Automated Solid-Phase Extraction of Wastewaters and Surface Waters for Polynuclear Aromatic Hydrocarbons and Phthalates for EPA Method 625

INTRODUCTION

EPA method 625 uses liquid-liquid extraction by Separatory funnel combined with gas chromatography with mass spectrometry detector for the analysis of PAH's and Phthalates in municipal and industrial waste water.

Unlike the drinking water methods, however, analysis of wastewaters and the streams they flow into need to contend with particulates. The Dionex AutoTrace® SPE instrument has features that allow analysis of samples even with high amounts of suspended solids.

The loading pumps are able to load samples at a given rate even if the bed begins to block. The solvent delivery syringe is able to deliver solvent under sufficient positive pressure to overcome most blockages. If the bed does get completely blocked, the sample is still in the original container from where it can be retrieved for further processing without loss.

AUTOTRACE EXTRACTION PROCEDURES

2.5 mL of methanol and 2 mL of concentrated sulfuric acid is added to 500 mL of sample. Surrogates for Method 625 are added and monitored for extraction efficiency. However, only 2-fluorobiphenyl, 2,4,6-tribromophenol and terphenyl-d14 are expected to perform well. More specifically, 500 mL of sample was spiked with 50 µL of 1000 ng/µL Base/Neutral Surrogate solution and 100 µL of 2000 ng/µL Acid Surrogate solution. Analytes were fortified into the samples by adding 25 µL of 2000 ng/µL Spiking Solution.

AutoTrace Instrument for Automated SPE

SPE Cartridge Used:

ISOLUTE® C18(EC) 500 mg in a 6 mL cartridge with a polyurethane plug prefilter, part no. 221-0050-CD.

AUTOTRACE EXTRACTION PROCEDURE

Process 6 Samples using the following procedure:

Step 1: Wash syringe with 2 mL of CH_3OH

Step 2: Rinse column with 5 mL of EtOAc into solvent waste

Step 3: Rinse column with 5 mL of DCM into solvent waste

Step 4: Condition column with 10 mL of CH_3OH into solvent waste

Step 5: Condition column with 10 mL of water into aqueous waste

Step 6: Pause and alert operator, resume when Continue is pressed

Step 7: Load 550 mL of sample onto column

Step 8: Dry column with gas for 10 min

Solvents	Flow Rate	
Water (reagent grade)	Cond. Flow:	40 mL/min
CH_3OH (methanol)	Load Flow:	20 mL/min
EtOAC (ethyl acetate)	Rinse Flow:	40 mL/min
DCM (dichloromethane)	Cond. Air Push:	15 mL/min
Unused (load with methanol)	Elute Flow:	20 mL/min
	Elute Air Push:	5 mL/min

Step 6 is added so that conditioning steps can be started before final sample preparations are completed. This would alleviate concern that the instrument will try to load samples before the operator is ready.

SAMPLE ELUTE PROCEDURE

Process 6 Samples using the following procedure:

Step 1: Collect 5 mL fraction into sample tube using EtOAc

Step 2: Manually Rinse sample container with 7 mL to collect

Step 3: Manually Rinse sample container with 10 mL to collect

Step 4: Collect 2 mL fraction into sample tube using DCM

Solvents	Flow Rate	
Water (reagent grade)	Cond. Flow:	40 mL/min
CH_3OH (methanol)	Load Flow:	3 mL/min
EtOAC (ethyl acetate)	Rinse Flow:	40 mL/min
DCM (dichloromethane)	Cond. Air Push:	15 mL/min
Unused (load with methanol)	Elute Flow:	20 mL/min
	Elute Air Push:	5 mL/min

Note change in Load Flow rate.

Manual Rinse Details

At Step 2, add 5 mL of ethyl acetate to sample container, at Step 3, add 5 mL of dichloromethane to sample container.

All channels that succeed in delivering solvent from its sample container are now finished. The channel's tension is released and the extracts removed, dried and concentrated to 1 mL or to a final volume as dictated by method 625 or Detection Limit requirements.

For any channel that fails to push the contents of the sample container through the SPE cartridge, load Block Shocker Program and run on effected channels.

BLOCKED SHOCKER PROGRAM:

Process 6 Samples using the following procedure:

Step 1: Collect 3 mL fraction into sample tube using EtOAc

Step 2: Manually Rinse sample container with 15 mL to collect

Step 3: Collect 2 mL fraction into sample tube using DCM

Do not add more solvent at Step 3. This program seeks to empty the sample container of solvent already contained.

If the loading pump is still unable to deliver solvent through SPE cartridge, Blocked Shocker can be run twice more. The solvent forced through by the piston will suffice to extract the analytes from the SPE cartridge.

Once all channels that successfully loaded have been eluted, resume work on samples that are blocked by starting at the Sample Load Program with a fresh SPE cartridge on a different channel, loading the remainder of the sample. Elute both cartridges as described above and combine the extracts into one.

DISCUSSION

The solvent delivery piston is more able to force liquid through the SPE than is the loading pump. Sometimes, the resistance is caused by residual water surface tension in the SPE bed. Forcing solvent through SPE bed with the syringe will reduce the resistance for the loading pump. If the sample contains sufficient solids to plug an SPE cartridge, the likelihood that analytes have adsorbed to the wall of the container and not been transferred to the cartridge is reduced, thus the solvent delivered by Blocked Shocker can be assumed to contain all of the analytes present in the sample.

Replicates (n=4) of two different river water samples were extracted by the above method. Similar samples from the same locations were used in a separate study looking at organochlorine pesticides. In AN 815, the use of the Blocked Shocker Program was needed. In this study it was not, possibly due to the sulfuric acid. In this case, filtration of the sample prior to any pretreatment may be required.

Analysis of Example Spike Recoveries

Samples were on a gas chromatograph equipped with mass selective detectors. The following results were obtained using a Hewlett Packard 6890 GC with an Agilent 5973 MSD on a 30 meter long, 0.25 mm ID capillary column with a 0.5 µm film coating.

Table 1. Iowa River Water, Iowa City, IA, Collected 9/13/02, Estimated Total Suspended Solids, 70 mg/L						
Compound	**S4786.2**	**S4786.3**	**S4786.5**	**S4786.6**	**Mean**	**Std. Dev.**
SS:2-Fluorobiphenyl	57.6%	71.1%	65.2%	77.2%	67.6%	8.4%
SS:Terphenyl-d14	87.1%	85.6%	91.2%	96.5%	90.1%	4.9%
SS:2,4,6-Tribromophenol	100.3%	109.2%	101.7%	105.2%	104.1%	4.0%
Acenaphthylene	40.4%	53.2%	45.7%	59.9%	50.0%	8.4%
Acenaphthene	36.1%	48.0%	44.7%	54.7%	45.8%	7.7%
Anthracene	61.1%	72.1%	74.5%	74.6%	70.6%	6.4%
Benzo[a]anthracene	69.8%	79.6%	86.2%	80.9%	79.1%	6.8%
Benzo[b]fluoranthene	67.6%	78.8%	84.6%	80.0%	77.8%	7.2%
Benzo[k]fluoranthene	72.6%	78.9%	86.6%	79.3%	79.4%	5.8%
Benzo[g,h,i]perylene	70.5%	66.7%	84.8%	80.1%	75.5%	8.4%
Benzo[a]pyrene	69.5%	79.8%	86.4%	80.8%	79.1%	7.0%
Chrysene	86.5%	92.1%	99.2%	94.3%	93.0%	5.3%
Dibenza[a,h]anthracene	67.1%	72.3%	82.1%	77.2%	74.7%	6.4%
Fluoranthene	60.8%	70.1%	72.2%	66.7%	67.4%	5.0%
Fluorene	42.8%	57.1%	53.9%	55.9%	52.4%	6.6%
Indeno[1,2,3-cd]pyrene	69.7%	71.4%	85.6%	80.4%	76.8%	7.5%
Naphthalene	35.9%	57.1%	41.4%	63.6%	49.5%	13.0%
Phenanthrene	58.4%	69.2%	70.2%	69.9%	66.9%	5.7%
Pyrene	70.1%	76.0%	84.6%	86.7%	79.4%	7.7%
Diethylphthalate	88.8%	94.0%	87.2%	91.2%	90.3%	3.0%
Dimethylphthalate	83.8%	86.9%	81.6%	88.4%	85.2%	3.1%
Di-n-butylphthalate	85.6%	90.3%	94.2%	92.0%	90.5%	3.6%
Di-n-octylphthalate	77.2%	94.4%	98.3%	109.9%	94.9%	13.6%

SUMMARY

Solid Phase Extraction provides reduced solvent consumption and sample handling when compared to traditional liquid-liquid extraction techniques. The AutoTrace Work station automates the conditioning loading rinse and eluting steps of Manual SPE that normally require extensive involvement by an operator. AutoTrace provides both automation of the SPE stepsand positive pressure flow of sample and solvent through the cartridges. Positive pressure flow of the sample and solvent ensure an uniform extraction and elution.

These features contribute greatly to increased laboratory productivity and reliable results for determination of organic pollutants in wastewater.

Table 2. Cedar River Water, Rochester, IA, Collected 9/13/02, Estimated Total Suspended Solids, 64 mg/L						
Compound	**S4786.2**	**S4786.3**	**S4786.5**	**S4786.6**	**Mean**	**Std. Dev.**
SS:2-Fluorobiphenyl	55.3%	66.5%	59.2%	61.6%	60.6%	4.7%
SS:Terphenyl-d14	74.5%	62.4%	60.1%	77.9%	68.7%	8.8%
SS:2,4,6-Tribromophenol	104.7%	94.5%	101.3%	96.2%	99.2%	4.7%
Acenaphthylene	50.1%	48.1%	52.6%	46.7%	49.4%	2.6%
Acenaphthene	45.3%	43.8%	49.4%	43.0%	45.4%	2.9%
Anthracene	60.4%	52.4%	63.3%	31.7%	51.9%	14.3%
Benzo[a]anthracene	70.1%	58.5%	70.5%	69.8%	67.2%	5.8%
Benzo[b]fluoranthene	68.8%	56.2%	69.2%	70.8%	66.2%	6.7%
Benzo[k]fluoranthene	68.5%	60.3%	69.4%	67.2%	68.2%	5.9%
Benzo[g,h,i]perylene	74.1%	58.6%	61.6%	73.0%	65.4%	6.8%
Benzo[a]pyrene	70.0%	59.2%	68.6%	82.8%	67.7%	6.0%
Chrysene	81.7%	70.7%	80.9%	80.1%	79.0%	5.6%
Dibenza[a,h]anthracene	86.6%	72.0%	75.3%	57.3%	78.5%	6.3%
Fluoranthene	51.4%	46.4%	56.4%	47.4%	52.9%	5.0%
Fluorene	47.7%	44.6%	52.9%	66.5%	48.2%	3.4%
Indeno[1,2,3-cd]pyrene	75.4%	61.9%	66.5%	44.4%	68.7%	5.8%
Naphthalene	48.5%	43.0%	44.4%	43.3%	44.5%	2.8%
Phenanthrene	55.2%	49.4%	58.2%	57.1%	55.0%	3.9%
Pyrene	68.3%	57.8%	66.8%	68.5%	65.3%	5.1%
Diethylphthalate	87.6%	82.5%	85.5%	82.3%	84.5%	2.5%
Dimethylphthalate	82.5%	80.9%	81.9%	79.2%	81.1%	1.5%
Di-n-butylphthalate	75.6%	71.5%	76.2%	75.3%	74.7%	2.1%
Di-n-octylphthalate	90.5%	79.8%	89.6%	96.5%	89.1%	6.9%

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