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Microwave mediated distillation with solid-phase microextraction: determination of off-flavors, geosmin and methylisoborneol, in catfish tissue

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Abstract

Presented is a rapid distillation device for use with solid-phase microextraction (SPME). We apply this device specifically for determining two semivolatile off-flavor compounds, methylisoborneol and geosmin, in channel catfish. The presence of these two compounds in channel catfish filets results in unwelcome tastes. In the presented procedure, a catfish tissue sample is placed within a sample container located inside the microwave device. Microwave radiation is applied and distillates formed migrate through a condenser via a purge gas and are collected in a sample vial. A SPME fiber is placed within the stirred collected distillate and methylisoborneol and geosmin are extracted. Qualitative and quantitative results of these extractions are obtained using a gas chromatograph-ion trap mass spectrometer. This solventless technique results in detection limits far below the human threshold for these off-flavor compounds in channel catfish.

Keywords: Off-flavour compounds; Catfish; Distillation device; Geosmin; Methylisoborneol

1. Introduction

Microwave extraction processes have become popular for the determination of many compounds including polynuclear aromatic hydrocarbons (PAHs) [1], phenols [2] and pesticides [3] in a wide variety of matrices including soils, sediments and animal tissues. The popularity of this technique is due to the rapid rates of heat transfer provided by microwave heating, which allows quicker times of analysis of analytes in complex samples compared to more traditional Soxhlet or liquid extractions but with similar results realized [4]. In this technique, microwave radiation is applied to a sample in a container containing an extraction solvent. Only the sample absorbs an appreciable amount of the applied microwave radiation.

The heat generated allows analytes to rapidly partition from the sample matrix into the extraction solvent. To achieve necessary limits of detection, analytes in the solvent are typically concentrated. This involves evaporating the solvent into the atmosphere via a flow of inert gas. Current trends in sample preparation are to reduce or eliminate the use

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of organic solvents in the laboratory due to their associated costs and hazards.

Recently, water at subcritical conditions has been used successfully as an alternative to organic solvents as an extraction solvent. Comparable results versus organic solvent extraction were obtained for the recovery of PAHs from soil and air particulate matter [5] and flavor ingredients in food products [6]. Solid-phase microextraction (SPME), [7,8] because of its ability to extract organic compounds from an aqueous matrix, was used to recover the analytes prior to instrumental determination. The benefits of using water as an extraction solvent are obvious: plentiful, non-toxic and inexpensive.

In steam distillation procedures, water is also utilized as an extraction solvent. This paper presents a rapid steam distillation–extraction technique mediated by microwave radiation. A sample of high aqueous content is placed in a sample container located within a microwave oven. Microwave radiation is applied thus forming steam, which extracts analytes from the sample, together forming a distillate. This distillate is condensed outside the microwave oven into an aqueous extract. Analytes are subsequently extract onto a SPME fiber, are then separated and detected with gas chromatography-ion trap detection.

Of particular interest is the determination of off-flavor compounds in catfish tissue. Off-flavor compounds, geosmin (GEO) and methylisoborneol (MIB), are responsible for earthy and musty taste characteristics, respectively, in food items such as channel catfish [9,10]. These off-flavor compounds may bioaccumulate from culture pond water into the tissue of channel catfish. Concentrations of MIB and GEO above the low parts-per-billion (ppb) level may render a catfish filet inedible and consequently significantly reduce its economic value.

Determination of these compounds in catfish tissue at these levels requires a method of low detectability. Microwave mediated distillation with solid phase extraction has been successfully applied for the determination of GEO and MIB in catfish tissue with detection limits at the sub ppb level [11].

The modification presented here replaces the solid phase extraction (SPE) step with solid phase micro extraction (SPME), thus eliminating the use of organic solvents. A catfish tissue sample is rapidly heated in a sample container located within a temperature controllable microwave oven. Distillates formed migrate from the sample container to a condenser located outside the microwave oven. The distillate is collected and GEO and MIB are concentrated on a SPME fiber. Detection limits for both off-flavor compounds are at the sub ppb level.

2. Experimental

2.1. Chemicals and materials

GEO was purchased from Wako Pure Chemicals (Osaka, Japan) and MIB was prepared by the organic synthesis group at the National Center For Toxicological Research (NCTR), Jefferson, AR according to the procedure of Wood and Snoeyink [12]. The internal standard, *cis*-decahydro-1-naphthol (DHN), was purchased from Aldrich Chemical Company (Milwaukee, WI). The solid-phase microextraction (SPME) device was acquired from Supelco, Inc (Bellefonte, PA).

2.2. Instrumentation

A Varian (Walnut Creek, CA) Saturn system, which consists of a 3400 capillary gas chromatograph coupled to an ion trap detector (GC–ITD) was used. The gas chromatograph was equipped with a septum programmable injector (SPI). The capillary column (JW Scientific, Folsom, CA) was 30 m×0.25 mm ID, with a DB5MS stationary phase 0.25 μm in thickness.

2.3. Microwave set-up

The microwave mediated distillation–solid-phase microextraction apparatus is depicted in Fig. 1. The microwave oven used was a CEM (Matthews, NC) Model 300. The original use of this oven was for rapid ashing of samples. In our design, the insulation was removed to allow a sample container to fit inside the oven chamber. In efforts to prevent damage to the magnetron, microwave absorbing elements, part of the original microwave ashing configuration, were kept inside the chamber to collect radiation not being absorbed by the catfish. A small hole was drilled on

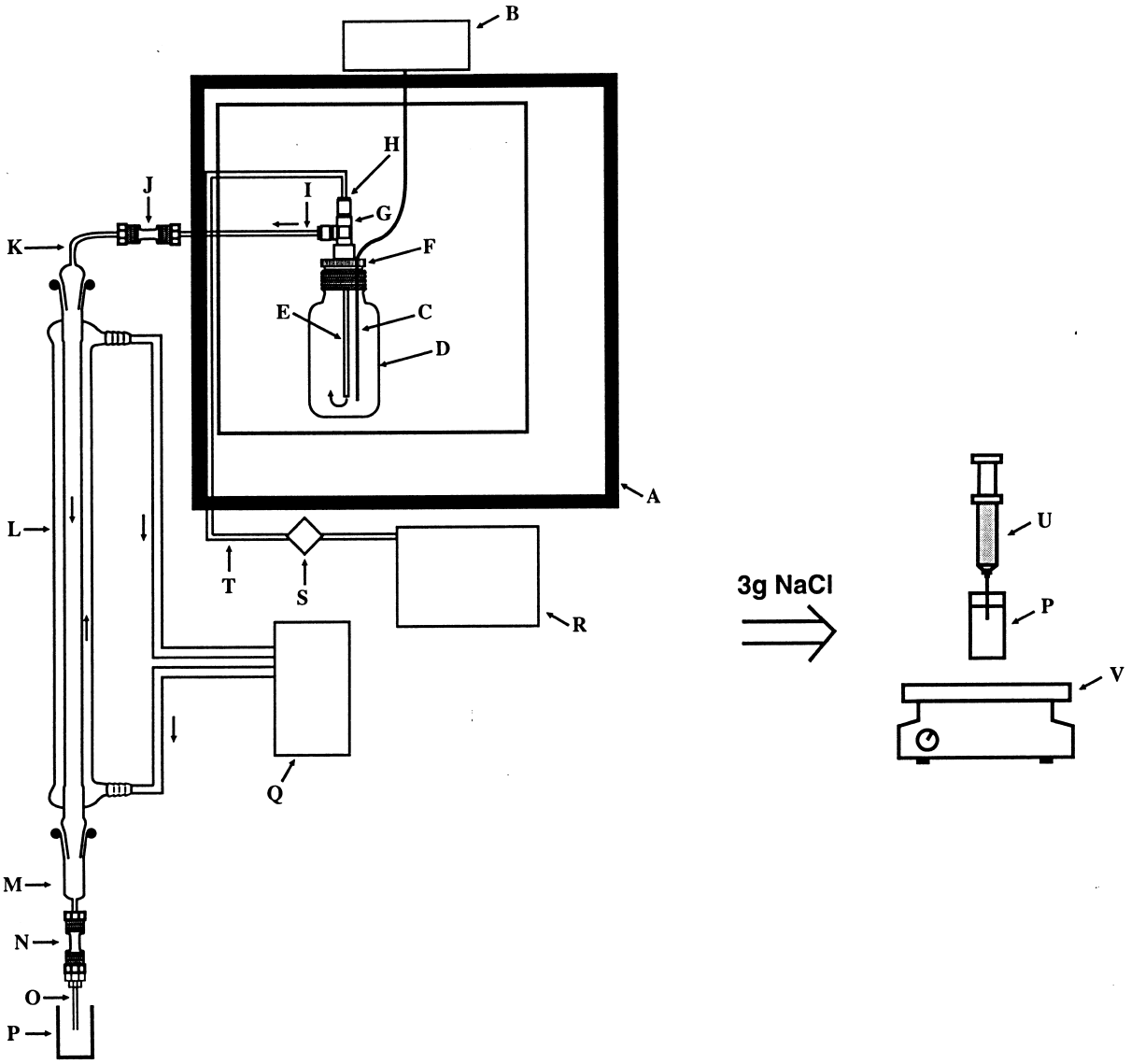

Fig. 1. Microwave mediated distillation solid-phase microextraction set-up.

the side of the microwave that just accommodated a 1/8 in. (1 in.=2.54 cm) PTFE tube (*T*) for the argon purge flow. This microwave opening was sealed with silicone glue. The argon supply (*R*) was regulated by a rotometer. A check valve (*S*) was placed in-line to prevent the reverse flow of distillates. The catfish sample was enclosed within a No. 25 Ace-Tred hydrogenation flask (*D*) (Ace Glass, Vineland, NJ) and a No. 25 Ace-Tred PTFE nut (*F*) (Ace Glass) equipped with a Teflon O-ring. A Swagelok PTFE Teflon male tee (*G*) (Evansville Valve and Fitting, Evansville, IN) was attached by threads to a center opening in component *F*. The argon sparge gas tube (*T*) was fitted through component *G* and secured with a 1/8 in. nut and ferrule. The Teflon tube, *T*, was extended to the bottom of the container. An opening was drilled in component *F* to secure a thermocouple (*C*) that extended into container *D*. A

1/4 in. Teflon tube (*I*) was attached to the male tee with a 1/4 in. nut and ferrule. Another opening was drilled in the microwave oven to just accommodate tube *I* which directed the flow of distillates and argon into the condenser. This opening was also sealed with silicone glue. Outside the microwave oven, tube *I* was connected by a Swagelok Teflon 1/4 in. union (Evansville Valve and Fitting) to a laboratory home made Pyrex adaptor (*K*). This adaptor allowed for the 90 connection of tube to the condenser (*L*), having a 14/20 ground glass coupling. The temperature of the condenser (*L*) was controlled by a bench top recirculating cooling unit (*Q*) (Model RB2055AO, FTS Systems, Stone Ridge, NY). At the bottom of the condenser a Pyrex fitting (*M*) which tapered to a 1/4 in. tube, connected to a 1/4 in.–1/8 in. PTFE reducer (*N*) (Evansville Valve and Fitting). Secured to the male end of the ground glass 14/20 joints were Teflon sleeves that ensured a leak-tight seal. Connected to the 1/8 in. end of this reducer was a 3 in. section of Teflon tubing (*O*). A 10 mL glass vial (*P*) was placed directly below this tube to collect distillate formed from the extraction process. Components in the distillate were extracted with a SPME device (*U*) equipped with a 100 polydimethylsiloxane (PDMS) fiber using a stirring plate (*V*).

2.4. Procedure

The microwave set-up was attached according to Fig. 1. The temperature of the condenser was set at 5°C using the recirculating refrigerator unit. Ten grams of a catfish tissue filet, spiked at 1 ppb DHN, was placed concentrically around the tip of the thermocouple. The temperature controller was set at 120°C and the argon purge flow was set at 25 mL/min. The microwave was activated for 6 minutes, during which time distillates formed from the catfish tissue migrated to the condenser and became a liquid. The liquid distillate was collected in a 10 mL glass vial. Two grams of sodium chloride were added to the vial along with a micro stirrer. The PDMS fiber was submerged into the stirred container, containing saturated NaCl distillate, for 25 min. Before desorbing into the SPI, which was set at 250°C, the fiber was briefly rinsed with distilled water to remove excess sodium chloride. Desorption time was held constant at 3 min. Between extractions, component J was released, and the set-up was rinsed with soapy water and followed by distilled water.

The GC oven was programmed at 60°C for 4 min, then ramped to 200°C at 8.5°C/min. The oven was held at 200°C for 16.5 min then ramped to 250°C at 20°C/min. and held for 3 min. The GC–ITD transfer line was held constant at 250°C. The ion trap was operated in the electron impact (EI) ionization mode with a filament current of 10 μA and a mass scan range of 60 to 250 daltons.

3. Results and discussion

3.1. Analytes and internal standard

Fig. 2 depicts the molecular structure of MIB, GEO and the internal standard, DHN. MIB is a [2.2.1] bicyclic tertiary endo-positioned alcohol. GEO is a two-fused six-membered ring system compound with a tertiary axial alcohol at a fused ring carbon. DHN is also a fused two-membered ring system compound having a secondary alcohol moiety

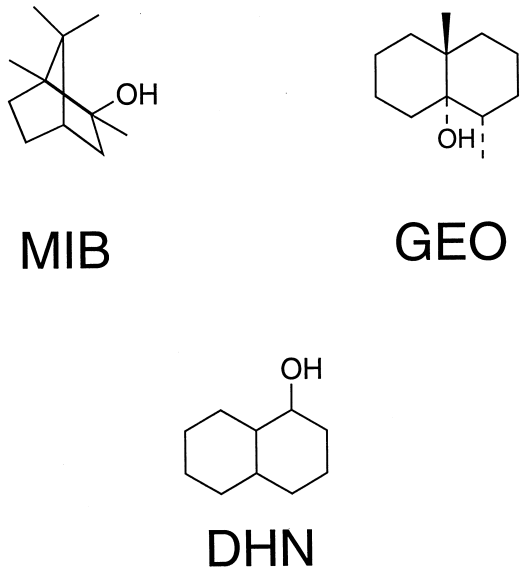

Fig. 2. Structures of the off-flavor Compounds, methylisoborneol (MIB) and geosmin (GEO) and the internal standard, *cis*-decahydro-1-naphthol (DHN).

and was chosen as the internal standard due to its structural similarity to the analytes.

3.2. Optimization of microwave

Based upon our previous observations [11], the drier the catfish sample after microwave distillation extraction, the better the percent recovery of analytes. It was noticed that the sample became more dry as the length of the microwave processing time increased. Microwave time was limited in our previous design due to pyrolysis of the catfish tissue, creating an oily extract, which when injected into the GC, contaminated the inlet and column. Using our current temperature control arrangement, pyrolysis has never been experienced. The microwave magnetron shuts down when the set temperature is met (120°C) and turns back on as lower temperatures are measured. Six minutes of microwave time sufficed in removing a significant portion of water from the fish tissue. After this time no observable distillate migrated to the condenser. The final sample had the same consistency as the optimized time in our previous work.

3.3. Optimization of argon flow

Though the argon flow rate was optimized in a previous design, the presented design differs. The condenser in this design is open, thus the flow rate is not optimized in, terms of trapping efficiency as in our previous SPE design. The argon flow rate was varied from 0 to 60 mLs/min and the area response recorded for GEO, MIB and DHN. Flow rates greater than 0 mL/min display no statistical difference in analyte recovery. This is due to a physical limitation at 0 mL/min. At 0 mL/min, the sample container allows only an exit of gas. Gas formed from the distillation of the catfish tissue expands rapidly, thus forcing distillate into the condenser. However, during microwave off cycles the gas will cool, thus reducing the pressure inside the container. With this change, the sample is forced back into the sample container. It was observed at 0 mL/min argon flow that the volume of distillate was less than at higher flow rates, because of this reversal of distillate flow. These higher flow rates provided a similar sample volume for their experimental trials. Incoming argon provides a steady flow of gas that prevents or minimizes reverse migration of distillate, by offsetting the pressure drop that occurs. A 25 mL/min argon flow rate was chosen in this study.

3.4. Optimization of SPME extraction

A graph of area response versus extraction time for the analytes and internal standard was prepared (Fig. 3) for spiked extracts at 1 ppb, assuming 100% recovery (10 ng of each component). From 0 to 30 minutes there is an observable rise in area count. After 30 minutes relative area responses of GEO, MIB and DHN begin to plateau. A SPME extraction time of 25 min was chosen in a trade-off between response and analysis time. The 25 min extraction time coincided nicely with the GC analysis time. During a GC run, a sample could be extracted maximizing efficiency for the analysis of many samples.

3.5. Calibration of response

Amounts of GEO and MIB were spiked into 10.0 g of catfish filet tissue having background concentrations of the analytes below the detection limit. Amounts spiked corresponded to concentrations in the range of 0.10 to 100 ppb. Both analyte and internal standard spiking solutions were prepared in a solvent of methanol and spiked volumes did not exceed 10. The internal standard, DHN, was spiked into the catfish tissue maintaining a constant concentration of 1 ppb. The height response for MIB, GEO and the internal standard, DHN, were measured on their respective ion trace chromatograms of $m/z=95$, $m/z=112$ and $m/z=136$, respectively. For MIB and GEO, these ions are base peaks in their spectra. $m/z=136$ was chosen due to this ion's low background in the fish sample and it is a relatively abundant peak in the spectrum of DHN. The average heights from three trials were used as a data point. From the data obtained, internal standard calibration plots were prepared. The linear regression for the average heights response of MIB/IS versus concentration of MIB (ppb) was $y=0.009+2.39x$ ($r^2=0.999$). For the GEO/IS height ratio versus concentration of GEO (ppb), the, linear regression was $y=0.152+1.27x$ ($r^2=0.999$).

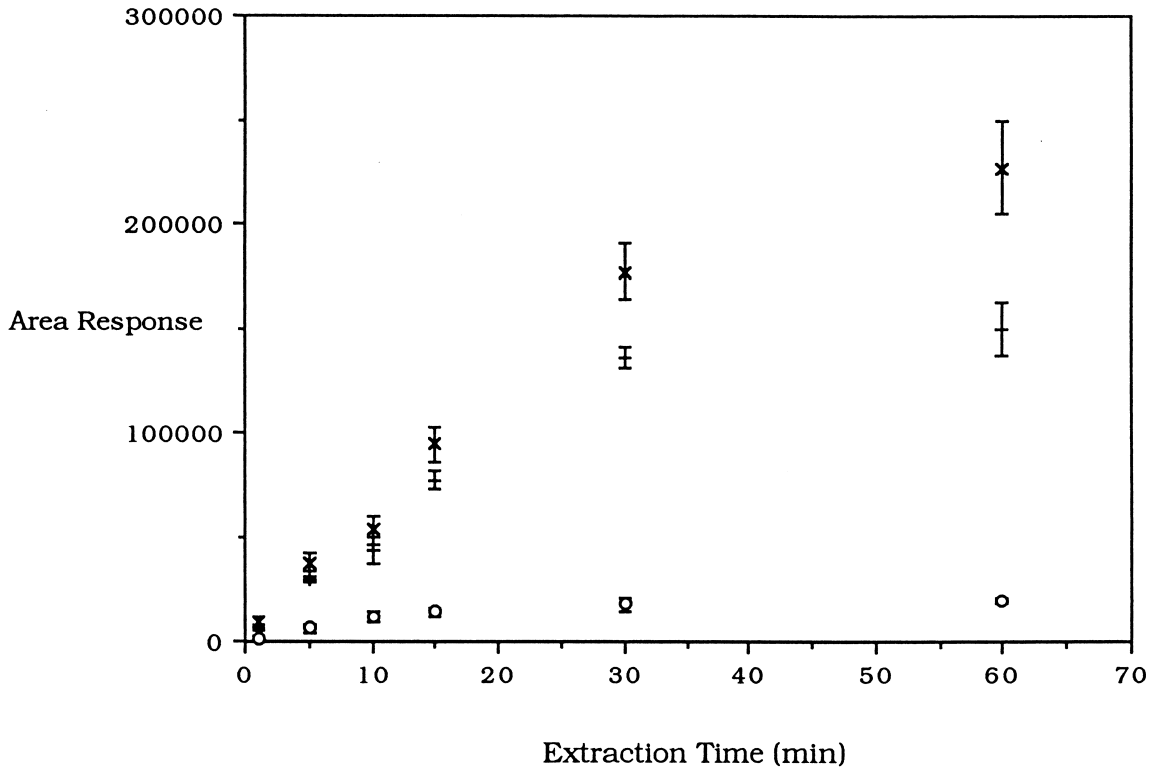

Fig. 3. SPME extraction time versus area response (MIB (+), GEO (x) and DHN (○))

3.6. Detection limits

The integration software for the Varian Saturn system provides signal-to-noise ratios (S/N) for peaks in the chromatogram based on heights. The S/N average for both MIB and GEO were calculated for each of the trials ($n=15$) used for the calibration of response. For this work, the detection limit is defined as that signal equal to three times the noise level. The average of the noise for both analytes were multiplied by three and the concentrations corresponding to these heights, the detection limits, were calculated using linear regression equations of the response of each analytes height versus concentration. The detection limits were calculated to be 0.043 for MIB and 0.008 ppb for GEO. These detection limits are well below the human threshold concentrations of 0.8 ppb for MIB and 8.0 ppb for GEO [13].

3.7. Recovery of GEO, MIB and DHN onto the SPME fiber

In SPME, analytes partition between the aqueous and organic (SPME coating) phases. Once equilibrium is established, the amounts of analyte on the fiber and in solution remain constant. The nature of physical partitioning will result in less than 100% recovery for the analyte in the SPME coating phase from the aqueous phase. The percent recovery using SPME in saturated NaCl conditions was measured for a distillate solution that would result in 100% recovery at 1 ppb for the analytes and IS. The amount calculated from a linear regression equation prepared from data of direct injections from 1 to 10 ng was compared to 10 ng of spiked amounts of GEO, MIB and DHN ($n=3$). The percent recovery of analytes onto the PDMS–SPME fiber was 15.6±0.8% for MIB, 36.9±1.4% for GEO and

20.2±0.1% for DHN. The effect of saturated NaCl on percent recovery was examined, by conducting the same set of experiments without NaCl. Percent recoveries obtained were 3.7±0.1% for MIB, 37.4±5.7% for GEO and 1.1±0.1% for DHN. Interestingly, under saturated NaCl conditions, the recovery response, for MIB increases 24% and the recovery of DHN improves 5%, while GEO has no statistical improvement.

3.8. Recovery of GEO, MIB and DHN from Microwave Extraction.

Each of the analytes and the internal standard were spiked at 1 ppb into the catfish tissue and analyzed according to the procedure. The average peak heights from each trial ($n=6$) were recorded. A calibration of distillate extracts were prepared by spiking solutions of MIB, GEO and DHN into 7 mL of distilled water, which is the approximate volume of the collected distillate. A calibration of response from 1 to 0.1 ppb of each analyte versus peak height was obtained in this manner. Concentrations were calculated using the obtained linear response equation from the average heights of the spiked catfish trials. Percent recoveries calculated were 81.4±5.4% for MIB, 30.4±5.3% for GEO and 42.9±6.6% for DHN. The microwave extraction efficiencies for the analytes are lower than those obtained with our previous SPE design. This difference is due to the open condenser not being able to quantitatively trap the analytes in the presented design. A higher efficiency is obtained in the SPE design because a SPE cartridge is directly connected to the exit of the

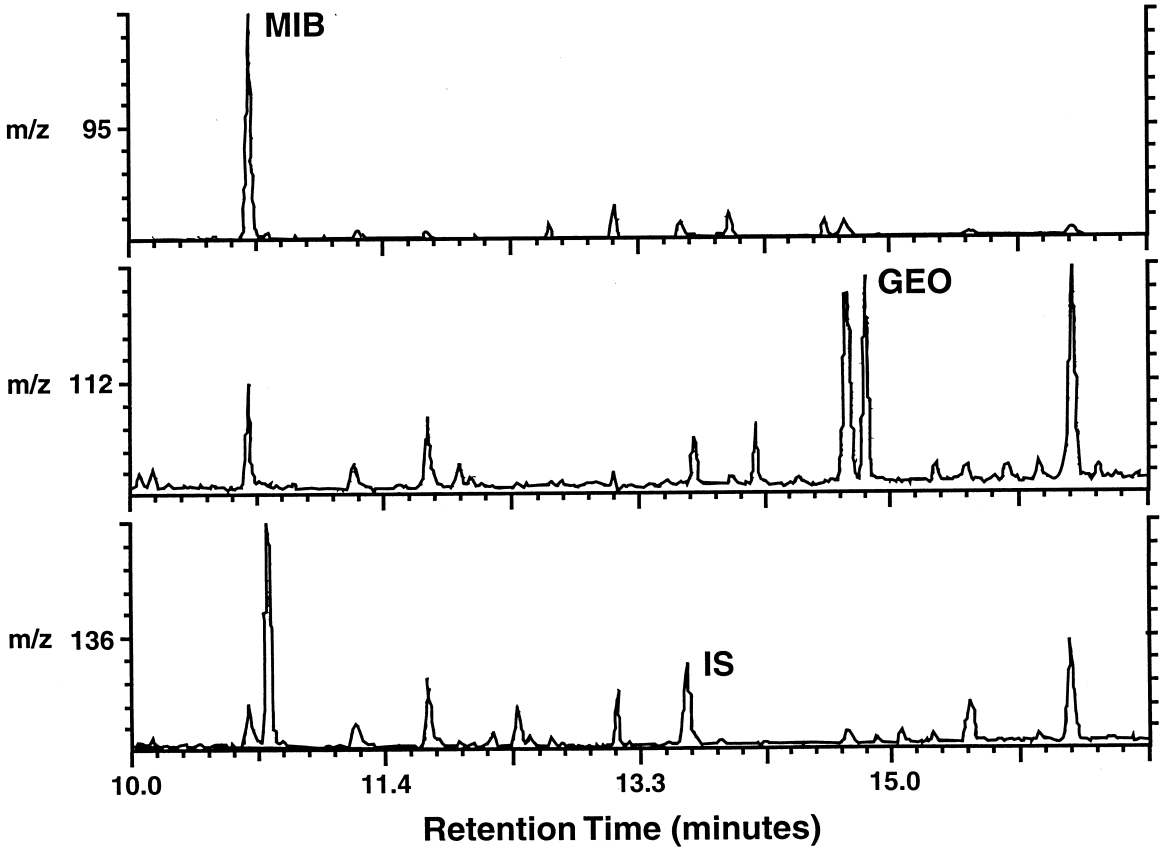

Fig. 4. Ion trace chromatograms from an ‘off-flavor‘ channel catfish extract (12.43 ppb MIB, 0.19 ppb GEO).

condenser, thus creating a more efficient trap for the analytes. Detection limits, however, are lower in the SPME set up and reproducibility is similar.

3.9. Application

A channel catfish taken from a commercial catfish pond known to be ''off-flavor'' was processed according to the described procedure. The resultant chromatogram is depicted in Fig. 4 as ion traces of $m/z=95$, $m/z=112$, and $m/z=136$ for MIB, GEO and the internal standard DHN, respectively. Quantitative information from integrated peak areas using internal standard plots revealed the concentration of the off-flavor compounds at 12.43 ppb MIB and 0.19 ppb GEO. The concentration of MIB, being well above the threshold limit, confirms the ''off-flavor'' classification of this channel catfish.

4. Conclusion

Combined microwave mediated steam distillation with solid-phase micro extraction offers unique advantages. Semivolatile organic analytes may be quantitatively extracted from complex samples having a high aqueous content without the use of organic solvents. The presented application reveals the very low limits of detection obtainable with this technique. This technique will find utility in other applications where traditional steam distillation has been used, but with a reduction in sample preparation time.

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