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Extraction Application Book

New extraction application methods

Dear Milestone Friend,

During the last few years we have clearly seen a growing interest for microwave solvent extraction.

Official methods, such as the US EPA 3546 ASTM D 5765 and the ASTM D6010 are now available, and this will certainly contribute to enhance the market opportunities for us.

For this reason, we have developed a **new library of extraction application methods.**

These methods will serve you as a **guide to focus onto the right market segments**, namely environmental, polymer, API (Active pharma ingredients) and food.

This is where you should mainly concentrate your efforts.

The new microwave extraction applications methods can be downloaded from the Milestone Intranet. Each method defines a precise working procedure, along with the analytical results achieved, and furthermore specifies a proper system configuration, which typically includes START E or ETHOS 1, fiber optic temperature control, magnetic stirring and the appropriate rotor.

The same could then be proposed to your customer as a 'Problem Solution' to their needs in solvent extraction.

We hope this information will be very fruitful for all of us!

Milestone Srl

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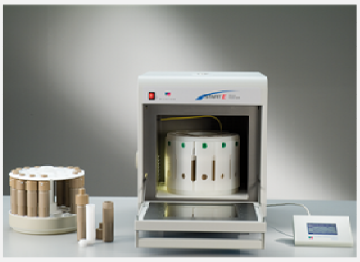
Environmental samples

Polymer samples

Active compounds

Food Samples

The following application notes are suitable <u>only</u> for the configuration described in each notes.



Application Method ME001

EPA 3546: Microwave Extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, phenoxyacid herbicides, substituted phenols, PCBs and PCDDs/PCDFs.

Introduction

EPA 3546 is intended to be guidance method which contains general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

Scope and Application

This method is a procedure for extracting water insoluble or slightly water soluble organic compounds from soil, clays, sediments, sludges and solid waste. This method was developed and validated on commercially-available solvent extraction system. Its procedure uses microwave energy to produce elevated temperature and pressure conditions in a close vessel containing the sample and organic solvent(s) to achieve analyte recoveries equivalent to those from Soxhlet procedure, using less solvent and taking significantly less time than the Soxhlet procedure.

This method is applicable to the extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, phenoxyacid herbicides, substituted phenols, PCBs and PCDD/PCDF, which may then be analyzed by a variety of chromatographic procedures. This method has been validated for solid matrices containing from 50 to 10000 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 10 to 5000 µg/kg organochlorine pesticides and chlorinated herbicides, 50 to 2500 µg/kg of substituent phenols, 100 to 5000 µg/kg of phenoxyacid herbicides, 1 to 5000 µg/kg of PCBs and 10 to 6000 ng/kg of PCDDs/PCDFs. This method may be applicabile to samples containing these analyte at higher concentrations and may be employed after adequate performance is demonstrated for the concentration of interest.

This method is only applicable to solid samples with small particle sizes. If practical, soil/sediment sample may be air-dried and ground to a fine powder prior to extraction. Alternatively, if worker safety or the loss of analytes during drying is a concern, soil/sediment samples may be mixed with anhydrous sodium sulphate or pelletized diatomaceous earth. (Drying and grinding samples containing PCDDs/PCDFs is not recommended, due to safety concerns). The total mass of material to be prepared depends on the specifications of the determinative method and the sensitivity needed for the analysis but an amount of 1-20 g of material is usually necessary and can be accommodated by this extraction procedure.

This method has been validated using a solvent mixture of Acetone/Hexane (1:1 v/v) from matrices such as soil, glass-fibers and sand. This solvent system or other solvent systems may be employed, provided that adequate performance is demonstrated for the analytes of interest.

Instrumentation

Microwave solvent extraction apparatus

The temperature performance specifications necessitate that the microwave extraction system be capable of sensing the temperature to within ± 2,5°C and automatically adjusting the microwave field output power within 2 sec of sensing. Temperature sensors should be accurate to ± 2°C. Temperature feedback control provides the primary performance mechanism for this method.

Microwave extraction vessel are needed. Vessels are available that can accommodate from 1 g to 20 g of sample. Vessels should be transparent to microwave energy, relatively inert to reagents and sample components and capable of withstanding 200°C and 200 psi.

Samples

Up to 20 g of soil, clays, sediments, sludges and solid waste.

Extraction solvent

30 ml Acetone / Hexane (1:1 v/v).

Reagent grade chemicals must be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

Samples should be extracted using a solvent system that gives optimum, reproducible recovery of the analytes of interest from the sample matrix, at the concentration of interest. The choice of extraction solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups.

Procedure

The sample preparation steps vary with the type of sample to be extracted. Where practical, samples should be air-dried and ground to a fine powder before extraction. However, where such steps are not practical because of concerns about loss of the analytes or potential contamination of the laboratory from high concentration samples, samples may be mixed with a drying agent such as sodium sulphate or pelletized diatomaceous earth prior to extraction.

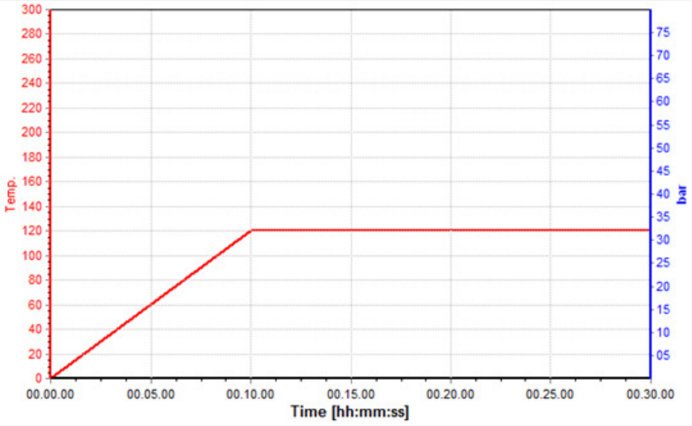
1. Samples are prepared for extraction by grinding them to a powder and loading them into the extraction vessel. If the sample is wet, mix the sample with an equal volume of anhydrous sodium sulphate or pelletized diatomaceous earth until a free-flowing powder is obtained. *Note*: The air-drying and grinding of samples containing PCDDs/PCDFs is not recommended due to safety concerns. Grinding may also be a concern for other more volatile analytes.
2. Add 30 ml of the solvent mixture into the extraction vessel and close it.
3. Heat the vessel to the extraction temperature in according to the microwave program and extracted for at least 10 min.
4. Cool the extraction mixture to room temperature.
5. Open the vessel and filter the content rinsing the solid material with the mixture of solvents used for the extraction.
6. If required, clean up the extract before performing analyte determination.

Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	10	Up to 1000*	120
2	20	Up to 1000*	120

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Comments

Rapid heating in combination with temperatures in excess of the atmospheric boiling point of organic solvents, reduce sample extraction times. Small amounts of solvents (30 ml) are used resulting in reduced sample preparation cost and time.

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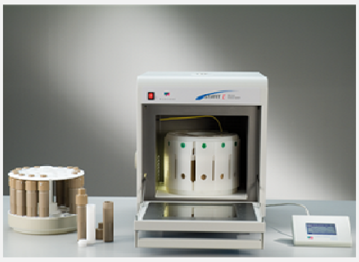


Application Method ME002

ASTM D-6010: Close vessel microwave solvent extraction of organic compounds from solid matrices.

Introduction

This practice describes the closed vessel microwave extraction of soils, sediments, sludges, and wastes for subquent determination of solvent extractable semivolatile and non-volatile organic compounds by such techniques as gas chromatography and gas chromatography-mass spectrometry. The detection limit and linear concentration range for each compound is dependent on the gas chromatograph or gas chromatograph-mass spectrometer technique employed.

Instrumentation

Microwave heating system: a laboratory microwave heating system capable of delivering a minimum of 900 W of microwave energy. The system should be capable of 1% power adjustments and 1-s time adjustments. The microwave unit must be capable of measuring and controlling solvent temperature within an extraction vessel. The microwave cavity should be constructed so as to prevent any possible metal to metal arcing from occurring within the cavity. The microwave cavity should be equipped with an exhaust ventilation and should contain a sensor capable of detecting solvent concentrations below their lower explosive limits and shutting the microwave source off. The cavity shall have a 360° oscillating turntable to ensure even sample heating and be capable of removing contained vessel-vented solvents. Safety interlocks to shut off the magnetron power output shall be contained in the cavity door opening mechanism.

Extraction vessel: the vessel consists of a fluoropolymer inner liner and cover to contain and isolate a solvent solution from a higher strength polymer outer pressure vessel body. The vessel assembly shall be transparent to microwave energy and capable of withstanding an internal pressure of 200 psi and a temperature of 200℃. The vessel assembly shall contain a safety pressure relief device that will prevent possible vessel rupture and be connected to a sealed venting system capable of removing vented solvent vapours from the microwave cavity.

Results

Typical analyte recoveries are shown in tables below.

Semivolatile analyte recovery from freshly spiked topsoil.

Analyte	**Spike Level** (mg/kg)	**Average recovery** (%)[a]	**RSD** (%)
Acenaphthene	5,0	97,6	9,8
Acenaphthylene	5,0	100	10
Acetophenone	5,0	92,2	12
Anthracene	5,0	108	9,2
Benzo(a)anthracene	5,0	113	9,4
Benzo(a)pyrene	5,0	110	8,6
Benzyl alcohol	5,0	96,1	9,0
Butyl benzyl phthalate	5,0	116	9,8
4-Chloroaniline	5,0	97,0	9,2
2-Chloronaphthalene	5,0	91,8	7,3
Dibenzo(a,h)anthracene	5,0	110	5,9
Dibenzofuran	5,0	98,8	9,9
Di-*n*-butyl phthalate	5,0	113	9,4
2,4-Dichlorophenol	5,0	97,5	8,0
Diethyl phthalate	5,0	111	8,0
Dimethyl phthalate	5,0	106	8,4
2,4-Dinitrotoluene	5,0	98,2	6,2
Fluorene	5,0	101	10
2-Nitroanilina	5,0	100	7,7

Analyte	**Spike Level** (mg/kg)	**Average recovery** (%)[a]	**RSD** (%)
Pentachlorobenzene	5,0	101	9,1
Phenanthrene	5,0	109	8,5
Phenol	5,0	97,3	9,2
Pyrene	5,0	113	8,4
2,3,6-Trichlorophenol	5,0	96,4	6,7
2,4,6-Tribromophenol	5,0	92,3	7,7

[a] The topsoil was dry when spiked. The number of determination was three. Determination were made by gas chromatography-mass spectrometry.

Semivolatile analyte recovery from ERA soil (lot 324)[b]

Analyte	**Certified concentration** (mg/kg)[c]	**Average recovery** (%)[d]	**RSD** (%)
Anthracene	1,01	68,6	4,7
Benzo(a)anthracene	2,03	103	6,7
Butyl benzyl phthalate	10,6	128	10,8
2-Chlorophenol	5,08	76,2	15,7
Dibenzofuran	6,79	88,8	1,9
2,4-Dinitrotoluene	5,0	83,0	4,2
Fluorene	6,06	72,1	1,0
Pentachlorophenol	12,2	85,0	6,8
Phenanthrene	1,57	110	6,8
Pyrene	8,03	110	12,8
2,4,5-Trichlorophenol	7,99	96,9	1,3
2-Fluorobiphenyl	6,0	102	8,8
2-Fluorophenol	20,0	96,0	13,1
2,4,6-Tribromophenol	20,0	94,8	3,9

[b] This soil was obtained from Environmental Resource Associates (ERA) in Arvada, CO, and has been certified for the compounds listed in the table. [c] Reported by ERA. [d] The number of determinations was four. The recoveries are based on the ERA certified values.

Chlorinated pesticide recovery from freshly spiked topsoil

Pesticide	**Spike Level** (µg/kg)	**Average recovery** (%)[e]	**RSD** (%)
Alachlor	200	87,6	2,3
α-BHC	20	94,4	4,1
β-BHC	20	90,4	3,6
Captan	200	105	1,7
α- Chlordane	20	80,0	3,9
Chloropropylate	100	86,0	5,6
DCPA	60	93,3	1,5
4-4’-DDE	20	84,7	3,1
4,4’-DDT	20	116	5,6
Diallate	200	98,6	4,1
Dichloran	60	110	9,3
Endrin	20	97,4	1,9
Etridiazole	100	92,6	14
Heptachlor	20	110	1,4
Isodrin	20	103	1,3
PCNB	100	94,5	1,8
Propachlor	100	91,5	8,4
Trifluralin	40	101	2,4

[e] The number of determination was three

Organophosphorus pesticide and industrial chemical compound recovery from freshly spiked topsoil

Pesticide	**Spike Level** (mg/kg)	**Average recovery** (%)[f]	**RSD** (%)
Aspon	1,0	91,8	3,4
Bolstar	1,0	103	1,8
Coumaphos	2,0	100	0,7
Demeton-O	1,0	98,7	7,1

Pesticide	**Spike Level** (mg/kg)	**Average recovery** (%)[f]	**RSD** (%)
Demeton-S	1,0	95,2	7,3
Dioxathon	2,0	99,0	1,7
Ethion	1,0	102	2,4
Famphur	1,0	101	1,7
Fonophos	1,0	102	3,3
Malathion	1,0	105	1,1
Parathlon ethyl	1,0	98,0	3,8
Phosmet	1,0	99,1	0,8
Ronnel	1,0	99,5	2,9
Tokuthion	1,0	99,0	3,0
Industrial chemicals			
Tri-*o*-cresylphosphate	1,0	100	1,6
Triazine herbicides			
Atrazine	1,0	100	0,9

[f] The number of determination was three.

PCB recovery from soil

Sample	**Certified value or spiked level** (mg/Kg)	**Aroclor type**	**Average recovery** (%)	**RSD** (%)
ERA	394 [f]	1260	89,9 [g]	2,6
Freshly spiked topsoil	0,100 [h]	1016	85,9 [i]	5,6
	0,100 [h]	1260	82,5 [i]	4,2

[f] Certified by Environmental Resource Associates (ERA).

[g] The number of determination was four.

[h] Spiked in the laboratory.

[i] The number of determination was three.

Sample

5 g of sample

Extraction solvent

30 ml of Acetone-Hexane (1:1 v/v)

Procedure

1. Take a 5 g portion of the sample, weigh to the nearest 0,1 mg and transfer into the extraction vessels.
2. Add 30 ml of Acetone-Hexane (1:1 v/v) to each sample.
3. Close each extraction vessel.
4. Place the closed extraction vessel onto the rotor body.
5. Place the rotor into the microwave cavity and make the proper connection to the reference vessel.

Note: If samples have different water contents, it is recommended that dry samples be extracted separately from wet samples, since dry and wet sample-solvent suspensions will heat at different rates, and thereby reach different temperatures.

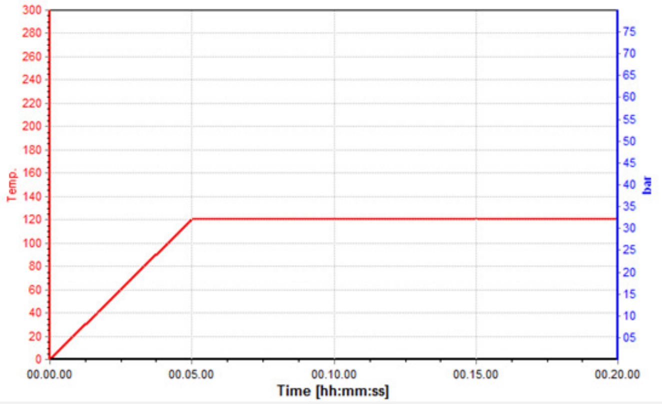
6. Heat the vessels according to the microwave program.
7. Allow the vessel to cool to room temperature.
8. Open the vessels, decant and filter or centrifuge to separate the solvent from the sample for further processing and cleanup if necessary before performing analyte determination.

Microwave program

Step	**Time** (min)	**Power** (W)	**Temperature** (℃)
1	10	Up to 1000*	120
2	10	Up to 1000*	120

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature profile



Reference

- D 1193 Specification for reagent water (Annual book of ASTM Standards, Vol 11.01).
- D 3976 Practice for preparation of sediment samples for chemical analysis. (Annual book of ASTM Standards, Vol 11.02).
- D 5368 Test method for evaluating solid waste volume 1A: Laboratory manual physical/chemical methods (Annual book of ASTM Standards, Vol 11.04).

The ASTM D-6010 can be downloaded for a fee at:
http://www.astm.org

Comments

Rapid heating in combination with temperatures in excess of the atmospheric boiling point of organic solvents, reduce sample extraction times. Small amounts of solvents (30 ml) are used resulting in reduced sample preparation cost and time.

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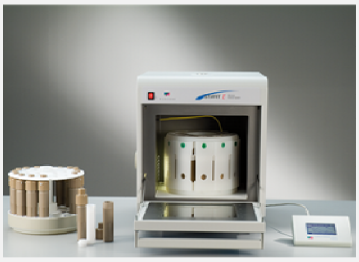


Application Method ME003

ASTM D-5765: Solvent extraction of Total Petroleum Hydrocarbons from soils and sediments using closed vessel microwave heating

Introduction

This practice covers the solvent extraction of total petroleum hydrocarbons (TPH) from soils and sediments, using closed vessel microwave heating, for subsequent determination by gravimetric or gas chromatographic techniques.

Instrumentation

Microwave heating system: a laboratory microwave heating system capable of delivering a minimum of 900 W of microwave energy. The system should be capable of 1% power adjustments and 1-s time adjustments. The microwave unit must be capable of measuring and controlling solvent temperature within an extraction vessel. The microwave cavity should be constructed so as to prevent any possible metal to metal arcing from occurring within the cavity. The microwave cavity should be equipped with an exhaust ventilation and should contain a sensor capable of detecting solvent concentrations below their lower explosive limits and shutting the microwave source off. The cavity shall have a 360° oscillating turntable to ensure even sample heating and be capable of removing contained vessel-vented solvents. Safety interlocks to shut off the magnetron power output shall be contained in the cavity door opening mechanism.

Extraction vessel: the vessel consists of a fluoropolymer inner liner and cover to contain and isolate a solvent solution from a higher strength polymer outer pressure vessel body. The vessel assembly shall be transparent to microwave energy and capable of withstanding an internal pressure of 400 psi and a temperature of 250°C. The vessel assembly shall contain a safety pressure relief device that will prevent possible vessel rupture and be connected to a sealed venting system capable of removing vented solvent vapours from the microwave cavity.

Sample

Up to 5 g of sample.

Extraction solvent

30 ml of Acetone-Hexane (1:1 v/v).

Results

Typical analyte recoveries are shown in tables below.

Total petroleum hydrocarbon recovery data

Material	**TPH Present** (%)	**TPH Recovered** (%)	**Measurement method**
Soil	1,34 [a]	1,32	Gravimetric
Soil [b]	0,214	0,223	Gravimetric
Soil [b]	0,099	0,094	GC-FID

[a] Value determined by Soxhlet extraction.

[b] Environmental Resource Associates (ERA) standard reference soil.

Procedure

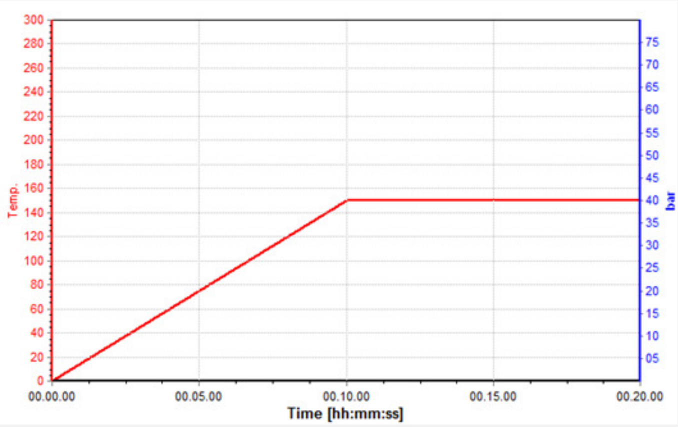
1. Take a 5 g portion of the sample, weigh to the nearest 0,1 mg and transfer into the extraction vessels.
2. Add 30 ml of Acetone-Hexane (1:1 v/v) to each sample.
3. Close each extraction vessel.
4. Place the closed extraction vessel onto the rotor body.
5. Place the rotor into the microwave cavity and make the proper connection to the reference vessel.
6. Heat the vessels according to the microwave program.
7. Allow the vessel to cool to room temperature.
8. Open the vessels, add 5 g of sodium sulphate and solvent mixture to the sample and then gently swirl the extraction mixture.
9. Filter the extract mixture through ashless filter paper rinsing and washing the extracted, sample, sodium sulphate and filter paper thoroughly with Acetone/Hexane (1:1 v/v) into an appropriate container.
10. Proceed with an appropriate procedure for gravimetric or gas chromatographic determination.

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (℃)
1	10	Up to 1000 *	150
2	15	Up to 1000 *	150

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Comments

Rapid heating in combination with temperatures in excess of the atmospheric boiling point of organic solvents, reduce sample extraction times. Small amounts of solvents (30 ml) are used resulting in reduced sample preparation cost and time.

Reference

- D 3856 Guide for good laboratory practices in laboratories engaged in sampling and analysis of water (Annual book of ASTM Standards, Vol 11.01).
- D 3976 Practice for preparation of sample containers and for preservation of organic constituents. (Annual book of ASTM Standards, Vol 11.02).
- D 5368 Test method for evaluating solid waste volume 1A: Laboratory manual physical/chemical methods (Annual book of ASTM Standards, Vol 11.04).

The ASTM D-5765 can be downloaded for a fee at: ***http://www.astm.org***

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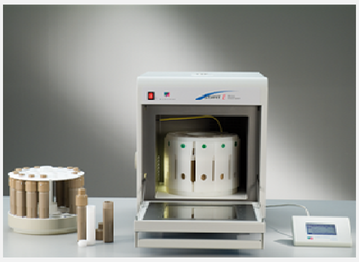


Application Method ME004

Extraction of Polycyclic Aromatic Hydrocarbons (PAHs) from sediments

(EPA Method 3546; ASTM D-6010)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. PAHs are one of the most widespread organic pollutants because of their high carcinogenicity and mutagenicity. In addition to their presence in fossil fuels they are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel or fat.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, ETHOS by Milestone using the SK-12 rotor. For HPLC determination of PAHs, a Waters (Milford, MA, USA) powerline system consisting of the Waters 600E system controller, Waters 486 tunable absorbance detector. For GC analysis a Hawlett-Packard (Palo Alto, CA, USA) HP 5890 series II gas chromatograph equipped with a MS detector was used.

Results

Comparison between Soxhlet extraction (SOX) and Microwave Assisted Extraction (MAE) using certified reference materials (CRM) HS-4 and HS-6.

PAH	**HS-4 Recovery** (%)		**HS-6 recovery** (%)	
	SOX	**MAE**	**SOX**	**MAE**
Naphthalene	73,3	80,0	74,4	96,1
Acenaphthylene	106,7	80,0	89,5	136,8
Acenaphthene	66,7	73,3	73,9	78,3
Fluorene	73,3	73,3	89,1	94,7
Phenanthrene	85,3	88,2	85,0	101,9
Anthracene	100,0	85,7	82,7	87,6
Pyrene	96,8	95,7	97,2	84,4
Benzo[*a*]anthracene	66,0	84,9	100,0	96,5
Chrysene	98,5	93,8	88,6	73,5
Benzo[*b*]fluoranthene	101,4	92,9	84,6	100,1
Benzo[k]fluoranthene	111,1	75,0	81,7	86,4
Benzo[*a*]pyrene	92,9	94,3	90,0	105,0
Indeno[1,2,3-*cd*]pyrene	98,0	92,2	96,4	78,9
Dibezo[*a,h*]anthracene	75,0	91,7	69,4	79,6
Benzo[*ghi*]perylene	82,4	86,3	88,8	84,3

Soxhlet: 5 g were extracted with dichloromethane for 16 h.

MAE: 5 g of sediment were extracted with 30 ml of acetone-hexane (1:1 v/v) for 30 min at 120°C.

Sample

5 g of sediments

Extraction solvent

30 ml of Acetone:Hexane (1:1 v/v)

Fluoranthene	78,4	88,0	81,6	85,6

Procedure

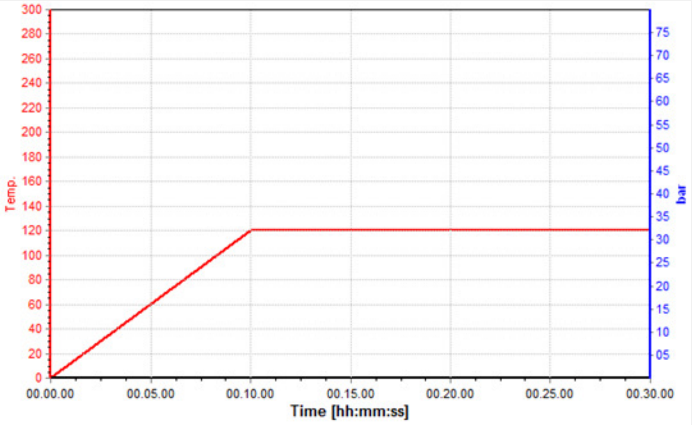
1. Place a TFM vessel on the balance plate, tare it and weigh the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead, if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the solvent, the Weflon and the magnetic stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (°C)
1	10	Up to 1000 *	120
2	20	Up to 1000 *	120

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (°C)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The results of LC-MS confirm that microwave extraction rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet extraction, and with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 16 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference

The ASTM D-6010 can be downloaded for a fee at: ***http://www.astm.org***

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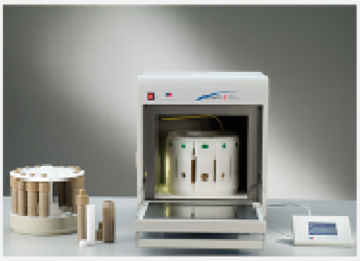


Application Method ME005

Extraction of polybrominated diphenylethers (PBDEs) in sludge

(EPA Method 3546; ASTM D-6010)

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in a variety of commercial products. The PBDEs congeners that have chemical structures close to polychlorinated diphenyls (PCBs) are suspected to be a potential threat to environmental and human health.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated PBDEs was carried out by an Agilent 6890 gas chromatograph equipped with 7683 automatic liquid sampler and a 5973 mass spectrometer (Agilent Technologies, Mississauga, ON, Canada).

Results

Recoveries after Microwave Assisted Extraction (MAE) from high spiked (HL) and low spiked (LL) samples.

PBDE	**HL Spiked** (ng/g)	**HL Recovery ±SD** (n = 3) (%)	**LL Spiked** (ng/g)	**LL Recovery ±SD** (n = 3) (%)
BDE 47	250	109 ± 6,4	10	106 ± 0,7
BDE 77	250	102 ± 5,3	10	112 ± 1,1
BDE 100	250	107 ± 5,7	10	98 ± 1,0
BDE 99	250	107 ± 6,4	10	108 ± 0,5
BDE 154	250	106 ± 6,8	10	99 ± 0,6
BDE 153	250	106 ± 6,3	10	102 ± 0,7
BDE 138	250	108 ± 6,6	10	95 ± 0,5
BDE 183	250	95 ± 8,8	10	106 ± 1,5
BDE 209	500	108 ± 8,1	20	101 ± 1,9

Samples

1 g of sludge.

Extraction solvent

30 ml Acetone / Hexane (1:3 v/v)

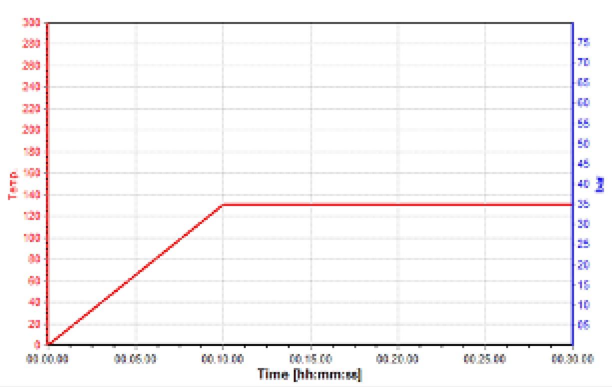
Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead, if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the mixture of solvents, the Weflon and the stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and purify the extract in order to remove the organic matter and the sulphur that can interfere during instrumental analysis.

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (℃)
1	10	Up to 1000*	130
2	20	Up to 1000*	130

Temperature Profile



Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (℃)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Comments

The results of GC confirm that microwave extraction rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet extraction, and with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 16 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference

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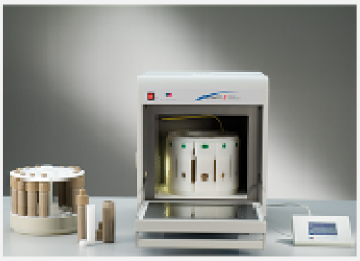


Application Method ME006

Extraction of Polychlorinated biphenyls (PCBs) from environmental samples

(EPA Method 3546; ASTM D-6010)

Introduction

PCB, well known as polychlorinated biphenyls, were used in many industrial applications like pesticides, flame retardant and paints. PCBs have been classified as dangerous substances to the human health and the environment due to their toxicity and bioaccumulation.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated PCBs was carried out by GC (Perkin-Elmer Autosystem) with an electron capture detector. Calibration was done by internal standardization with PCB 155.

Results

Microwave: 3 g of sediment was extracted with 30 ml of Acetone / Hexane (1:1 v/v) for 30 min at 120°C.

Soxhlet: 5 g of sediment was extracted with 150 ml Acetone / Hexane (1:1 v/v) for 7 h.

Compound	Spiked concentration (µg/g)	Microwave recovery (%)	Soxhlet recovery (%)
PCB 28	2,4	99,3 ± 5,9	117 ± 9,8
PCB 31	1,1	96,2 ± 4,4	117,5 ± 8,1
PCB 52	0,9	90,0 ± 3,4	84,6 ± 3,1
PCB 101	1,2	85,7 ± 2,8	90,6 ± 6,8
PCB 118	1,4	104,0 ± 10,8	109,3 ± 14,3
PCB 153	2,6	99,4 ± 2,4	104,4 ± 11,8
PCB 105	2,9	104,9 ± 7,9	111,4 ± 6,4
PCB 138	2,7	108,7 ± 2,7	108,7 ± 12,2
PCB 156	5,5	98,1 ± 3,4	92,9 ± 9,2

Samples

3 g of sediment.

Extraction solvent

30 ml Acetone / Hexane (1:1 v/v)

Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead, if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the mixture of solvents, the Weflon and the stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and remove the sulphur using activated copper.
9. Filtrate, add the internal standard and concentrate to dryness.
10. Dissolve in hexane and purify the extract through an alumina column (2 g of 6% hydrated alumina, 32 cm x 2 mm) in order to remove from the purified extract the polar compounds.

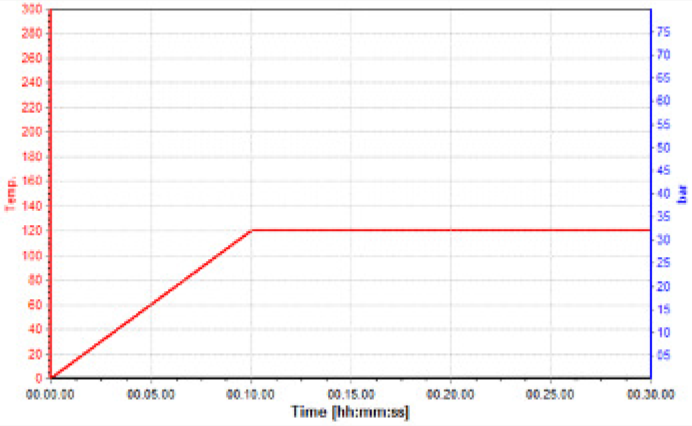
PCB 180	2,9	106,6 ± 3,8	108,6 ± 11,6

Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	10	Up to 1000*	120
2	20	Up to 1000*	120

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (℃)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The results of GC confirm that microwave extraction rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet extraction, and with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 7 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference .

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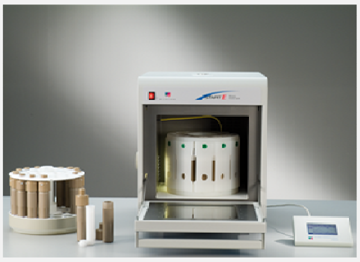


Application Method ME007

Extraction of total petroleum hydrocarbons (TPH) from soils

(EPA Method 3546; ASTM D-5765)

Introduction

Total petroleum hydrocarbons (TPH) is a term used to describe a large family of several hundred chemical compounds that originally come from crude oil. Release of petroleum products to the environment is a serious and increasingly prevalent problem.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated TPH was carried out by gas chromatography, using HP Agilent 6890 gas chromatograph equipped with a FID detector.

Results

Three methods for the extraction of TPH are compared. The techniques used are Soxhlet, Microwave Assisted Extraction (MAE) and CEN shake extraction.

CEN shake: 5 g of soil was shaken for 1 h with 40 ml of acetone and 20 of heptane at room temperature.

Soxhlet: 5 g of soil was extracted for 20 h with 40 ml of acetone and 20 ml of heptane.

MAE: 5 g of soil was extracted with 20 ml of acetone and 20 ml of heptane for 20 min at 150°C.

Recoveries obtained for standard oil mixture (n = 3):

Method	**Certified Value** (mg/l)	**Recovery** (mg/l)	**RSD** (%)
CEN shake	1866	1498 ± 161	11
Soxhlet	1728	1246 ± 65	5
MAE	1928	1915 ± 57	3

Recoveries obtained for spiked soil samples (n = 3):

Method	**Certified Value** (mg/g)	**Recovery** (mg/g)	**RSD** (%)
CEN shake	6,75	6,4 ± 0,3	6
Soxhlet	6,75	7,4 ± 1,3	20
MAE	6,75	7,9 ± 3,0	40

Samples

5g of soil

Extraction solvent

40 ml Acetone / Heptane (1 : 1)

Procedure

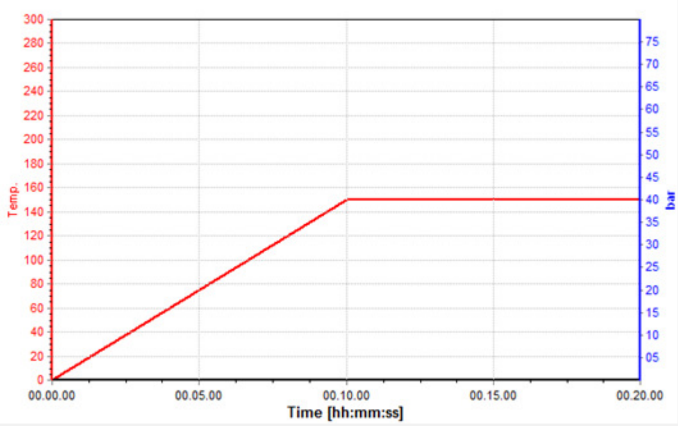
1. Place a TFM vessel on the balance plate, tare it and weigh of the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead, if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the solvent, the Weflon and the magnetic stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	10	Up to 1000*	150
2	10	Up to 1000*	150

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Comments

The results of GC analysis on the extracts confirm that microwave extraction (MAE) rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet and shake extraction, with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 20 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (℃)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Reference

1) A. Agazzi, C. Pirola, *Microchemical Journal*; 41 (2000) 1-3, 337 – 341.

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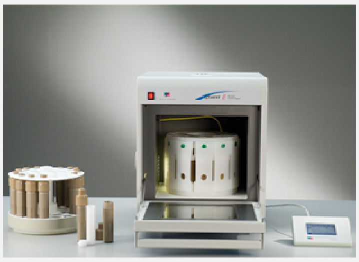

Application Method ME008

Extraction of triclosan and related chlorophenols in sludge and sediment

(EPA Method 3546; ASTM D-6010)

Introduction

A procedure for the determination of 2-(2,4-dichlorophenoxy)-5-chlorophenol (Triclosan) and two possible transformation compounds, 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP), in sludge from sewage treatment plants (STP) and sediment is presented. Triclosan (TCS) is extensively employed as an additive in many products because its bactericide and antimicrobial properties. Once in the aquatic environment, TCS undergo a series of transformation reactions to produce more toxic and/or bio-accumulative compounds.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, ETHOS by Milestone using the SK-12 rotor. Analytes were determinatedby gas-chromatography with tandem mass spectrometry (GC-MS/MS). The employed system consist of a Varian CP 3900 Gas Chromatograph (Walnut Creek, CA, USA) connected to an ion-trap mass spectrometer (Varian Saturn 2100).

Results

Recoveries, extraction plus clean-up, were evaluated using aged spiked samples corresponding to primary, biological and disinfected sludge, as well as river sediment. Chlorophenols were added to samples at different concentration levels, 10 and 300 ng/g. TCS was spiked at two levels, 10 and 300 ng/g in the case of sediments, and at 0,9 and 5 µg/g to sludge samples.

Sample	**Addition level** (ng/g)	**Recoveries ± RSD (%)**	
		2,4-DCP	**2,4,6-TCP**
River sediment	10	81,8 ± 5,7	96,0 ± 4,9
River sediment	300	79,1 ± 8,2	92,0 ± 8,9
Primary sludge	300	79,1 ± 8,2	98,9 ± 3,2
Disinfected sludge	300	106,6 ± 7,5	88,3 ± 5,0
Disinfected sludge	40	82,9 ± 9,2	97,4 ± 13,0

Sample	**Addition level** (ng/g)	**Recoveries ± RSD** (%)
		TCS
River sediment	10	98,7 ± 8,5
River sediment	300	99,7 ± 6,5
Primary sludge	5000	81,7 ± 6,5
Secondary sludge	5000	94,0 ± 6,5
Disinfected sludge	5000	82,2 ± 11,5
Disinfected sludge	900	97,3 ± 10,5

Below is shown the comparison of concentrations (ng/g) of the analytes in non-spiked sludge using Soxhlet extraction and Microwave-Assisted Extraction (MAE).

Sample	**Method**	**Mean ± SD**		
		2,4-DCP	**2,4,6-TCP**	**TCS**
Primary sludge	MAE	79,9 ± 8,5	19,4 ± 0,3	2543 ± 50
Primary sludge	Soxhlet [a]	89,8 ± 8,4	21,6 ± 1,5	2696 ± 270
Biological sludge	MAE	316 ± 33	38,1 ± 9,2	5400 ± 125
Biological sludge	Soxhlet [a]	349 ± 12	37,7 ± 2,5	5388 ± 316

Secondary sludge	300	78,3 ± 8,1	87,6 ± 5,1

Sample

1 g for sediments or 0,5 g for sludge samples

Extraction solvent

30 ml of Acetone:Methanol (1:1)

Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the solvent and the magnetic stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique[1].

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (°C)
1	10	Up to 1000 *	130
2	20	Up to 1000 *	130

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile

a: 100 ml of Acetone:Methanol (1:1) for 24h

Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (°C)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The results of LC-MS confirm that microwave extraction rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet extraction, and with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 24 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference

1) S. Morales, P. Canosa, I. Rodeiguez, E. Rubi, R. Cela; *Journal of Chromatography A*, 1082 (2005) 128-135.

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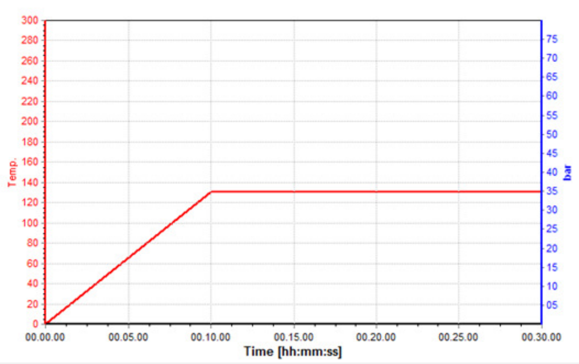300 280 260 240 220 200 180 160 140 120 100 80 60 40 20 0 Temp. 75 70 65 60 55 50 45 40 35 30 25 20 15 10 05 bar 00.00.00 00.05.00 00.10.00 00.15.00 00.20.00 00.25.00 00.30.00 Time [hh:mm:ss]	

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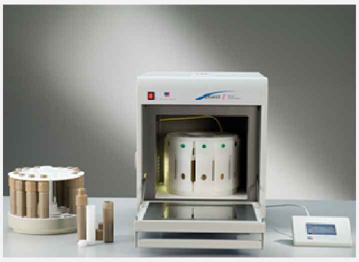


Application Method ME009

Extraction of Dioxins and Furans from fly ash

(EPA Method 3546; ASTM D-6010)

Introduction

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are grups of polyalogenated compounds which are significant because they act as environmental pollutants. PCDDs and PCDFs can be formed by pyrolysis or incineration, at high temperature, of chlorine containing products, such as PVC, PCBs and other organochloride.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated PCDDs and PCDFs was carried out in according US-EPA 1613. A 60 m DB 5ms column (J&W) was coupled to a high resolution mass spectrometer (Thermofinnigan MAT 95 XL).

Sample

2 g of Fly ash sample CRM 490 from BCR

Extraction solvent

30 ml Toluene

Results

Compound	Certified Value (ng/g)	Microwave Extraction (ng/g)	Extraction time (min)
2378 TCDD	0, 169	0,170	45
12378 PCDD	0,670	0,715	45
123478HxCDD	0,950	0,767	45
123789HxCDD	2,840	2,357	45
123678HxCDD	4,800	4,010	45
2378TCDF	0,900	0,919	45
12378PCDF	1,710	1,788	45
23478PCDF	1,850	2,461	45
123478HxCDF	2,370	2,363	45
123678HxCDF	2,640	2,668	45
234678HxCDF	2,470	2,992	45

Compound	Soxhlet (ng/g)	Time (h)	START E (ng/g)	Time (min)
2378 TCDD	0,180	24	0,170	45
12378 PCDD	0,769	24	0,715	45
123478HxCDD	0,814	24	0,767	45
123789HxCDD	2,742	24	2,357	45
123678HxCDD	4,628	24	4,010	45
2378TCDF	0,879	24	0,919	45
12378PCDF	1,716	24	1,788	45
23478PCDF	2,608	24	2,461	45
123478HxCDF	2,279	24	2,363	45
123678HxCDF	2,633	24	2,668	45
234678HxCDF	2,924	24	2,992	45

Procedure

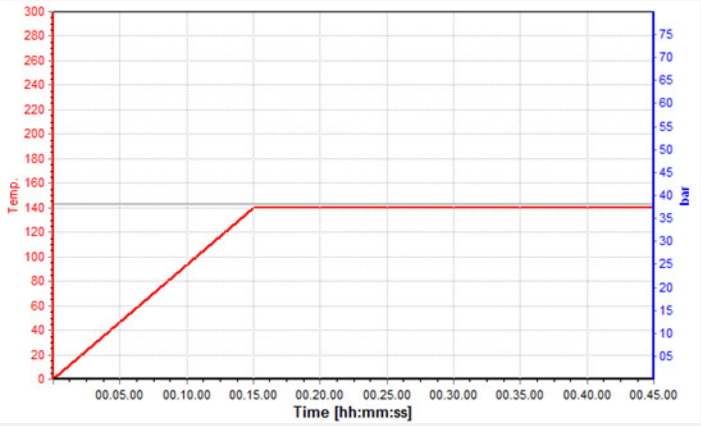
1. Place a TFM vessel on the balance plate, tare it and weigh of the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the toluene, the Weflon and the magnetic stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (°C)
1	15	Up to 1000 *	140
2	30	Up to 1000 *	140

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (°C)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The results of LC-MS confirm that microwave extraction rates were found to be significantly faster, with recoveries comparable or superior to the traditional Soxhlet extraction, and with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to 24 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference

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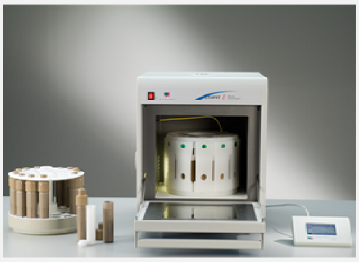


Application Method ME010

Extraction of 4-nonylphenol (NP) and 4-nonylphenol ethoxylates (NPE) from sediments (EPA Method 3546; ASTM D-6010)

Introduction

NPE are a class of the broader group of compounds known as alkylphenol ethoxylates (APE). APE have been used as detergents, emulsifiers, wetting agents and dispersing agents in many applications. NP has been reported to cause a number of estrogenic responses in a variety of aquatic organisms.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the PRO-24 rotor. The determination of the investigated NP and NPE was carried using an HPLC system composed of a gradient PU-1580 pump (Jasco Corporation, Tokyo, Japan) with a 10 µl sample loop and a FP-920 fluorescence detector. The separation was achieved on a C-18 LiChrospher 60 RP-select B, 250 x 4.6 mm, 5 µm, (Merck, Darmstadt, Germany) at 25℃.

Results

Results expressed as mean ± SD. Number of replicates in brackets.

Microwave extraction: 40 ml of methanol at 120℃ for 20 min.

Soxhlet extraction: 400 ml of methanol for 10 h.

Pressurised liquid extraction: 2 cycles with methanol (15 ml) at 100℃, 100 atm, in static mode (flush 60%, purge 90 s) for 10 min.

Sample 1: low polluted sample

Compound	**Microwave** (ng/g)	**Soxhlet** (ng/g)	**Pressurised liquid** (ng/g)
NP	0,34 ± 0,04 (n = 3)	0,45 ± 0,30 (n = 4)	0,33 ± 0,11 (n = 5)
NPE	1,4 ± 0,1 (n = 3)	2,9 ± 0,8 (n = 6)	1,5 ± 0,5 (n = 5)

Sample 2: moderately polluted sample

Compound	**Microwave** (ng/g)	**Soxhlet** (ng/g)	**Pressurised liquid** (ng/g)
NP	2,2 ± 0,4 (n = 3)	2,8 ± 0,8 (n = 4)	2,9 ± 0,6 (n = 5)
NPE	11,4 ± 0,9 (n = 3)	11,2 ± 2,5 (n = 4)	10,4 ± 0,6 (n = 5)

Sample

5 g of sediments

Extraction solvent

40 ml of methanol

Procedure

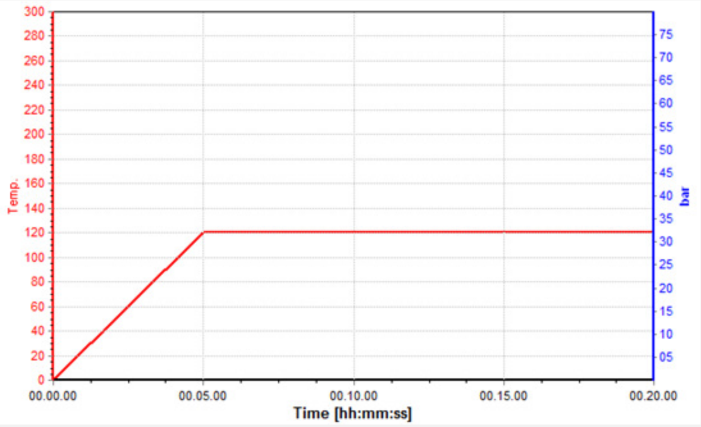
1. Place a TFM vessel on the balance plate, tare it and weigh the sample. If the sample contains a low content of water, add a drying agent like diatomaceous earth with a ratio sample-drying agent of 2:1; instead if the content of water is high use a ratio sample-drying agent of 1:1. Mix the mixture until the sample become homogeneous.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add 40 ml of methanol and the magnetic stirrer.
4. Close the vessel and introduce it into the rotor.
5. Connect the temperature sensor to the reference vessel.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and clean-up the solution according to the analytical technique[1].

Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	5	Up to 1000 *	120
2	15	Up to 1000 *	120

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (℃)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

Microwave extraction is a suitable alternative extraction method for the 4-NP and 4-NPE determination, showing an accuracy and precision comparable to that obtained with pressurised liquid extraction and better than those obtained with Soxhlet. The small amount of solvent consumption, reduced extraction time and the treatment up to 41 vessels simultaneously, are the most important advantages of microwave extraction. These advantages offer a great opportunity for laboratories with high sample throughput.

Reference

1) V. Croce, S. Paggio, A. Pagnoni, S. Polesello, S. Valsecchi; analisi chimica, 93, 2003, by Società Chimica Italiana.

The ASTM D-6010 can be downloaded for a fee at: ***http://www.astm.org***

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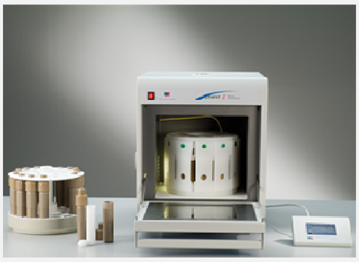

Application Method ME011

Extraction of organophosphate (Ops) flame retardants from indoor dust

(EPA Method 3546, ASTM D-6010)

Introduction

Several esters of phosphoric acid are extensively employed as plasticizers and flame retardant additives in textiles, wallpapers, varnishes and polymeric materials. In most cases, these species are not chemically bounded to host materials; therefore, they can be easily emitted to the surrounding areas. OPs are ubiquitous pollutants in sewage water and indoor atmospheres.

Instrumentation

Samples were extracted using an Ethos microwave system equipped with SK-12 rotor. OPs were quantified using a HP 5890 Series II GC System (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a NPD detector. A DB-5 (5% phenylmethylpolysiloxane) type column (30m × 0.32mm I.D., d.f. 0.25_m), purchased from Agilent (Wilmington, DE, USA), was used for the separation of OPs.

Results

[a] Spiked only with TiBP, TBP, TCEP and TPPO at the 1 µg/g

[b] Spiked with all Ops at different levels (form 0,2 to 10 µg/g)

OPs	Pooled dust sample[a]		Discrete dust sample[b]	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
TiBP	92	13	98	3
TBP	98	10	100	5
TCEP	89	7	88	9
TCPP	-	-	93	4
TDCP	-	-	94	6
TPP	-	-	104	6
TBEP	-	-	97	6

Comparison of OPs levels obtained for three non-spiked samples using Microwave Assisted Extraction and Soxhlet. n.q. under quantification limit: 0,04 µg/g for TiBP, TBP, TCEP and TCPP; 0,05 µg/g for the rest of Ops. Values in µg/g, n=3 replicates.

OPs	Sample 1			
	MAE (mean ± SD)	Time (min)	Soxhlet (mean ± SD)	Tmie (h)
TiBP	n.q.	30	n.q.	15
TBP	0,046 ± 0,0009	30	0,053 ± 0,006	15
TCEP	1,5 ± 0,1	30	1,79 ± 0,08	15
TCPP	4,2 ± 0,4	30	4,0 ± 0,3	15
TDCP	4,1 ± 0,3	30	3,9 ± 0,3	15
TPP	1,6 ± 0,2	30	1,62 ± 0,09	15
TBEP	17,5 ± 0,7	30	16,6 ± 0,3	15
TPPO	n.q.	30	n.q.	15

OPs	Sample 2			
	MAE (mean ± SD)	Time (min)	Soxhlet (mean ± SD)	Tmie (h)
TiBP	n.q.	30	n.q.	15
TBP	0,162 ± 0,005	30	0,163 ± 0,008	15
TCEP	3,2 ± 0,3	30	3,4 ± 0,3	15
TCPP	3,8 ± 0,4	30	4,0 ± 0,3	15
TDCP	0,107 ± 0,006	30	0,119 ± 0,003	15
TPP	0,57 ± 0,03	30	0,57 ± 0,06	15
TBEP	11.1 ± 0,9	30	12,3 ± 1,1	15

TPPO	91	11	85	11

OPs	Sample 3			
	MAE (mean ± SD)	Time (min)	Soxhlet (mean ± SD)	Tmie (h)
TiBP	0,16 ± 0,01	30	0,15 ± 0,01	15
TBP	0,15 ± 0,02	30	0,14 ± 0,02	15
TCEP	0,26 ± 0,03	30	0,30 ± 0,03	15
TCPP	1,8 ± 0,1	30	1,7 ± 0,1	15
TDCP	0,86 ± 0,05	30	0,84 ± 0,03	15
TPP	0,60 ± 0,04	30	0,55 ± 0,04	15
TBEP	11.2 ± 0,4	30	10,7 ± 0,8	15
TPPO	n.q.	30	n.q.	15

Legend

TiBP: Triisobutyl phosphate
TBP: Tributyl phosphate
TCEP: Tris(2-chloroethyl) phosphate
TCPP: Tris(chloropropyl) phosphate
TDCP: Tris(2-chloro,1-chloromethylethyl) phosphate
TPP: Triphenyl phosphate
TBEP: Tris(butoxyethyl) phosphate
TPPO: Triphenyl phosphine oxide

Microwave Program

Step	Time (min)	Power (W)	Temperature (°C)
1	10	Up to 1000 *	130
2	20	Up to 1000 *	130

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile

TPPO	0,068 ± 0,006	30	0,075 ± 0,004	15

Sample

0,5 g of dust.

Extraction solvent

20 ml of acetone

Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add 10 ml of acetone and the magnetic stirrer.
4. Close the vessel and introduce it into the rotor.
5. Connect the temperature sensor to the reference vessel.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and clean-up the solution according to the analytical technique [1].

Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (°C)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The extraction efficiency of this technique was similar to that achieved using the Soxhlet method, with the advantages of a reduced consumption of solvent (20 ml in MAE and 75 ml in Soxhlet) and the possibility of processing up to 41 samples simultaneously.

Reference

1) M. Garcia, I. Rodriguez, R.Cela; Journal of Chromatography A, 1152 (2007) 280 - 286.

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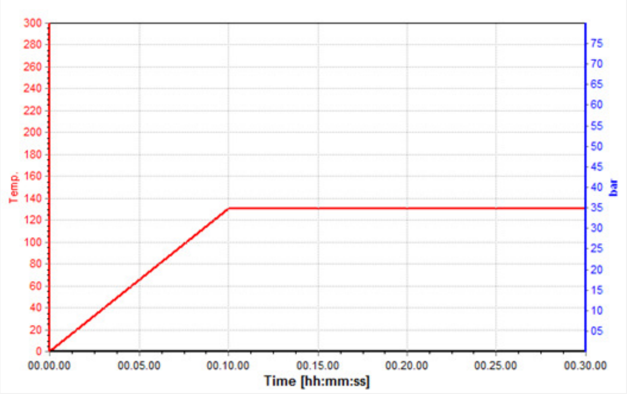

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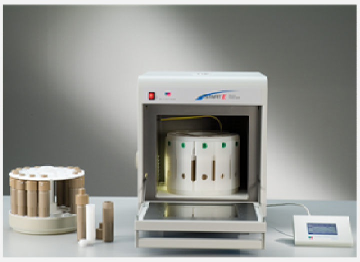


Application Method ME012

Extraction of antioxidants in polyolefins

Introduction

To ensure non-degradative processing and long-term stability, polyolefins are stabilized by antioxidants mainly composed of sterical hindered phenols in combination with phosphites. A physical loss of the stabilizer can occur by diffusion, photochemical reactions and degradation to smaller fragments. The amount of unreacted antioxidant in the polymeric material is an indicator of how far the oxidation has come.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, ETHOS by Milestone using the SK-12 rotor. The determination of the investigated antioxidants was carried out on a Waters 2695 (Waters, Milford, MA, USA).

Results

Irgafos 168, *Tris* (2,4-di-*tert*-butylphenyl) phosphate, concentrations (given by the sum of Irgafos 168 and its oxidative degradation product, Irgafos 168 phosphate) and related recoveries.

Polymer	**Expected** (ppm)	**Found** (ppm)	**Recovery** (%)
PP	1000	1040	> 95
HDPE	800	820	> 95
LLDPE	1000	1020	> 95
HECO	1000	1040	> 95
Supersoft	1000	1100	> 95
PB	2000	2020	> 95

Legend

PP: Polypropylene; HDPE: High density polyethylene; LLDPE: Linear low density polyethylene; HECO: Ethylene-propylene heterophasic copolymer; Supersoft: Ethylene-propylene heterophasic copolymer and ethylene-propylene amorphous copolymer; PB: Polybutylene

Microwave extractions were performed with 25 ml Ethyl acetate- n-Hexane (75/25 v/v) for 20 min using 1000W. The extraction temperatures were: 120°C for PP, HDEP and HECO; 100°C for LLDPE and Supersoft; 80 °C for PB.

HPLC duplicate analysis results of Irgafos 168 and its by-products extracted from PP by different procedures (concentration of stabilizer added to original polymer: 1000 ppm; amount of sample treated: 2,5 g).

Extraction technique	**Irgafos 168** (ppm)	**Irgafos 168 phosphate** (ppm)	**2,4-DTBP**[*] (ppm)	**Time**
Microwave[a]	810 ± 20	195 ± 4	5	20 min
Ultrasonication[b]	800 ± 20	190 ± 4	6	1 h
Soxhlet[c]	720 ± 18	210 ± 6	25	6 h

[*] 2,4-DTBP: 2,4-di-*tert*-butylphenol

a) Microwave: 25 ml Ethyl acetate- n-Hexane (75/25 v/v) for 20 min at 120°C using 1000W.

b) Ultrasonication: 50 ml of anhydrous n-hexane at room temperature for 1 h.

c) Soxhlet: 40 ml of chloroform for 6 h.

Samples

2,5 g of polyolefin

Extraction solvent

25 ml Ethyl acetate / n-Hexane (75/25 v/v)

Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the solvent, the Weflon and stirring bars.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

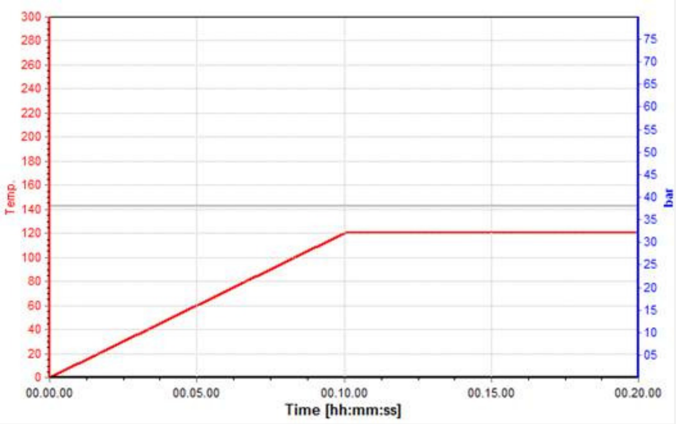
Microwave Program

Step	Time (min)	Power (W)	Temperature (°C)
1	10	Up to 1000*	120^{**}
2	10	Up to 1000*	120^{**}

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

** The extraction temperature depends on the kind of polyolefin, in this case is shown the microwave program for Polypropylene extraction.

Temperature Profile



Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (°C)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The proposed microwave methods in comparison with traditional extraction methodologies allow several advantages like reduced extraction time and solvent volume; in addition using the available rotors for the treatment up to 41 vessels simultaneously, offer a great opportunity for laboratories with high sample throughput.

Reference

1) P. Molander, K. Haugland, D.R. Hegna, E. Ommundsen, E. Lundanes, T. Greibrokk; *Journal of Chromatography A*, 864 (1999) 103-109.

2) M.S. Dopico Garcia, J.M. Lopez, R. Bouza, M.J. Abad, E. Gonzalez Rodriguez; *Analytica Chimica Acta,* 521 (2004) 179-188.

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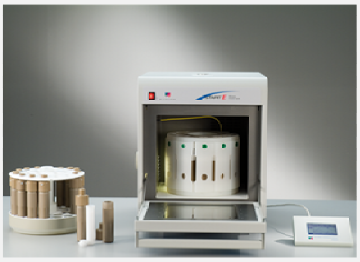


Application Method ME013

Extraction of adipate and phthalate plasticizers from PVC

Introduction

Some organic compounds that are used as additives in plastics (e.g. plasticizers such as phthalates and adipates) can be toxic by contact with skin, ingestion or inhalation. Recently, some European Union Directives have limited the use of these substances, warning the risk.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated plasticizers was carried out by GC-9A gas chromatograph (Shimadzu, Kyoto, Japan). The supercritical fluid extractor (SFE) system employed for comparison was a SFX 220 (ISCO, UK), equipped with a SFX 200 controller and type D syringe pump.

Results

Di-2-ethylhexyl adipate (DEHA) recoveries by Microwave Assisted Extraction (MAE) for different plasticizer concentrations.

DEHA concentrations (%)	**Recoveries** (%)	**RSD** (%) (n = 3)
1,07	86 ± 1	0,5
0,33	93 ± 4	1,7
0,11	104 ± 15	5,9
0,036	125 ± 17	5,5

MAE conditions: 0,5 g of sample extracted with 25 ml of methanol at 120°C for 15 min using 500 W

Comparison between MAE and SFE for different adipate plasticizer.

Plasticizer	**MAE**		**SFE**	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DMA	84 ± 6	3,1	62 ± 5	3,5
DEA	86 ± 3	1,4	65 ± 8	4,8
DBA	96 ± 5	2,2	81 ± 9	4,3
DIBA	96 ± 8	3,4	81 ± 8	3,8
DEHA	94 ± 5	2,0	80 ± 7	3,7
DIDA	78 ± 7	3,7	84 ± 7	3,3

Comparison between MAE and SFE for different phthalate plasticizer.

Plasticizer	**MAE**		**SFE**	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DEHP	103 ± 9	3,4	88 ± 9	4,3
DINP	93 ± 8	3,5	84 ± 9	4,2
DIDP	95 ± 9	4,9	86 ± 7	3,2

MAE conditions: 0,5 g of sample extracted with 25 ml of methanol at 120°C for 15 min using 500 W.

SFE conditions: 0,2 g of sample extracted at 95°C for 30 min with a pressure of 7000 psi using methanol as solvent.

Legend

DMA: dimethyl adipate; DEA: diethyl adipate; DBA: di-n-butyl adipate; DIBA: diisobutyl adipate; DIDA: diisodecyl adipate; DEHP: di-2-ethylhexyl phthalate; DINP: diisononyl phthalate; DIDP: disodecyl phthalate

Samples

0,5 g of PVC

Extraction solvent

25 ml methanol

Procedure

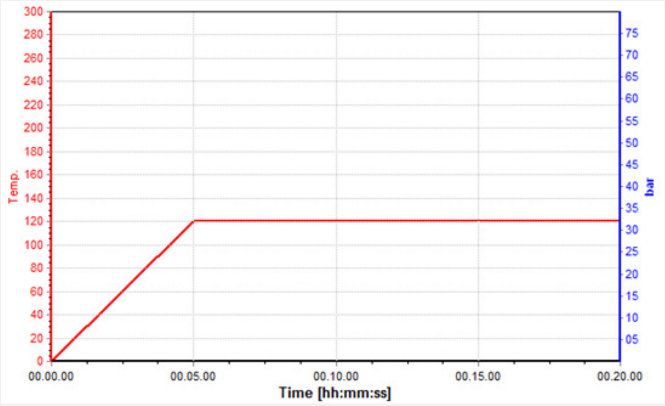
1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the methanol and the stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (℃)
1	5	Up to 1000*	120
2	10	Up to 1000*	120

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (℃)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The results of GC confirm that microwave extraction were found to be significantly faster, with recoveries comparable or superior to the traditional supercritical fluid extraction. Mainly the short extraction time compared to SFE and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

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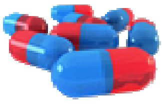

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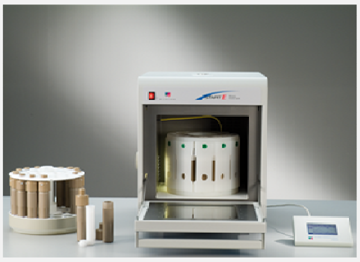


Application Method ME014

Extraction of phenolic compounds in plant material

Introduction

Phenolic compounds constitute a large group of naturally occurring compounds showing a broad spectrum of biological activities. There is currently much interest in these phytochemicals as bioactive components in foodstuffs. The compounds can play an important role in reducing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, ETHOS SEL by Milestone using the SK-12 rotor. The determination of the investigated phenolic compounds was carried out by an HP 1100 liquid chromatograph (Wilmington, DE, USA) with diode array detector working at 280 and 320 nm.

Results

Yield (%) of phenolic compounds for Microwave-Assisted Extraction (MAE) and for Soxhlet using HCl 2 mol/l.

Phenolic Compounds	**MAE** [a] (%)	**Soxhlet** [b] (%)
Protocatechuic acid	95,1	64,0
3,4-dihydroxybenzaldehyde	100	85,3
p-Hydroxybenzoic acid	89,5	0,0
Chlorogenic acid	100	53,7
Vanillic acid	100	100
Caffeic acid	100	68,1
Vanillin	100	0,0
p-Coumaric acid	100	27,0
Ferulic acid	100	47,9
Benzoic acid	42,6	97,7
Rosmarinic acid	100	69,0
m-Coumaric acid	92,5	90,1

a: Microwave extraction was performed with 1 g of plant material at 50℃ for 30 min using 500 W and 15 ml of HCl 2 mol/l; the RSDs were 4,5-5,7 %

b: Soxhlet extraction was performed with 1 g of plant material for 1 h with 40 ml of HCl 2 mol/l; the RSDs were 5,2-8,3 %.

Samples

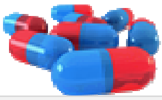
1 g of cocksfoot (*Dactylis glomerata*)

Extraction solvent

15 ml of HCl 2 mol/l

Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the solvent and the stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique[1].

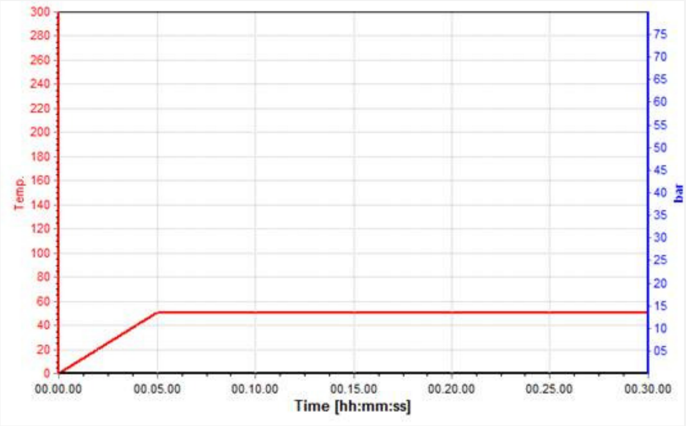


Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	5	Up to 1000*	50
2	25	Up to 1000*	50

* Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (℃)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

Microwave extraction has been found to be more suitable due to the shortened extraction time and better RSDs due to more stable conditions (temperature, time) during the extraction. An additional advantage of the microwave extraction with the Milestone units is the possibility to extract up to 41 samples simultaneously.

Reference

1) D. Štěrbovă D. Matějiček, J. Vlček, V. Kubáň; *Analytica Chimica Acta*, 513 (2004) 435-444.

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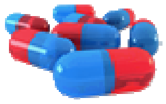
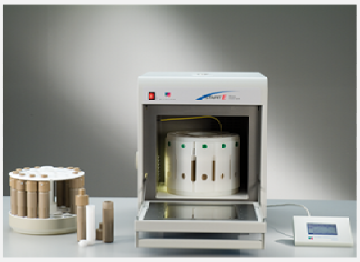




Application Method ME015

Extraction of triterpenoid saponins from plants

Introduction

Triterpenoid saponins are triterpenes which belong to the group of saponin compounds. Triterpenes are well know thanks to their therapeutic properties such as hypolipidaemic, anti-atherosclerotic, antihypertensive, antimicrobial and anticoagulant. In this application note is shown the procedure and the results on the extraction of triterpenoid saponins from *Ganoderma atrum*.

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, START E by Milestone using the SK-12 rotor. The determination of the investigated triterpenoid saponins was carried out by Double beam UV/VIS spectrophotometer (TU-1901, PGENENAL, Beijing, China).

Results

Comparison of Microwave Assisted Extraction (MAE) with other conventional methods: Shaking extraction, Ultasonic extraction, Heat reflux extraction and Supercritical Fluid Extraction (SFE).

Methods	**Extraction Time** (min)	**Solvent volume** (ml)	**Yield** (%)
Shaking	12 h	400	2,58
Ultrasonic	30 min	200	1,72
Heat reflux	2 h	200	2,22
SFE	3 h	60	1,52
MAE	20 min	30	5,11

Shaking: 10 g of sample was extracted twice for 6 h with 200 ml of Ethanol (95% v/v) at room temperature.

Ultrasonic: 4 g of sample was extracted twice for 15 min with 100 ml of ethanol (95% v/v).

Heat Reflux: 4 g of sample was extracted twice for 1 h at

SFE: 80 g of sample was extracted for 3 h with carbon dioxide and 60 ml of ethanol (95% v/v) at 55°C and 55 MPa.

MAE: 3 g of sample was extracted for 20 min with 30 ml of ethanol (95% v/v) at 80 °C.

Samples

3 g of plant material.

Extraction solvent

30 ml of ethanol (95% v/v)

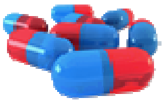
Procedure

1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the of solvent and the magnetic stirring bar.
4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and further manipulate the solution according to the analytical technique.

Microwave Program

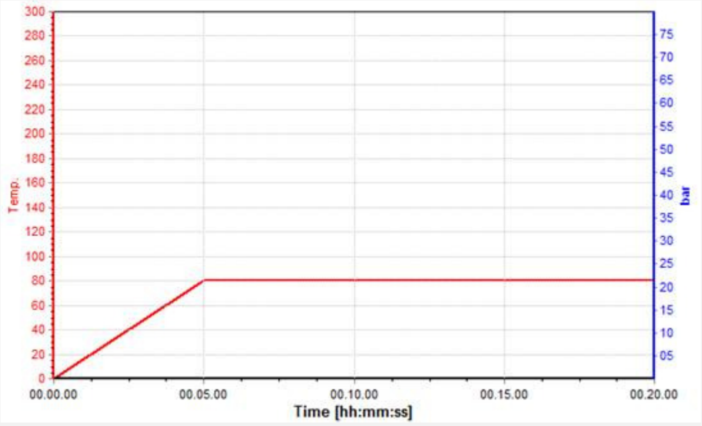
Step	**Time** (min)	**Power** (W)	**Temperature** (°C)
1	5	Up to 1000*	80
2	15	Up to 1000*	80

* Use up to 500 Watt for operations with 3 or less vessels



78℃ with 100 ml of ethanol (95% v/v).

Temperature Profile



Comments

The results confirm that microwave extraction were found to be significantly faster, with recovery superior to the conventional methods, with a substantial reduction of the amount of solvent required. Mainly the short extraction time compared to the other extraction techniques and the possibility to process up to 41 vessels simultaneously offer a great opportunity for laboratories with high throughput.

simultaneously.

Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (℃)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

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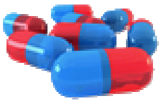

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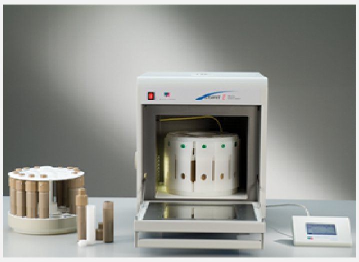


Application Method ME016

Extraction method for determination of drugs in human saliva

Introduction

Over the past two decades, saliva has been increasingly employed to monitor drug use in the workplace and schools and for criminal purposes. In this method is shown the comparison of two liquid-liquid extraction procedures: Toxitubes A® and microwave-assisted extraction (MAE)

Instrumentation

Analyses were performed on a model 2695 chromatograph from Waters® (Milford, MA) connected to a model 996 photodiode array detector, also from Waters®.

Results

The between-day precision and accuracy of the method were estimated by analysis of five saliva samples containing the drugs at two different concentrations (0,1 and 1,0 µg/ml). The precision was calculated as the relative standard deviation (RSD) and the accuracy was expressed as a percentage of the difference between the measured concentration and the true value for the same concentration.

Drug	**Extraction method**	**RSD (%)** (n=5)	**Relative error (%)** (n=5)	**Recovery (%)**
Morphine	MAE	3,22	3,63	83
	Toxitubes A	2,91	2,70	67
6AM	MAE	3,23	1,50	90
	Toxitubes A	2,79	1,18	53
BEG	MAE	1,89	3,13	89
	Toxitubes A	4,55	2,55	69
Cocaine	MAE	4,51	3,23	94
	Toxitubes A	3,20	5,05	86
Cocaethylene	MAE	2,23	2,73	100
	Toxitubes A	3,64	5,80	95
EDDP	MAE	2,29	5,70	94
	Toxitubes A	4,19	3,05	72
Methadone	MAE	2,53	2,40	96
	Toxitubes A	4,24	1,93	77

6AM: 6-acetylmorphine

BEG: benzoylecgonine

EDDP: 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine

Sample

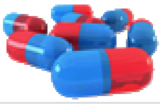
1 ml of saliva.

Extraction solvent

10 ml of chloroform.

Procedure

1. Place in the TFM vessel 1ml of saliva
2. Introduce the TFM vessel into the HTC safety shield.
3. Add 10 ml of chloroform with the magnetic stirrer and the Weflon button.
4. Close the vessel and introduce it into the rotor.
5. Connect the temperature sensor to the reference vessel.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and clean-up the solution according to the analytical technique[1].

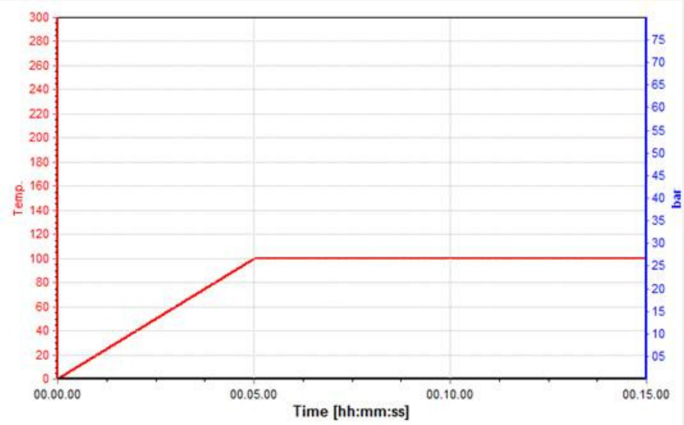


Microwave Program

Step	Time (min)	Power (W)	Temperature (℃)
1	5	Up to 1000 *	100
2	10	Up to 1000 *	100

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	Nr. of vessels	Vessel volume (*ml*)	Max. T (℃)	Max. P (*bar*)	Sample amount (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

Combining the HPLC-DAD technique and Microwave-Assisted Extraction (MAE) to prepare the saliva samples provide a good sensitivity and specificity for the determination of drugs of abuse in saliva, a biological matrix which has proved to be effective and useful in diagnosing overdose. The extraction efficiency of MAE was similar to that achieved using the Toxitubes A, with the possibility of processing up to 41 samples simultaneously.

Reference

1) P. Fernandez, L. Morales, C. Vàzquez, M. Lago, A.M. Bermejo; *Journal Of Applied Toxicology* 2008; 28: 998-1003

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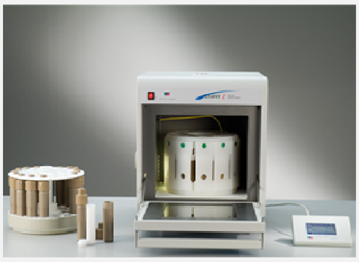


Application Method ME017

Extraction of PAHs from fish

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are widespread environmental pollutants. Adverse effects of PAHs on living organism have been studied for decades. Some of them have been classified as carcinogenic and mutagenic; 16 PAHs were listed as priority pollutants by the U.S. Environmental Protection Agency (EPA).

Instrumentation

The extraction was performed in a dedicated microwave extraction unit, ETHOS E by Milestone equipped with SK-12 rotor. Chromatographic separations were developed in a system composed by a 600E pump, with a gradient controller (Waters, Milford, MA, USA), UV-VIS diode array and fluorescence detectors in series (HP Series 1100, Agilent, Waldbrom, Germany).

Results

Measured concentrations and confidence intervals compared to certified one in NIST SRM 2977 mussel tissue.

Compound	**Certified** (ng/g)	**Measured** (ng/g) (ẋ ± 2 SD) (n=5)	**Extraction time** (min)
B[a]A	20,34 ± 0,78	20,4 ± 1,4	27
B[b]F	11,01 ± 0,28	10,2 ± 1,3	27
B[a]P	8,35 ± 0,72	7,0 ± 2,2	27
DB[a,h]A	1,41 ± 0,19	1,5 ± 0,6	27
I[1,2,3-cd]P	4,84 ± 0,81	4,1 ± 0,8	27

Legend

B[a]A: benz[a]anthracene, **B[b]F**: benzo[b]fluoranthene

B[a]P: benzo[a]pyrene **DB[a,h]A**: dibenz[a,h]anthracene

I[1,2,3-cd]P: indene[1,2,3-cd]pyrene

Sample

0,2 g NIST SRM 2977 mussel tissue.

Extraction solvent

4 ml of saturated methanolic potassium hydroxide solution and 10 ml of n-hexane.

Procedure

The method was developed to process 1 g of fresh fish sample or 0,200 g of lyophilized materials reconstituted with 0,8 ml of ultrapure water.

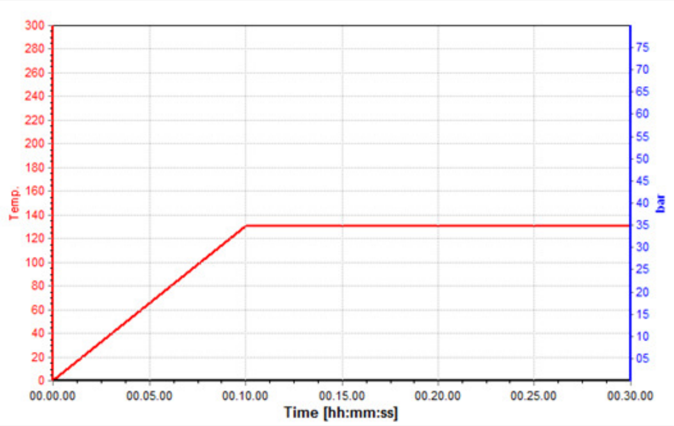
1. Place a TFM vessel on the balance plate, tare it and weigh the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add 4 ml of saturated methanolic potassium hydroxide solution (it is used to digest the lipids in the fish samples in order to avoid to interfere with the analytes during chromatographic separation) and 10 ml of n-hexane with the magnetic stirrer.
4. Close the vessel and introduce it into the rotor.
5. Connect the temperature sensor to the reference vessel.
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and clean-up the solution according to the analytical technique[1].

Microwave Program

Step	**Time** (min)	**Power** (W)	**Temperature** (℃)
1	10	Up to 1000 *	130
2	17	Up to 1000 *	130

*Use up to 500 Watt for operations with 3 or less vessels simultaneously.

Temperature Profile



Choice of Vessels

Rotor type	**Nr. of vessels**	**Vessel volume** (*ml*)	**Max. T** (℃)	**Max. P** (*bar*)	**Sample amount** (*g*)
SK-6LV	6	270	200	10	30
SK-12	12	100	260	35	15
PRO-16	16	75	200	30	10
PRO-24	24	75	200	30	10
Multiprep	41	65	200	20	5

Comments

The developed procedure allows the quick and efficient extraction of all regulated PAHs in fish samples by simultaneously hydrolizing the fats in samples and extracting the hydrocarbons of interest in hexane. Mainly the short extraction time compared to 24 hours Soxhlet extraction and the treatment up to 41 vessels simultaneously offer a great opportunity for laboratories with high sample throughput.

Reference

1) T. Pena, L. Pensado, C. Casais, C. Mejuto, R. Phan-Tan-Luu, R. Cela; *Journal of Chromatography A*, 1121 (2006) 163-169

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