Enhanced Method Development Capabilities with Automated SPME Extraction Optimization

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Overview:

Purpose:
- Automate the selection of the most suitable type of solid phase micro extraction (SPME) fiber for the analysis of a number of contaminants in corn oil and water samples.
- Optimize extraction conditions (agitation time and temperature) for different types of SPME fibers for both head space and liquid immersion applications.
- Analyze extracted samples by GC/MS.
- Minimize the time required for method development.

Methods:
- 2 g of corn oil was spiked with 1 µg of 15 different analytes in 3 groupings for headspace extraction.
- 18 mL of water was spiked with 2 µg of 15 different analytes in 3 groupings for liquid immersion extraction.
- SPME with 4 common types of SPME fibers.
- PAL RTC robotic sampler handler for automated SPME extraction, incl. automated change of fibers and injection.
- GC/MS analysis of extracts with an Agilent 5975C GC-MS.

Results:
- A workflow is described that enables the automated selection of the most suitable extraction conditions (type of fiber, extraction time and temperature) for the GC/MS analysis of a number of contaminants in oil and water samples.
- Applying the automated workflow described above the total time to identify the most suitable fiber and then optimize conditions was 4.5 days. Compared to performing the experiments manually which is estimated to be15 days, this represents of time saving of 10 days.

Introduction:

- SPME (solid phase micro extraction; s. ref 1) is an analyte enrichment method commonly used for the analysis of gaseous or liquid samples in conjunction with gas chromatography/mass spectrometry.
- An important part of method optimization is the selection of the appropriate fiber. This normally requires the manual changing of fibers. In practice, little fiber optimization work is actually done and most methods used rely on analyst experience.
- A new type of robotic sample handler allows for the automatic change of tools. This opens the possibility for the automated selection of the best suited SPME fiber as well as the optimization of key process parameters.

Methods:
- A novel PAL RTC robotic sample handler was used to perform extraction with SPME fibers and injection into the GC.
- PAL Sample Control Software v. 1.0 controlled the RTC and data acquisition with the Agilent GC/MS instrument. GC/MS methods were written with Chemstation v. E.02.01.1177.

SPME headspace experiments:
- Samples of 2.00 ± 0.02 grams corn oil were transferred into 20 mL sample vials sealed with magnetic caps and then spiked with 1µL of each spiking solution (see below) in separate vials.
- The spike level is approximately 0.5 ppm for each analyte in the corn oil.
- The spiking solutions were prepared at approx. 25.0 mg of each analyte and diluted with 25 mL of dichloromethane (DCM).
- Blanks of the corn oil were prepared and any signal for an analyte of interest was subtracted from the sample.

Table 1:
Masses and CAS numbers of compounds added to Spiking Solution #1 for condition optimization testing.

Compounds in Spiking Solution #1	CAS #	Mass (mg)
Furfuryl butyrate	623-21-2	25.8
2,3-Hexanedione	3848-24-6	25.6
Octyl aldehyde	124-13-0	25.2
Valeraldehyde	110-62-3	24.9

Table 2:
Masses and CAS number of compounds added to Spiking Solution #2 for condition optimization testing.

Compounds in Spiking Solution #2	CAS #	Mass (mg)
Acetic acid	64-19-7	27.0
Octanoic acid	124-07-2	24.9
Octanol	111-87-5	25.3
Ethanol (Everclear)	64-17-5	25.5

Table 3:
Masses and CAS number of compounds added to Spiking Solution #3 for condition optimization testing.

Compounds in Spiking Solution #3	CAS #	Mass (mg)
4-Nitroaniline	100-01-6	25.3
2,4,6-Trichloroanisole	87-40-1	25.6
4-Nitrophenol	100-02-7	25.6
2,3-Dimethylpyrazine	5910-89-4	26.4
Di-2-ethylhexyl phthalate (DEHP)	117-81-7	28.3
Dibutyl disulfide	629-45-8	25.8

- The vials were robotically transferred to the agitator and the SPME fiber was exposed in the head space of the vials for 30 minutes under agitation @250rpm with 5 sec on and 2 sec off for enrichment at 50 and 65°C, respectively.
- Desorption of analytes was achieved in the GC injector for 5 minutes at 270°C.
- After each series of analyses the fiber was exchanged robotically.

Liquid immersion extraction of water samples with SPME fibers.
- Water samples were prepared by pipetting 18 mL of water in 20 mL sample vials sealed with magnetic caps and spiked with 2 µL of each spiking solution into 3 different vials.
- Blanks water samples were prepared and responses for analytes of interest were subtracted from the sample respones.
- The amount of time in which the SPME fiber was exposed to the liquid sample was varied (15 and 30 minutes).
- Samples were agitated @250rpm with the agitator 5 sec on and 2 sec off at 30°C.
- Fibers were immersed in the water for the designated amount of time to enrich analytes.
- Desorption in the GC was for for 5 minutes at 270°C.

SPME fibers and conditioning
Four different SPME fibers were used to determine the most effective fiber for each set of conditions:
- 75 µm Carboxen(CAR)/ Polydimethylsiloxane(PDMS) Blue fibe
- 50 µm Divinylbenzne(DVB)/CAR/PDMS Gray fiber
- 85 µm Polyacrylate(PA) White fiber
- 65 µm PDMS/DVB Pink fiber

- Each SPME fiber was conditioned at a specific temperature for 30 minutes as indicated by the manufacturer and underwent a 5 minute conditioning before being used for each set samples which consisted of a blank vial and a vial containing each spiking solution in corn oil or water.

GC-MS analysis was performed on an Agilent 5975C GC-MS

GC Method
- Initial temperature of 10°C, held for 3 minutes
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- The helium gas flow was 1ml/min
- Injection temperature was 280°C in split less mode.
- Column used was an Agilent DB-5MS (30 m x 0.250 mm X 1µm)

MS parameters:
- Scan Mode 29-350 amu
- Source Temperature 220°C
- Transfer Line Temperature 280°C

Results:

Head Space Extraction
The results from the head space analysis of the corn oil samples are shown below.
The response values in Figures 1 and 2 have been corrected for any response observed for each analyte in the blank corn oil.
The analytes Ethanol, DEHP, Octanoic acid, and Nitroanaline were not detected.

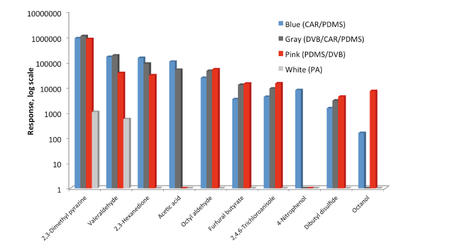

Figure 1: Comparison of compound responses from different SPME fibers by head space extraction using an enrichment temperature of 50°C.

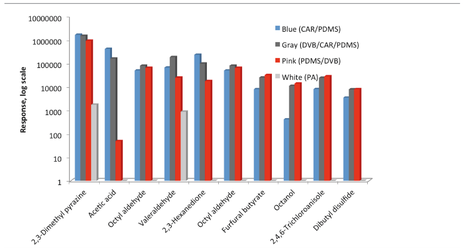

Figure 2: Comparison of compound responses from different SPME fibers by head space extraction using an enrichment temperature of 65°C.

Liquid Immersion Extraction
The data obtained from the liquid immersion experiment is shown in Figure 3 for the samples analyzed where the SPME fibers was exposed to the solution (Enrichment) for 15 minutes and Figure 4 for the samples that were enriched for 30 minutes. Both sets of data were taken with the agitator set at 30°C with an incubation time of 15 minutes. The responses have been corrected for any response observed in the blank water sample for the analytes.

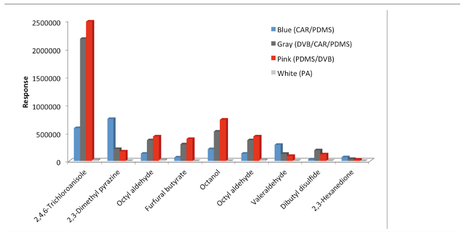

Figure 3: Comparison of compound responses from different SPME fibers by liquid immersion extraction using a 15 minute enrichment times.

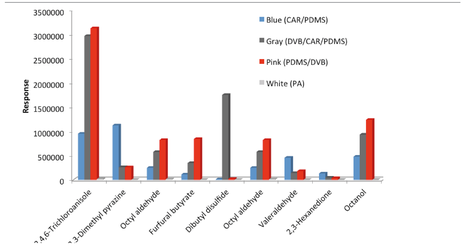

Figure 4: Comparison of compound responses from different SPME fibers by liquid immersion extraction using a 30 minute enrichment times.

Enrichment time
A time course study was also performed by using the best suited fiber selected in the automated workflow described above (75 µm Carboxen(CAR)/ Polydimethylsiloxane(PDMS) Blue) under the same conditions as described for the head space extraction method. The data was acquired using an agitator temperature of 65°C and varying the amount of time that the blue SPME fiber was exposed in the head space of the oil sample during the enrichment process. The enrichment times used were 5, 30, 60 and 120 minutes. The processed data values obtained for the time dependent head space study are shown in Figure 5 and shows the comparison of compound responses with the blue SPME fiber at the different enrichment times. Again any response observed in the blank for an analyte was subtracted from the sample response.

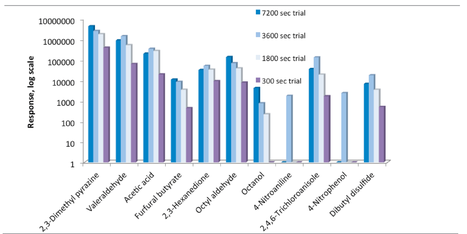

Figure 5: Comparison of compound responses with the blue CAR/PDMS SPME fiber by head space extraction using 5, 30, 60 and 120 minute enrichment times.

In some cases the 120 min extraction time response was greater than the 60 min extraction time and in some cases the 60 min response was greater. This could be a result of adsorption/desorption of the fiber over the extraction time period. To look at that question the vial heating time and extraction time were varied. The results are show in figures 6 and 7.

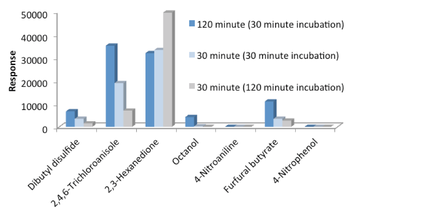

Figure 6: Comparison of compound responses with the blue CAR/PDMS SPME fiber by changing the vial heating time and enrichment times.

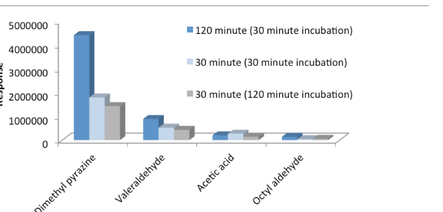

Figure 7: Comparison of compound responses with the blue CAR/PDMS SPME fiber by changing the vial heating time and enrichment times.

Conclusions:

- A workflow has been described enabling the automated selection of the most suitable extraction conditions (type of fiber, extraction time and temperature) for the GC/MS analysis of a number of contaminants in oil and water samples.
- Among the four fibers tested the 75 µm Carboxen (CAR)/ Polydimethylsiloxane (PDMS) Blue fiber was identified as the most suitable. The optimal (time vs. response) extraction conditions identified were 60 min. at 65°C.
- Applying the automated workflow described above the total time to identify the most suitable fiber and then optimize conditions was 4.5 days. Compared to performing the experiments manually which is estimated at 15 days this represents of time saving of 10 days.

Reference: Arthur, C. L.; Pawliszyn, J. Anal. Chem. 1990, 62, 2145-2148