analytes with a high affinity to glass walls.[34] It is clear, though, that the drawback of the Flushback effect is a decrease in the evaporation speed. The better the Flushback effect, the slower the evaporation process.

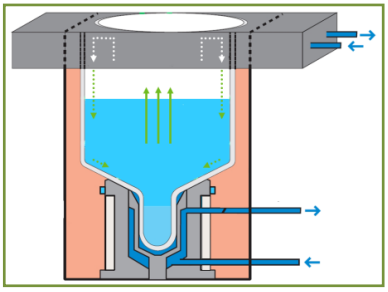


Figure 13: Flushback Module shows partial refluxing of the vapor which yields higher recoveries.

1.7.3 Optimizing the evaporation parameters

To achieve the best evaporation conditions, four parameters, the temperature of the heating plate, the boiling point, the cooling water temperature, and the rotational speed of the orbital movement, must be attuned to one another. In addition to speed, high analyte recoveries are also important. These two goals exist in opposite. Therefore, particularly in trace analysis, an optimized evaporation process primarily yields high recoveries at the expense of speed. In contrast to rotary evaporation, in parallel evaporation speed is generally less important since the bottleneck is usually shifted to the analysis due to the simultaneous evaporation. The ultimate goal of the following recommendations is therefore to avoid analyte loss.

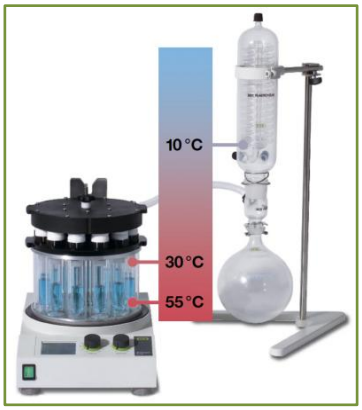


Figure 14: Illustration of the Δ25/20 °C rule.

The Δ25/20 rule

A simple rule of thumb can be used to find the optimum settings for the three temperature parameters: the Δ25/20 °C rule.[35] This rule specifies the temperature difference between the three different zones,

[34] J. Cavegn, B. Haag, R. Hartmann, Trace analysis of PAHs: evaluation of concentration and comparison of two extraction methods (EPA 3541 and EPA 3545), best@buchi no. 51/2008.

[35] When using a rotary evaporator, the rule should be modified to Δ20/20 °C.

i.e., the heating bath, the vapor temperature, and the cooling temperature. For example, when the temperature on the instrument is set to 55 °C, the vacuum should be set so that a boiling point of 30 °C results, i.e., a ΔT of 25 °C lower. In order to achieve sufficient condensing, the cooling temperature is another ΔT of 20 °C lower, i.e., 10 °C (see **Figure 14**).

Temperature and rotation of the heating plate, T_i

The evaporation speed mainly depends on the amount of heat supplied by the heating plate. It is important to note, though, that liquids cannot assume a temperature higher than their boiling point. The boiling point T_{bp} is solely determined by the applied vacuum. Hence, there is no correlation between the set temperature on the instrument T_i and the temperature of the sample T_s. Higher instrument temperatures T_i only result in faster evaporation, but not in higher sample temperatures T_s. When the sample is boiling, T_s always equals T_{bp}.

Instrument temperature T_i
$T_i = T_{bp}$ + 25 °C
Condensate covers approx. 1/2 of the condenser

The only critical point is at the end of an evaporation to dryness process. Because at this point the sample is no longer cooled by the loss of evaporation energy, so the sample now sticks to the glass wall of the sample vessel and is therefore directly exposed to the temperature of the heating plate. This results in an immediate increase of the sample temperature T_s from T_{bp} to T_i at the end of the evaporation process (see **Figure 15**). To avoid this sudden rise in temperature, the evaporation process should be stopped immediately when the last sample is evaporated, and the sample should be taken out of the heating bath. Using the Multivapor™, this is easily achieved with the transfer rack.[36] The Syncore® Analyst on the other hand, overcomes this problem by concentrating the sample in a cooled appendix (see Section 1.7.2).

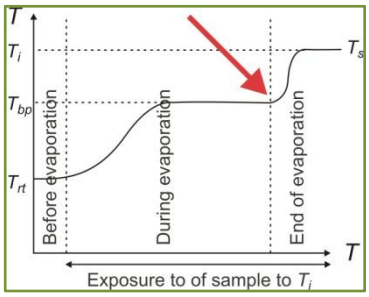


Figure 15: Change of the sample temperature during the evaporation process with a sudden rise in temperature at the end of the process.

Another important factor that influences the speed and recoveries is the surface area of the sample. The larger the surface, the faster is the evaporation. The surface area is determined by the rotational speed of the orbital moving heating plate. Faster rotations increase the surface area and hence accelerate the process. But as shown in **Figure 16**, it also distributes the sample on a larger glass area which usually reduces the recovery.

Figure 16: 0.2 g hydrocarbon grease evaporated at 170 rpm (left) and at 300 rpm (right).

Generally, better results in terms of recovery are achieved by generating a smooth vortex with the lowest possible rotations. For the Analyst, this is typically the case at 120 – 180 rpm, for the Multivapor™ at position 4 – 6.

Temperature of the cooling medium, T_c

The heat of evaporation which is required for the transition from the liquid to the gas phase is withdrawn during condensation from the vapor.[37] In order to maintain a balanced difference in dynamic pressure,

Rotational speed	
Analyst	120 – 180 rpm
Multivapor	Pos. 4 - 6

[36] Please refer to the Multivapor brochure for more information (http://www.buchi.com/Multivapor-P-6-P-12.3156.0.html).

[37] This phenomenon is referred to as a thermal pump. See: The laboratory assistant, Büchi Labortechnik AG, second edition, p. 24.

it is important that the condensation speed and the evaporation speed be attuned to one another. Whenever more substance is vaporized than is condensed in the cooler, the pressure within the apparatus rises and the vacuum pump has to draw continually, pulling in the vaporized solvent and pumping it out into the environment. When working with hazardous chemicals, in particular, this must be avoided.[38] The use of a post-pump secondary condenser is highly recommended to further reduce harmful emission of solvents into the laboratory atmosphere.

According to the Δ25/20 °C rule, a good balance is achieved with a cooling temperature about 20 °C or less below the boiling point of the sample. When evaporated at relatively low temperatures, i.e., instrument settings of approx. 50 °C, a recirculating chiller is required. Büchi's B-740/8 800 W chiller provides sufficient cooling capacity for this purpose. Whenever the condensate covers approximately half of the height of the condenser, the evaporation is occurring optimally (see **Figure 17**). Higher condenser loadings usually have a negative impact on solvent recovery.

Figure 17: The vacuum and cooling conditions are ideal when the condensate covers approximately half of the height of the condenser.

Temperature of the boiling point, T_{bp}

Boiling is referred to as the state where the vapor pressure equals the outside pressure.[39] A vacuum is therefore applied to decrease the temperature of the boiling point.

The most convenient way to achieve the right setting is to use the Büchi vacuum controller V-800 or V-850. With this instrument, the pressure is either determined automatically using the EasyVac function, or it can be easily determined by means of an integrated temperature/pressure converter (the so-called Solvent Library) which is available for a variety of solvents. Moreover, with the V-850 pressure gradients can be programmed to ensure smooth and reproducible evaporation with the highest possible yields.[40]

Cooling temperature T_c
$T_c = T_{bp} - 20$ °C
Condensate covers approx. 1/2 of the condenser

In practice, the following procedure is recommended:

1. Set the heating bath to the desired temperature, for example 55 °C.
2. Determine the pressure using the Solvent Library so that a boiling point of Δ25 °C lower, i.e., 30 °C, results.
3. Set the cooling temperature another Δ20 °C, i.e., 10 °C, lower.

To avoid boiling retardation or loss of analyte at the initial state, program a pressure gradient. Start 500 mbar above the calculated pressure. Decrease the pressure for 350 mbar in 4 min. Decrease further for 100 mbar in 5 min and another 60 mbar in 10 min. Keep the pressure constant until the evaporation is finished. For very volatile compounds slow aeration over 5 min is recommended.

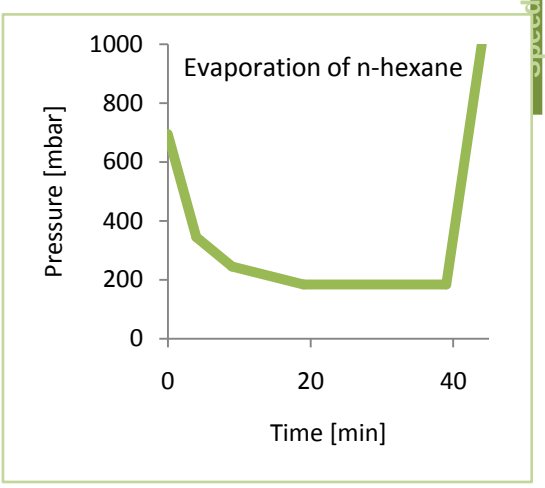
[38] It is estimated that worldwide approx. 160'000 liters of solvent per day are discharged into the air due to unbalanced evaporation and condensation speeds. See: The laboratory assistant, Büchi Labortechnik AG, second edition, p. 47.

[39] See *Clausius-Clapeyron* equation: $\frac{d \ln P}{dT} = \frac{L}{RT^2}$.

[40] Optimizing the evaporation using the Syncore® Analyst, recoveries of 94±3% were achieved for naphthalene in PAH determination of soil samples. See: J. Cavegn, B. Haag, R. Hartmann, Trace analysis of PAHs: evaluation of two extraction methods (EPA3541 and EPA3545) and optimization of subsequent concentration, best@buchi no. 51/2008.

Example: Pressure settings for evaporation of *n*-hexane.

Solvent		*n*-hexane
Desired boiling point, T_{bp}		25 °C
Pressure derived from the solvent list		195 mbar
Instrument temperature, T_i		55 °C
Pressure gradient		
Starting point	195 + 500 = 695 mbar	
First ramp	695 – 350 = 345 mbar	4 min
Second ramp	345 – 100 = 245 mbar	5 min
Third ramp	245 – 60 = 185 mbar	10 min
Constant	185 mbar	20 min
Aeration	185 + 805 = 1000 mbar	5 min



2 Collection of Short Notes

In contrast to the Technical Notes, the Short Notes deal with one particular analyte or type of sample and list all steps involved in the process, such as sample pre-treatment, packing of the cell, extraction parameters, further clean-up steps, parallel evaporation and analysis. It is a summary of a much the more detailed Application Note and is available on request from the local Büchi representative. The corresponding Application Note describes the step-by-step procedure which allows one to reproduce the application. Many of the following applications have been carefully developed by Büchi customers who have long-term experience in this field. Successful collaboration with many customers around the world is greatly acknowledged.

	Analyte	Matrix	Instrument	Page
2.1	Environmental analysis			27
	PAH	Sediment	E-916	27
	PCB	Sediment	E-916	29
	PBDE	Sediment	E-914	30
	Dioxines, furans	Soil	E-916	32
	PFC	Soil, sewage sludge	E-916	34
2.2	Trace analysis in foodstuff			36
2.3	Fat determination in foodstuff			37
	Fat	Shortbread	E-916	37
	Fat	Hydrolyzed food/feed	E-916	38
	Fat	Hydrolyzed food/feed	E-914	39
2.4	Plastics and their additives			41
	PCB, phthalates	Waste polymer	E-916	41
2.5	Pharmaceuticals ad natural products			43
	Total polyphenol	Edelweiss	E-916	43
	Total polyphenol	Genépi	E-916	44
	Costunolide	Genépi	E-916	46
	Hypericin	St. John's Wort	E-916	47

2.1 Environmental analysis

Pressurized solvent extraction is used for a wide range of applications in environmental analysis. It meets the requirements of U.S. EPA method SW-846 3545 and SW-846 6860. The following analytes are particularly suitable: PAH, PCB, dioxins and furanes.

2.1.1 Extraction of Sediment using the SpeedExtractor E-916 for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAH) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. They are of concern because some compounds have been identified as carcinogenic, mutagenic and teratogenic. Sediment samples from a round robin program (SETOC 2008/4, Wepal, Wageningen University) were extracted according to EPA 3545A [1] with the SpeedExtractor E-916 and analyzed by GC-MS. The results correspond to the values found by the round robin testing and show high recovery and low variation.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. The lead substance benzo(a)pyrene is shown in **Figure 18**.

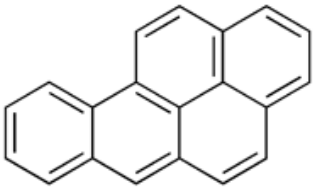


Figure 18: Chemical structure of benzo(a)pyrene.

PAHs occur in oil, coal and tar produced by cal carbonization, but not in bitumen. They can also be found in grilled meat, cigarette smoke and automobile exhaust. PAHs are persistent, ubiquitous and some of them have carcinogenic, mutagenic and teratogenic properties.

Experimental

Instrumentation: SpeedExtractor E-916 with 20 ml cells, GC-MS Varian 3000. Samples: 4 samples from the SETOC 2008/4 round robin program, Wepal, Wageningen University. Approx. 3 g of dry sample was mixed with 10 g of sand and filled into the extraction cell. After addition of the internal standard solution the samples were extracted using the parameters shown in the adjacent table. A four-fold extraction was performed. The received extracts were analyzed by GC-MS.

Temperature	100 °C
Pressure	120 bar
Solvent	Acetone 50%, hexane 50%
Cells	20 ml
Vials	240 ml
Cycles	2
Heat-up	1 min
Hold	10 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

Results

The results correspond to the values found by the round robin testing and show good recovery and low variation. The results for the lead substance benzo(a)pyrene are shown in **Table 3**. The total extraction time was about 40 min, and approx. 50 ml of solvent per cell was used.

Table 3: Values for benzo(a)pyrene, SpeedExtractor: RSD for four-fold extraction, SETOC: RSD for results from the participating 43 laboratories of the round robin.

	SpeedExtractor E-916		SETOC	
	Mean Value [µg/kg]	RSD % (n=4)	Mean Value [µg/kg]	RSD % (n=43)
Sample 1	495	9	463	13
Sample 2	239	10	211	22
Sample 3	1046	8	989	10
Sample 4	1484	7	1330	14

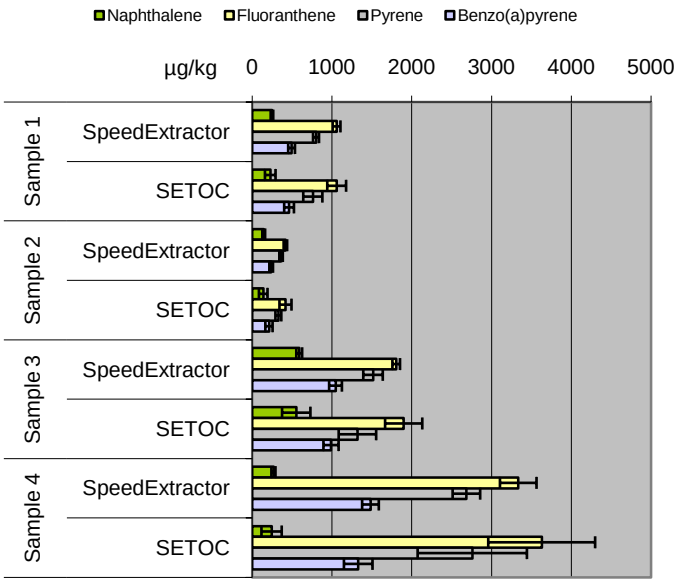


Figure 19: Graphical representation of the determination of a selection of PAH.

Conclusion

The extraction by SpeedExtractor E-916 represents a reliable and comparable procedure for the determination of PAH in sediments.

Acknowledgement

We greatly acknowledge Labor Veritas in Zurich, Switzerland, namely Peter Egli for the analytics and their support for the development of this application note.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

[2] SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 008/2009

2.1.2 Extraction of Sediment using the SpeedExtractor E-916 for the Determination of Polychlorinated Biphenyls (PCBs)

PCBs were used as coolants and dielectric fluid in transformers, as flame retardants, hydraulic fluids and additives in plastics and for many other applications until the 1980s. They are toxic, persistent and bioaccumulate in terrestial and aquatic biosystems and due to atmospheric effects they are ubiquitous in the environment. Sediment samples from a round robin program (SETOC 2008/4, Wepal, Wageningen University) were extracted according to EPA 3545A [1] with the Buchi SpeedExtractor E-916 and analyzed by GC-MS. The results correspond to the values found by the round robin testing and show high recovery and low variation.

Introduction

Poychlorinated Biphenyles (PCB) are a class of organig compounds whose biphenyl skeleton is substituted with 1-10 chlorine atoms. To simplify description they are usually specified with a number from 1 to 209.

Until the 1980s PCBs were used as coolants and dielectric fluid in transformers, as flame retardants, hydraulic fluids and additives in plastics and for many other applications. Today, production and use are banned nearly worldwide.

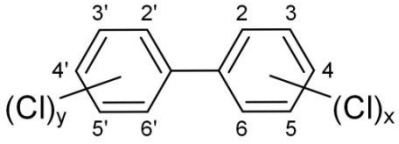


Figure 20: Chemical structure of the polychlorinated byphenyls (PCBs) skeleton.

Experimental

Instrumentation: SpeedExtractor E-916 with 20 ml cells, GC-MS Varian 3000. Samples: 3 different samples from the SETOC 2008/4 round robin, Wepal, Wageningen University. Approx. 3 g of dry sample was mixed with 10 g of sand and filled into the extraction cell. After addition of the internal standard solution the samples were extracted using the parameters shown in the adjacent table. A four-fold extraction was performed. The collected extracts were analyzed by GC-MS.

Temperature	100 °C
Pressure	120 bar
Solvent	Acetone 50%, hexane 50%
Cells	20 ml
Vials	240 ml
Cycles	2
Heat-up	1 min
Hold	10 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

Results

The results correspond to the values found by the round robin testing and show good recovery and low variation.

Table 4: Values for PCB in µg/kg, SpeedExtractor: n=4, SETOC round robin: n≥33, RSD in brackets

	Sample 1		Sample 2		Sample 3	
	E-916	SETOC	E-916	SETOC	E-916	SETOC
PCB 28	6.3 (16)	7.1 (12)	8.0 (24)	5.4 (17)	25.1 (25)	20.6 (27)
PCB 52	8.0 (14)	6.7 (35)	3.2 (40)	4.1 (21)	29.5 (8)	30.6 (23)
PCB 101	10.3 (29)	9.2 (21)	11.2 (27)	6.3 (23)	50.4 (13)	47.9 (19)
PCB 118	6.5 (5)	6.0 (15)	6..0 (26)	4.4 (17)	34.5 (7)	29.7 (30)

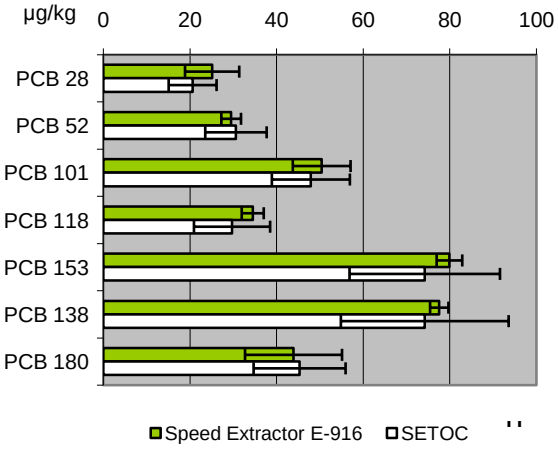


Figure 21: Values for sample 3 of SETOC 2008/4

PCB 153	12.1 (16)	12.6 (10)	12.6 (14)	9.6 (25)	79.9 (4)	74.2 (23)
PCB 138	10.7 (21)	10.9 (16)	9.5 (17)	8.4 (28)	77.6 (3)	74.2 (26)
PCB 180	5.1 (26)	6.0 (28)	6.2 (26)	4.9 (20)	43.9 (26)	45.3 (23)

Conclusion

Extraction of sediments with the SpeedExtractor E916 is a reliable and powerful method for the extraction of PCBs in sediment.

Acknowledgement

We greatly acknowledge Labor Veritas in Zurich, Switzerland, namely Peter Egli for the analytics and their support for the development of this application note.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

[2] SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 009/2009

2.1.3 Determination of PBDEs in Sediment Samples using the SpeedExtractor E-914

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in a myriad of applications and are monitored worldwide because of health concerns. In this application, PBDE congeners were extracted from two sediment samples (certified sample and sediment core from Lake Maggiore) according to EPA 3545A [1] using the SpeedExtractor E-914 and determined by GC-MS/GC-ECD. The results were comparable to the certified values and showed high reproducibility and recovery.

Introduction

PBDEs are brominated flame retardants used in a myriad of applications (**Figure 22**: Chemical structure of the PBDE). They are monitored worldwide because of health concerns. Bay and estuary sediments store toxic compounds and are therefore very appropriate sites to evaluate the contamination level of PBDEs in the environment.

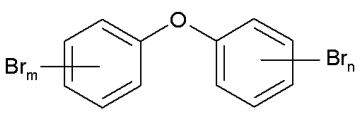


Figure 22: Chemical structure of the PBDE skeleton.

This application note describes the extraction of PBDEs from two sediment samples using the SpeedExtractor E-914: one certified sediment sample BROC-02 (RIVO, The Netherlands), one real sample from lake Maggiore, Italy. The PBDE congeners were determined by GC-MS/GC-ECD.

Experimental

Instrumentation: SpeedExtractor E-914 with 40 ml cells, Trace 2000 GC / PolarisQ ion-trap mass spectrometer (Thermo), Trace GC Ultra / ECD-40 detector (Thermo). Sample: Certified reference material (CRM) BROC-02 from the Research Institute for Fisheries (RIVO), The Netherlands, sediment sample collected from Pallanza Bay, Lake Maggiore, Italy. The four-fold extraction of both samples was performed using the parameters given in the adjacent sample. This extraction method requires an overall time of 26 min and consumes approx. 30 ml of solvent per position.

The extract was concentrated and cleaned on a multi-layer silica gel column. The determination of tri- to hepta-BDEs was performed by GC-MS. The congener BDE-209 was determined by GC-ECD.

Results

The mean values (n=4) obtained for BROC-02 are displayed in **Table 5**. They are comparable to the certified values. The calculated RSDs are generally below 20%. The recoveries of the ^{13}C-labeled internal standards fall in the range of 69-97% (data shown in Application Note 010/2009).

Table 5: Mean values (n=4) (ng/g) for eleven PBDE congeners

	Certified sample BROC-02		
	E-914		Cert. values
Compound	[ng/g]	RSD [%] (n=4)	[ng/g]
BDE-28/33	0.52	23	0.63
BDE-47	10.7	8	10.1
BDE-49	2.63	8	2.75
BDE-66	0.18	11	0.29
BDE-85	0.68	10	0.66
BDE-99	12.9	12	14.2
BDE-100	3.03	13	3.04
BDE-153	1.24	7	1.93
BDE-154	1.46	3	1.71
BDE-183	0.39	15	0.45
BDE-209	1’207	8	1’164

The mean concentrations (n=4) obtained for the sediment sample from Pallanza Bay are displayed in

Table 6. They range from 0.43 to 3.6 ng/g with RSDs below 15%.

Table 6: Mean values (n=4) (ng/g) for 4 PBDE congeners.

	Sediment sample from Pallanza bay	
Compound	Content [ng/g]	RSD % (n=4)
BDE-47	0.43	14
BDE-99	0.43	10
BDE-100	0.16	13
BDE-209	3.60	14

SpeedExtractor

Temperature	100 °C
Pressure	100 bar
Solvent	Hexane 75%, acetone 25%
Cells	40 ml
Vials	240 ml
Cycles	1
Heat-up	3 min
Hold	13 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

Conclusion

The SpeedExtractor E-914 delivered results with high accuracy, reproducibility and recovery. The data show that this procedure is perfectly suitable for the extraction of PBDEs in sediment.

Acknowledgement

We sincerely thank the CNR Water Research Institute in Italy for the analytics and development of this application note.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

[2] SpeedExtractor E-914 operation manual

For more detailed information refer to Application note 010/2009

2.1.4 Determination of Dioxins and Furans in Soil using the SpeedExtractor E-916

Polychlorinated dibenzo dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs) are persistent organic pollutants (POPs) banned by the Stockholm Convention and they are therefore monitored worldwide. PCDDs and PCDFs were extracted from a soil sample with the SpeedExtractor E-916 and an ASE® 200 system (Dionex) according to the U.S. EPA Method 3545A. Dioxin and furan congeners were determined by GC-HRMS. Data showed that the SpeedExtractor E-916 delivered extractions equivalent to those obtained with the ASE® 200.

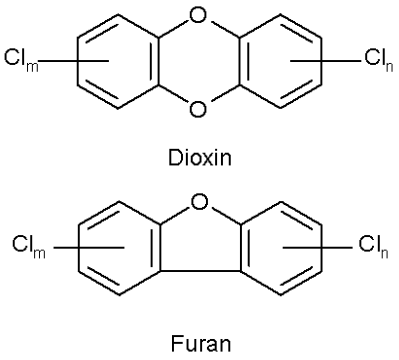


Figure 23: Chemical structure of the dioxin and furan skeleton.

Introduction

PCDDs and PCDFs are persistent organic pollutants (POPs). They are unwanted by-products of combustion released by chemical manufactures and municipal and industrial waste incinerators. Due to their toxicity they are monitored worldwide.

This application note describes the procedure used to extract PCDDs and PCDFs from a soil sample using the SpeedExtractor E-916 in accordance with the U.S. EPA Method 3545A [1].

Experimental

The soil sample was extracted using the SpeedExtractor and a commonly used ASE® method [2]. Instrumentation: SpeedExtractor E-916 with 10 ml cells, HP 5890 high resolution GC coupled with a MAT-90 Finnigan mass spectrometer (Thermo). Sample: Approx. 1 g of soil sample was mixed with sand and filled into the extraction cells. After addition of the 13C-labeled PCDD/PCDF internal standards a three-fold extraction was performed using the parameters given the adjacent table. The extracts were cleaned-up by flash chromatography on silica

Temperature	130 °C
Pressure	100 bar
Solvent	Toluene
Cells	10 ml
Vials	240 ml
Cycles	4
Heat-up	1 min
Hold	2 min
Discharge	2 min
Flush with solvent	2 min
Flush with gas	2 min

gel and alumina and analyzed by GC-HRMS. PCDD and PCDF concentrations were determined by the isotopic dilution method.

Results

The analytical results based on three extractions with the SpeedExtractor E-916 and two extractions with the ASE® 200 are displayed in **Table 7**. The data are comparable. Relative standard deviations (RSDs) ranged from 2 to 29%. The relative standard deviation of the WHO-TEQ was calculated to be 6%.

Table 7: Mean values in pg/g and RSDs for dioxins and furans

	SpeedExtractor E-916		ASE® 200	
	Content [pg/g]	RSD % (n=3)	Content [pg/g]	RSD % (n=3)
2378-TCDF	154	17	158	11
2378-TCDD	7	29	7	5
12378-PeCDF	504	2	439	6
23478-PeCDF	370	15	325	18
12378-PeCDD	47	4	51	5
123478-HxCDF	657	9	557	9
123678-HxCDF	311	14	265	15
234678-HxCDF	276	21	208	2
123789-HxCDF	190	10	201	1
123478-HxCDD	35	14	32	12
123678-HxCDD	58	5	54	3
123789-HxCDD	33	9	39	3
1234678-HpCDF	2807	11	2068	13
1234789-HpCDF	529	5	484	15
1234678-HpCDD	672	5	519	7
OCDF	2006	2	1191	23
OCDD	2003	10	1509	17
WHO-TEQ	476	6	424	4

Conclusion

The SpeedExtractor E-916 delivers extractions equivalent to those obtained with the ASE® 200 providing high recovery and high reproducibility.

Acknowledgement

We sincerely thank the Fraunhofer Institute for Process Engineering and Packaging IVV in Freising, Germany, namely Dr. L. Gruber, Dr. M. Schlummer and G. Wolz for the analytics and the development of this application note.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

[2] Cleres, S. et al., "Parallel Pressurized Solvent Extraction of PCDD/F, PBDE and PFC from Soil, Sludge and Sediment Samples" 29th International Symposium on Halogenated Persistent Organic Pollutants, 2009, Beijing, China.

[3] SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 012/2009

2.1.5 Determination of Perfluorinated Compounds (PFCs) in Soil and Sewage Sludge using the SpeedExtractor E-916

Perfluorinated Compounds (PFCs) are fluorocarbons used in many commercial and domestic applications. They are persistent pollutants and are therefore monitored worldwide. Twelve PFC congeners were extracted from soil and sewage sludge samples using the SpeedExtractor E-916 and an ASE® 200 system (Dionex). The PFC congeners were analyzed by HPLC-ESI-MS/MS. The data showed that the SpeedExtractor E-916 delivered extractions equivalent to those obtained with the ASE® 200.

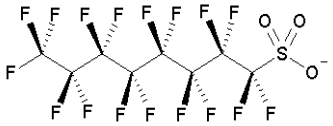


Figure 24: Chemical structure of PFOS.

Introduction

PFCs are fluorocarbons used in many commercial and domestic applications to make items stain-, oil- and water- resistant. They are very stable chemicals and accumulate in the environment. Due to health concerns they are monitored globally. Perfluorooctane sulfonate (PFOS) was recently banned by the Stockholm Convention [1].

This application note describes the extraction of PFCs from soil and sewage sludge samples using the SpeedExtractor E-916.

Experimental

The soil and sludge samples were extracted using the SpeedExtractor E-916 method described below and a commonly used ASE® 200 method [2]. Instrumentation: SpeedExtractor E-916 with 40 ml cells, HPLC Alliance 2695 (Waters) coupled with a Quattro-LC mass spectrometer (Waters). Samples: 2 soil samples and 1 sewage sludge sample provided by the Fraunhofer Institute IVV, Germany.

Approx. 1 g of soil or sewage sludge sample was mixed with silica gel and filled into the extraction cells. ^{13}C-labeled and ^{18}O-labeled internal standards were added to the mixture and extracted according to the parameters given in the adjacent table. The extracts were purified by SPE using Oasis WAX cartridges (Waters). The cleaned extracts were then subjected to HPLC-ESI-MS/MS analysis. Quantification was done by the isotope dilution method.

Temperature	80 °C
Pressure	100 bar
Solvent	Methanol
Cells	40 ml
Vials	240 ml
Cycles	4
Heat-up	1 min
Hold	5/20/20/20 min
Discharge	2 min
Flush with solvent	2 min
Flush with gas	3 min

Results

The analytical results based on two extractions with the SpeedExtractor E-916 and one extraction with the ASE® 200 are

displayed in **Table 8**. The results obtained with both instruments are very similar.

Table 8: Concentrations of PFCs in ppm; E-916 (n=2) and ASE (n=1)

Compound		**PFTeA**	**PFPeA**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**
Soil 1	E-916	<0.5	<0.5	<0.5	<0.5	2.8	0.2
	ASE®	<0.5	<0.5	<0.5	<0.5	1.7	0.2
Soil 2	E-916	<0.5	<0.5	<0.5	<0.5	2.1	<0.5
	ASE®	<0.5	<0.5	<0.5	<0.5	1.5	<0.5
Sludge	E-916	<0.5	<0.5	2.2	1.7	17.7	0.6
	ASE®	<0.5	<0.5	2.5	1.0	15.5	0.9
Compound		**PFDA**	**PFUnA**	**PFDoA**	**PFHxA**	**PFOS**	**PFDS**
Soil 1	E-916	0.2	1.2	<0.5	<0.5	0.4	0.5
	ASE®	0.2	<0.5	2.3	<0.5	0.4	<0.5
Soil 2	E-916	0.2	1.4	<0.5	<0.5	0.5	<0.5
	ASE®	0.2	<0.5	0.7	<0.5	0.6	0.2
Sludge	E-916	8.0	3.2	9.5	<0.5	1065	29.3
	ASE®	7.4	2.6	4.8	<0.5	1043	24.8

Conclusion

Twelve PFC congeners were extracted from soil and sewage sludge samples with the SpeedExtractor E-916 and an ASE® 200 system. The results show that the SpeedExtractor delivered extractions equivalent to those obtained with the ASE® 200.

Acknowledgement

We sincerely thank the Fraunhofer Institute for Process Engineering and Packaging IVV in Freising, Germany, namely Dr. L. Gruber, Dr. M. Schlummer and G. Wolz for the analytics and the development of this application note.

References

[1] Stockholm Convention on Persistent Organic Pollutants, COP4, 4-9 May 2009

[2] Cleres, S.; Gruber, L.; Lastennet, G.; Schlummer, M.; Wolz, G. “Parallel Pressurized Solvent Extraction of PCDD/F, PBDE and PFC from Soil, Sludge and Sediment Samples” 29th International Symposium on Halogenated Persistent Organic Pollutants, Beijing, China, Dioxin 2009.

[3] SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 013/2009

2.2 Trace analysis in foodstuff

In preparation.

2.3 Fat determination in foodstuff

Fat determination is traditionally performed with Soxhlet or hot extraction. The following sections describe how hydrolyzed or non-hydrolyzed samples can be extracted with the SpeedExtractor to determine the fat content.

2.3.1 Fat Determination in Shortbread using SpeedExtractor E-916

The determination of fat in food and feed is a routine procedure for quality assurance and labeling. Fat was determined in butter shortbread after homogenization of the sample and extraction with the SpeedExtractor E-916. The total fat content was determined gravimetrically after the extract has been dried to a constant weight. The fat content of 10.21% corresponds to the value determined with classic Soxhlet.

Figure 25: Shortbread

Introduction

The determination of fat in food and feed is a routine procedure for in quality assurance and labeling. Fat was determined in butter shortbread after homogenization of the sample and extraction with the SpeedExtractor E-916. The solvent was evaporated in parallel using the Multivapor P-6. The total fat content was determined gravimetrically after the extract has been dried to a constant weight.

Experimental

Instrumentation**:** Mixer B-400, SpeedExtractor E-916, Multivapor P-6 with Vacuum pump V-700 and Controller V-855, drying oven. The homogenized sample was mixed with quartz sand and extracted with the SpeedExtractor using the parameters shown in the adjacent table. The sample was extracted in triplicate.

Temperature	100 °C
Pressure	100 bar
Solvent	Hexane 100%
Cells	40 ml
Vials	240 ml
Cycles	3
Heat-up	1 min
Hold	5 min
Discharge	4 / 3 / 3 min (1^{st}/2^{nd} /3^{rd} cycle)
Flush with solvent	2 min
Flush with gas	4 min

The total time for the extraction is approx. 60 min and per position, approx. 50 ml solvent are used. The solvent was evaporated in parallel using the Multivapor P-6 (see **Figure 26**). The extracts were then dried to a constant weight in a drying oven (102 °C) and the fat content was calculated.

Results

The determined fat content (**Table 9**) of 10.20% corresponds to the content obtained when extracting the same sample with Soxhlet extraction. With a fat content of 10.20% (rsd=0.42%, n=3) was determined.

Table 9: Determined fat contents in short bread

Sample	Fat content [%]
Sample 1	10.25
Sample 2	10.14
Sample 3	10.24
Mean value	**10.21**
rsd %	0.60

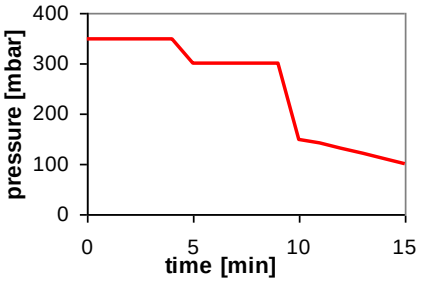


Figure 26: Pressure gradient for evaporating the solvent in the Multivapor P-6

References

SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 004/2009

2.3.2 Fat Determination in Food and Feed Products using Speed-Extractor E-916 after Acid Hydrolysis

The determination of fat in food and feed is a routine procedure in quality assurance and labeling. A simple and fast procedure for fat determination in various food and feed matrices is introduced below. The sample is hydrolyzed with the Hydrolysis unit E-416, followed by an extraction with the SpeedExtractor E-916. The total fat content in determined gravimetrically after the extract has been dried to a constant weight. The fat contents correspond to the values determined by Weibull-Stoldt.

Figure 27: The samples milk chocolate, cat feed and processed meat samples

Figure 28: Tortellini

Introduction

Fat determination is one of the key analyses performed in the food industry. Complex matrices such as food typically require acid hydrolysis prior to solvent extraction, to free bound lipids and to facilitate the extraction. An easy and reliable procedure to determine the fat content in different food and feed matrices is presented below.

Experimental

Instrumentation**:** Hydrolysis Unit E-416, SpeedExtractor E-916, Multivapor P-6, drying oven. Samples**:** Milk chocolate, cat feed (dry pellets), processed meat, and meat stuffed tortellini.

The homogenized samples were hydrolyzed with 4 mol/l HCl for 30 min, filtered, washed to a neutral pH, and dried before they were carefully transferred to the extraction cells and extracted using the parameters shown in the adjacent table. The samples were extracted six-fold. The solvent was evaporated in parallel using the Multivapor P-6. The extracts were then dried to a constant weight in a drying oven (102 °C) and the fat content was calculated.

The fat content of the samples was also determined by Weibull-Stoldt (includes acid hydrolysis and Soxhlet extraction), see corresponding Application Note [1]).

Temperature	100 °C
Pressure	100 bar
Solvent	Petroleum ether 100%
Cells	40 ml
Vials	240 ml
Cycles	2
Heat-up	1 min
Hold	5 min
Discharge	3 min
Flush with solvent	3 min
Flush with gas	5 min

Results

The determined fat contents are presented in **Table 10**. They correspond to the values determined with the classic method (Weibull-Stoldt) and have low relative standard deviations.

Table 10: Determined fat contents in food and feed samples, fat in g/100 g, (relative standard deviation in brackets)

Sample	SpeedExtractor E-916 (n=6)	Weibull-Stoldt (n=3)
Milk chocolate	34.92 (0.40%)	34.92 (0.07%)
Cat feed	12.80 (0.29%)	11.02 (0.23%)
Processed meat	20.49 (0.57%)	20.31 (0.13%)
Tortellini	5.88 (0.61%)	5.75 (0.80%)

Conclusion

The determination of the fat contents in hydrolyzed food and feed samples by solvent extraction using SpeedExtractor E-916 provides reliable and reproducible results that correspond to those obtained by Soxhlet. The total extraction time is approx. 45 min; per position approx. 60 ml solvent are used.

References

[1] Application note E-416-E-816-Sox-001: Fat determination according to Weibull-Stoldt - Standard application.

[2] SpeedExtractor E-916 operation manual

[3] Hydrolysis unit E-416 operation manual

For more details see Application note 007/2009

2.3.3 Fat Determination in Food and Feed Products using Speed-Extractor E-914 after Acid Hydrolysis

The determination of fat in food and feed is a routine procedure in quality assurance and labeling. A simple and fast procedure for fat determination in various food and feed matrices is introduced below. The sample is hydrolyzed with the Hydrolysis Unit E-416, followed by an extraction with the SpeedExtractor E-914. The total fat content is determined gravimetrically after the extract has been dried to a constant weight. The fat contents correspond to the values determined by Weibull-Stoldt.

Figure 29: The samples milk chocolate, cat feed and processed meat samples

Introduction

Fat determination is one of the key analyses performed in the food industry. Complex matrices such as food typically require acid hydrolysis prior to solvent extraction, to free bound lipids and to facilitate the extraction. An easy and reliable procedure to determine the fat content in different food and feed matrices is presented below.

Experimental

Instrumentation**:** Hydrolysis unit E-416, SpeedExtractor E-914, Multivapor P-6, drying oven. Samples**:** Milk chocolate, cat feed (dry pellets) and processed meat.

The homogenized samples were hydrolyzed with 4 N HCl for 30 min, filtered, washed to a neutral pH, and dried before they were carefully transferred to the extraction cells and extracted using the parameters shown in the adjacent table. The samples were extracted fourfold. The solvent was evaporated in parallel using the Multivapor P-6. The extracts were then dried to a constant weight in a drying oven (102 °C) and the fat content was calculated.

Temperature	100 °C
Pressure	100 bar
Solvent	Petroleum ether 100%
Cells	80 ml
Vials	240 ml
Cycles	2
Heat-up	5 / 1 min (1^{st}/2^{nd} cycle)
Hold	5 min
Discharge	4 / 3 min (1^{st}/2^{nd} cycle)
Flush with solvent	3 min
Flush with gas	5 min

The fat contents of the samples were also determined by Weibull-Stoldt (includes acid hydrolysis and Soxhlet extraction), see corresponding Application note.

Results

The determined fat contents are presented in **Table 11**. They are in accordance to the values determined with the classic method (Weibull-Stoldt) and have low relative standard deviations.

Table 11: Determined fat contents in food and feed samples, fat in g/100g, (relative standard deviation in brackets)

Sample	SpeedExtractor E-916 (n=6)	Weibull-Stoldt (n=3)
Milk chocolate	34.92 (0.40%)	34.92 (0.07%)
Cat feed	12.80 (0.29%)	11.02 (0.23%)
Processed meat	20.49 (0.57%)	20.31 (0.13%)
Tortellini	5.88 (0.61%)	5.75 (0.80%)

Conclusion

The determination of the fat contents in hydrolyzed food and feed samples by solvent extraction using SpeedExtractor E-916 provides reliable and reproducible results that correspond to those obtained by Soxhlet. The total extraction time is approx. 45 min; approx. 100 ml solvent are used per position.

References

[1] SpeedExtractor E-914 operation manual

[2] Hydrolysis Unit E-416 operation manual

[3] Application Note E-416-E-816-Sox-001: Fat determination according to Weibull-Stoldt - Standard application.

For more details see Application note 006/2009

2.4 Plastics and their additives

Classic extraction of polymers requires very long extraction times (12 – 24 hrs.) and large amounts of solvent. The SpeedExtractor is the ideal solution for the quality control of batch processes, as the extracts for an entire batch are available in a very short time. The method is particularly suitable for the extraction of softeners or additives, and also for the characterisation of polymer structures (branching ratios).

2.4.1 Determination of Polychlorinated Biphenyls (PCBs) and Phthalates in Waste Polymer Samples using the SpeedExtractor E-916

Polychlorinated biphenyls (PCBs) and phthalates are polymer additives present in a myriad of industrial and domestic applications. Due to health concerns PCB and phthalate levels are monitored worldwide. PCBs and phthalates were extracted from two waste polymer samples intended for mechanical recycling using the SpeedExtractor E-916 and an ASE® system. The PCB and phthalate congeners were determined by GC-MS. Data show that the SpeedExtractor E-916 delivered extractions equivalent to those obtained with the ASE®.

Introduction

PCBs and phthalates are polymer additives present in a myriad of industrial and commercial applications. Because of health concerns PCBs have been phased out of production in many parts of the world and the use of phthalates has been restricted. Today these chemicals are monitored worldwide.

This application note describes the extraction of PCBs and phthalates from two waste polymer samples intended for mechanical recycling using the SpeedExtractor E-916. This application meets requirements of U.S. EPA Method 3545A [1].

Experimental

Two polymer samples were extracted using the SpeedExtractor and a commonly used ASE® method [2]. Instrumentation: SpeedExtractor E-916 with 20 ml cells, GC-MS QP 5000 (Shimadzu) for PCBs, GC-MS TSQ 7000 (Thermo) for phthalates, Ultra Centrifugal Mill (Retsch). Sample: Two waste polymer samples sieved to < 0.7 mm

Approx. 0.6 g of ground polymer was mixed with pumice stone and filled into the extraction cell. After addition of ^{13}C- labeled internal PCB standards and 2 deuterated internal phthalate standards the samples were extracted in duplicate with the SpeedExtractor E-916 using the parameters given in the adjacent table. The extraction process required 45 min and approx. 40 ml of solvent were consumed per position. The extracts were cleaned up on a chromatography column containing acid and basic modified silica. PCBs and phthalates were determined by GC-MS.

Temperature	80 °C
Pressure	100 bar
Solvent	Hexane 90%, 2-propanol 10%
Cells	20 ml
Vials	240 ml
Cycles	3
Heat-up	1 min
Hold	5 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

Results

The mean concentrations of 7 PCB and 3 phthalate congeners are displayed in **Table 12** and **Table 13**. The data obtained by SpeedExtractor E-916 and ASE® are comparable.

Table 12: Concentrations (ppb) and RSDs (%, in brackets) for PCBs, (n=2)

PCBs	Sample 1 [ppb] (RSD)		Sample 2 [ppb] (RSD)	
	E-916	ASE®	E-916	ASE®
2,4,4'-TriCB	6'192 (3)	5'436 (3)	1'214 (1)	1'051 (1)
2,2',5,5'-TetraCB	1'172 (1)	1'063 (1)	241 (2)	230 (2)
2,2',4,5,5'-PentaCB	222 (1)	217 (4)	110 (1)	115 (3)
2,2',3,4,4',5'-HexaCB	98.0 (1)	112.8(19)	90.3 2()	95.6 (4)
2,2',4,4',5,5'-HexaCB	79.4 (6)	95.2 (22)	82.7 (10)	85.3 (3)
2,2',3,4,4',5,5'-HeptaCB	18.5 (1)	21.7 (34)	21.1 ()5	19.7 (4)
DecaCB	0.5 (120)	0.4 (27)	0.6 (16)	0.5 (20)

Table 13: Concentrations (ppm) and RSDs (%) for phthalates, (n=2)

Phthalates	Sample 1 [ppb] (RSD)		Sample 2 [ppb] (RSD)	
	E-916	ASE®	E-916	ASE®
DiBP Diisobutylphthalate	8.56 (22)	7.84 (5)	21.4 (1)	22.1 (1)
DBP Dibutylphthalate	5.72 (10)	5.82 (7)	22.7 (3)	21.4 (4)
DEHP Diethylhexylphthalate	1'257 (1)	1'272(20)	1'278 (13)	1'180 (4)

Conclusions

The results show that the SpeedExtractor E-916 delivers extractions equivalent to those obtained with the ASE® system and demonstrate that this extraction procedure is perfectly suitable for the extraction of PCBs and phthalates in polymer samples.

Acknowledgement

We sincerely thank the Fraunhofer Institute for Process Engineering and Packaging IVV in Freising, Germany, namely Dr. L. Gruber, Dr. M. Schlummer and G. Wolz for the analytics and the development of this application note.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

[2] Cleres, S. et al., "Determination of PCBs and Phthalates in Waste Polymer Samples Intended for Mechanical Recycling" 29th International Symposium on Halogenated Persistent Organic Pollutants, 2009, Beijing, China.

[3] SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 011/2009

2.5 Pharmaceuticals and natural products

The wide pressure and temperature range and the ability to make up solvent mixtures as required are of great advantage, especially for the identification of actives in pharmaceutical products or for natural product extraction, since the extraction can be performed gently and quickly. Typical applications are quality control of actives in natural products or pharmaceutical products or research on new actives in plant materials.

2.5.1 Extraction of Edelweiss (*Leontopodium alpinum*) using the Speed-Extractor E-916 for the Determination of Total Polyphenol Content

Edelweiss (Leontopodium alpinum) grows in alpine areas and is also cultivated for its valuable extract. Ground Edelweiss was extracted with the SpeedExtractor E-916 using an alcohol-water mixture and the total polyphenol content was determined photometrically using the Folin-Ciocalteu method. The determined total polyphenol content, expressed as gallic acid, was 54.8 mg/g which corresponds to the values reported in literature [2].

Figure 30: Edelweiss (*Leontopodium alpinum*)

Introduction

Edelweiss (*Leontopodium alpinum*) grows in alpine areas between 1800 and 3000 meters above sea level. It is also cultivated for their valuable extract, rich in polyphenols and antioxidizing agents. The extract is used in cosmetics, facial creams and sun screen.

The sum parameter of total polyphenol content is commonly used in plant analysis to quantify the power of the antioxidizing effect. An efficient extraction method to determine the total polyphenol content in Edelweiss using the SpeedExtractor E-916 is presented below.

Experimental

Instrumentation**:** SpeedExtractor E-916, ultra centrifugal mill, microplate reader. The dried and ground blossoms (<1 mm) were mixed with diatomaceous earth and extracted with the SpeedExtractor E-916 using the parameters shown in the adjacent table. The sample was extracted in triplicate.

The polyphenolic compounds in the diluted extracts were determined photometrically according to the Folin-Ciocalteu procedure [1], using gallic acid as standard substance. The absorption is measured at 750 nm, and each extract was analysed twice.

Temperature	50 °C
Pressure	100 bar
Solvent	Water 60%, ethanol 40%
Cells	40 ml
Vials	240 ml
Cycles	3
Heat-up	1 min
Hold	9 min
Discharge	5 min
Flush with solvent	3 min
Flush with gas	5 min

Results

The results (**Table 14**) correspond to the values found in literature from 50 up to 60 mg/g [2].

Table 14: Determined total polyphenol content expressed as content of gallic acid (n=2)

Sample	Gallic acid [mg/g]
Sample 1	56.9
Sample 2	54.1
Sample 3	53.4
Mean value	**54.8**
rsd %	3.44

Conclusion

The extraction of Edelweiss using SpeedExtractor E-916 for the determination of the total polyphenol content represents a powerful tool for the study of plant materials. The results are in correspondence with literature. The short total extraction time of approx. 1 h 10 min and the small solvent volume used of approx. 60 ml are further benefits of this procedure.

Acknowledgement

We greatly acknowledge the University of Applied Science of Western Switzerland in Sion/Valais namely Dr. Wilfried Andlauer and Julien Héritier for the analytics and their support for the development of this application note.

References

[1] Folin, O.; Ciocalteu, J. (1927) J. biol. Chem. 73, 627

[2] Rey, Ch.; Slacanin, I. (1999) Approche culturale et phytochimique de l'edelweiss. Revue Suisse Vitic. Arboric, Hortic. 31 (2): 89-96

[3] SpeedExtractor E-916 operation manual

For more detailed information refer to Application Note 002/2009

2.5.2 Extraction of Genépi (*Artemisia umbelliformis*) using the Speed-Extractor E-916 for the Determination of Total Polyphenol Content

The Genépi plant (Artemisia umbelliformis) grows in alpine areas. It is cultivated and used to produce a herb-liquor. Ground Genépi was extracted with the SpeedExtractor E-916 using an alcohol-water mixture and the total polyphenol content was determined photometrically using the Folin-Ciocalteu method. The determined total polyphenol content, expressed as gallic acid, was 38.8 mg/g.

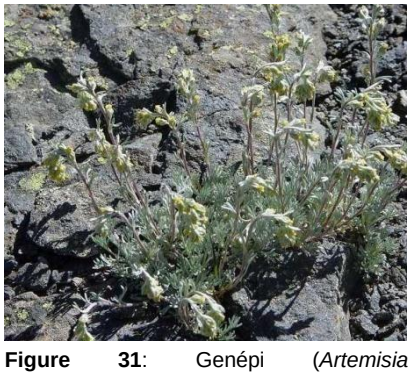


Figure 31: Genépi (*Artemisia umbelliformis*)

Introduction

The Genépi plant (*Artemisia umbelliformis*) grows in alpine areas between 2000 and 3700 meters above sea level. The plant is cultivated

and used to produce a herb liquor, a local specialty in the region of the Valais Alps of Switzerland, Northern Italy and Western France.

The sum parameter of total polyphenol content is commonly used in plant analysis to quantify the power of the antioxidant effect. An efficient extraction method to determine the total polyphenol content in the Genépi plant using the SpeedExtractor E-916 is presented below.

Experimental

Instrumentation**:** SpeedExtractor E-916, ultra centrifugal mill, microplate reader. The dried and ground plants (< 1 mm) were mixed with diatomaceous earth and extracted with the SpeedExtractor E-916 using the parameters shown in the adjacent table. The sample was extracted in triplicate.

Temperature	50 °C
Pressure	100 bar
Solvent	Water 60%, ethanol 40%
Cells	40 ml
Vials	240 ml
Cycles	3
Heat-up	1 min
Hold	9 min
Discharge	5 min
Flush with solvent	3 min
Flush with gas	5 min

The polyphenolic compounds in the diluted extracts were determined photometrically according to the Folin-Ciocalteu procedure [1], using gallic acid as standard substance. The absorption is measured at 750 nm, and each extract was analysed in duplicate.

Results

The results (**Table 15**) correspond to the values determined at the University of Applied Science in Sion/Valais using their established method (37.2 mg/g) [2].

Table 15: Determined total polyphenol content expressed as content of gallic acid (n=2)

Sample	Gallic acid [mg/g]
Sample 1	39.0
Sample 2	39.7
Sample 3	37.7
Mean value	**38.8**
rsd %	2.60

Conclusion

The extraction of Genépi using SpeedExtractor E-916 for the determination of the total polyphenol content represents a powerful tool for the study of plant materials. The short total extraction time of approx. 1 h 10 min and the small solvent volume used of approx. 60 ml are further benefits of this procedure.

Acknowledment

We greatly acknowledge the University of Applied Science of Western Switzerland in Sion/Valais namely Dr. Wilfried Andlauer and Julien Héritier for the analytics and their support for the development of this application note.

References

[1] Folin, O.; Ciocalteu, J. (1927) J. Biol. Chem. 73, 627

[2] Simmonet, X. et al. (2006) Stade phénologique et qualité des hampes florales du genépi blanc. Revue Suisse Vitic. Arboric. Hortic. 38(3) 189-193

[3] SpeedExtractor E-916 operation manual

For more detailed information refer to Application Note 003/2009

2.5.3 Extraction of Genépi (*Artemisia umbelliformis*) using the SpeedExtractor E-916 for the Quantification of Costunolide

The Genépi plant (Artemisia umbelliformis) grows in alpine areas. It is also cultivated and used to produce a herb liquor. Costunolide is a sesquiterpene lactone and the main compound responsible for the bitterness of the Genépi plant. Ground Genépi was extracted with the SpeedExtractor E-916 using an alcohol-water mixture and subsequently analyzed on HPLC. The determined costunolide content in the samples was 9.52 mg/g and corresponds to the values reported in literature [2], [3].

Figure 32: Genépi (*A. umbelliformis)*

Introduction

The Genépi plant (*Artemisia umbelliformis*) grows in alpine areas between 2000 and 3700 meters above sea level. The plant is cultivated and used to produce a herb liquor, a local specialty in the region of the Valais Alps of Switzerland, Northern Italy and Western France.

Costunolide is a sesquiterpene lactone and the main compound responsible for the bitterness of the Genépi plant and much desired for its distinguished taste. Costunolide has also been reported to have an anti-cancer effect [1] and as a remedy for stomach cramps and loss of appetite [2]. The amount of costunolide found in Genépi varies based on the time of harvest [3].

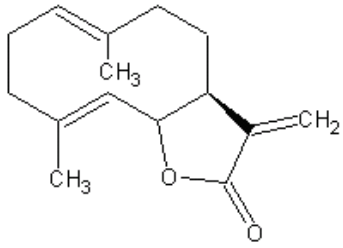


Figure 33: Chemical structure of costunolide.

Experimental

Instrumentation**:** SpeedExtractor E-916, ultra centrifugal mill, HPLC system with UV detector (210nm).

The dried and ground plants (<1 mm) were mixed with diatomaceous earth and extracted with the SpeedExtractor E-916 using the parameters shown in the adjacent table. The sample was extracted in triplicate.

Costunolide was quantified by HPLC (equipped with UV detector at 210 nm), using a calibration based on an external standard.

Temperature	50 °C
Pressure	100 bar
Solvent	Water 60%, ethanol 40%
Cells	40 ml
Vials	240 ml
Cycles	3
Heat-up	1 min
Hold	9 min
Discharge	5 min
Flush with solvent	3 min
Flush with gas	5 min

Results

The results (see **Table 16**) correspond to the values reported in literature from 5.6 mg/g up to 29.1 mg/g [2], [3]. The value of sample two is not an outlier according to the Grubbs test (significance 95%).

Table 16: Determined amount of costunolide

Sample	Costunolide [mg/g]
Sample 1	9.80
Sample 2	8.85
Sample 3	9.91
Mean value	**9.52**
rsd %	6.10

Conclusion

The extraction of Genépi plants using Speed Extractor E-916 represents a very powerful method to prepare extracts for the quantification of important plant compounds such as costunolide. The values determined are in correspondence with literature. The short total extraction time of approx. 1 h 10 min and the small solvent volume used of approx. 60 ml are further benefits of this procedure.

Acknowledgment

We acknowledge the University of Applied Science of Western Switzerland in Sion/Valais namely Dr. Wilfried Andlauer and Julien Héritier, for the analytics and their support inr the development of this application note.

References

[1] Choi, S. et al (2005) Inhibitory effects of costunolide on the telomerase activity in human breast carcinoma cells. Cancer Lett. 227(2), 153-162.

[2] Rey, Ch.; Slacanin, I. (1997) Domestication du genépi blanc. Revue Suisse Vitic. Arboric. Hortic. Vol.29 (3) I-VIII.

[3] Simmonet, X. et al. (2006) Stade phénologique et qualité des hampes florales du genépi blanc. Revue Suisse Vitic. Arboric. Hortic. 38(3) 189-193.

[4] SpeedExtractor E-916 operation manual

For more detailed information, refer to Application note 004/2009

2.5.4 Pre-Extraction and Extraction of Hypericin in St. John's Wort (*Hypericum perforatum*) using the SpeedExtractor E-916

St. John's Wort herb and capsules with dry extract are widely used in herbal medicine. For quality control reason the amount of hypericin in the products is determined by extraction and photometric quantification. The quantification by photometry is interfered by co-extracted chlorophyll. Classically, chlorophyll is removed by a time-consuming manual procedure. A convenient removal of chlorophyll followed by quantitative extraction of hypericin is presented, based on pressurized solvent extraction using the SpeedExtractor E-916.

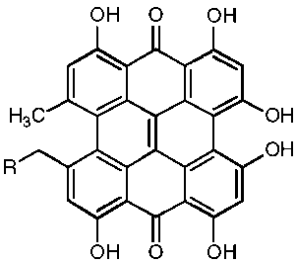


Figure 34: Hypericin: R=H, Pseudohypericin: R=OH

Introduction

In herbal medicine St. John's Wort herb and capsules with dry extract are widely used for the treatment of depressions. In this products hypericin is determined for quality reasons. The determination of the total amount of hypericin can be done by extraction and photometric quantification at 590 nm. The quantification by photometry is interfered by co-extracted chlorophyll. Removal of the interfering chlorophyll was achieved by a pre-extraction with dichloromethane. The remainings were then extracted with methanol to quantify hypericin.

Table 17: Extraction parameters for the two consecutive extraction methods.

	Pre-extraction	Main-extraction
Temperature	80 °C	80 °C
Pressure	100 bar	100 bar
Solvent	Dichloromethane	Methanol
Cells	10 ml	10 ml
Vials	240 ml	240 ml
Cycles	2	2
Heat-up	1 min	1 min
Hold	4 min	2 min
Discharge	2 min	2 min
Flush with solvent	5 min	5 min
Flush with gas	4 min	4 min

Experimental

Instrumentation: SpeedExtractor E-916, Photometer: Thermo Helios, Ultrazentrifugal mill: Retsch, ZM 200 with distance sieve 0.5 mm. Samples: Dried and cut St. John's Wort herb and capsules containing 425 mg dry extract of St. John's Wort. Approx. 0.6 g of the ground herb or approx. 0.05 g of the dry extract was mixed with quartz sand and extracted in two consecutive extractions with the SpeedExtractor using the parameters shown in **Table 17**. The samples were extracted in triplicate.

After completing to 200 ml, photometric quantification at 590 nm was done. Absorption coefficient: $E_{cm}^{g/100ml} = 870$

Results

By performing a pre-extraction with dichloromethane the chlorophyll can be efficiently removed from the samples without affecting the hypericin content. Only a negligible amount of chlorophyll remains, with insignificant interference with hypericin at 590 nm (**Figure 35** and **Figure 36**). Found concentrations in herb and capsules correspond with the declared values (**Table 18**).

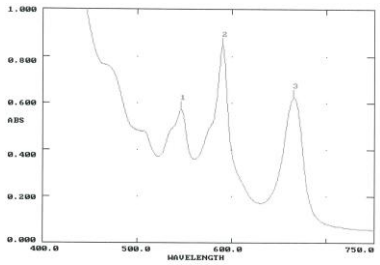


Figure 35: Spectrum without pre-extraction: 2 = hypericin, 3 = chlorophyll

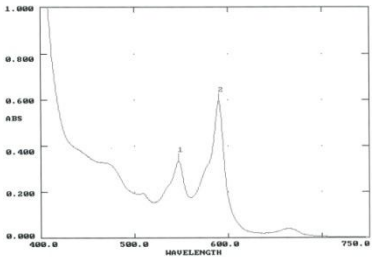


Figure 36: Spectrum with pre-extraction; 2 = hypericin

Table 18: Determined content of hypericin, n=3

Sample	Hypericin [rsd]	Declared value by the supplier
Herb	0.8 mg/g [1.6%]	Min. 0.8 m
Capsules	1.18 mg/capsule [1.9%]	Min. 0.75 mg/capsule

Conclusion

Application of two consecutive extractions is a fast and reliable way for the determination of total hypericin in St. John's Wort herb and capsules.

References

[1] PhEur 6.2, 07/2008:1438, Hyperici her.

[4] SpeedExtractor E-916 operation manual

For more detailed information, refer to Application note 015/2009