Determination of Geosmin and Methylisoborneol in Catfish Tissue (*Ictalurus punctatus*) by Microwave-Assisted Distillation−Solid Phase Adsorbent Trapping

Eric D. Conte,[†] Chun-Yi Shen,[†] Peter W. Perschbacher,[‡] and Dwight W. Miller*[,†]

Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079, and University of Arkansas at Pine Bluff, Pine Bluff, Arkansas 71601

Methylisoborneol (MIB) and geosmin (GEO) are algal off-flavor compounds, which when present in catfish tissue create undesirable taste and odors in the prepared products. These undesirable taste and odor problems are not limited to catfish aquaculture. A procedure was developed for the determination of off-flavor compounds in channel catfish tissue that involves microwave radiation distillation with solid phase trapping. This is a modification of a microwave distillation−cold trapping procedure but without the need of a cryogen or a liquid−liquid extraction step. A channel catfish fillet sample is placed in a container located within a microwave oven. This container, which is directly connected to a thermostated condenser containing a solid phase adsorbent, is continually purged with argon gas. The trapped distillate components are eluted with ethyl acetate and then injected into a gas chromatograph−ion trap mass spectrometer for analysis. This technique offers a rapid and sensitive means of off-flavor analysis in fish tissue and improved recovery for MIB from 73 ± 3% (50 ppb) to 85 ± 5% (10 ppm) compared to 62 ± 6% for microwave−cold trap collection. The method detection limits are 1.7 and 1.1 ppb for MIB and GEO, respectively.

Keywords: *Off-flavors; methylisoborneol; geosmin; catfish; microwave-assisted distillation*

INTRODUCTION

Aquaculture is the newest and one of the fastest growing segments of agriculture. In the United States, aquaculture is approaching a $1 billion industry, with better than half of this industry resulting from channel catfish culture. The catfish production from aquaculture is dependent on a "pond production system". The pond ecosystem relies on the phytoplankton community to help oxygenate the water. These phytoplankton species can produce compounds that, when absorbed by the aquaculture product, will result in an off-flavored product. Off-flavor in cultured fish (Lovell and Sackey, 1973; Martin, 1992) and shellfish (Perschbacher and Martin, 1992) has been tied to cyanobacteria (blue-green algae) that release methylisoborneol (MIB), producing a musty taste, or geosmin (GEO), producing an earthy, pond-bottom taste. Off-flavor has been estimated to cost an average of $0.08/kg by delaying harvest (Keenum and Waldrop, 1988).

Processing plants for channel catfish employ sensory analysis for acceptance or rejection of fish. Sensory analysis involves cooking the fish tissue in a plastic bag for 5 min with a microwave oven, followed by smelling and/or tasting the cooked fish to determine the presence of any off-flavor. This method is manpower intensive and requires the analysis of large numbers of samples on a regular basis to maintain proficiency. Limitations associated with sensory taste and odor evaluation are described by McGuire *et al.* (1981).

Other means of off-flavor compounds analysis in channel catfish tissue utilize vacuum distillation (Lovell *et al.*, 1973) or microwave−cold trap collection (Martin *et al.*, 1987) followed by gas chromatography−flame ionization detection (GC−FID) or gas chromatography−mass spectrometry (GC−MS). Vacuum distillation utilizes a vacuum pump connected in series with collection cold traps and a sample container. A fish tissue sample is placed in a heated container and volatiles are trapped, under a vacuum, in two cryogenically cooled traps. A liquid−liquid extraction step is required for removal of analytes from the collection cold traps. This technique is time-consuming, requiring approximately 1 h of preparation per sample. Vacuum distillation also requires considerable sample manipulation (Lovell *et al.*, 1973). For instrumental determination of MIB and GEO in fish tissue, the method of choice by researchers is microwave-mediated distillation of semivolatiles under a flow of nitrogen followed by cryogenic trapping of the off-flavors water as a condensate (Martin *et al.*, 1987). The off-flavors are extracted from the condensate with hexane prior to analysis by GC−FID or GC−MS. The microwave−cold trap technique offers the advantage of rapid sample preparation time, but is somewhat labor intensive. As in the vacuum distillation method above, the microwave distillation method also requires a time-intensive liquid−liquid extraction step in the sample preparation prior to chromatographic analysis.

The method described here was based on Martin's microwave−cold trapping procedure and adapted to solid phase trapping and extraction methodologies. Solid phase adsorbent analysis offers the advantages of selectivity, collection efficiency, ease of sample recovery over liquid−liquid extraction based procedures, a reduction of time required for sample preparation, and a reduction in the total volume of organic solvent used for an analysis.

MATERIALS AND METHODS

Chemicals. MIB (99.5% by GC−MS) was synthesized according to the procedure of Wood and Snoeyink (1977), and GEO (99%+ by GC−MS) was purchased from Wako Pure Chemicals (Osaka, Japan). The internal standard for GEO,

[†] National Center for Toxicological Research.
[‡] University of Arkansas.

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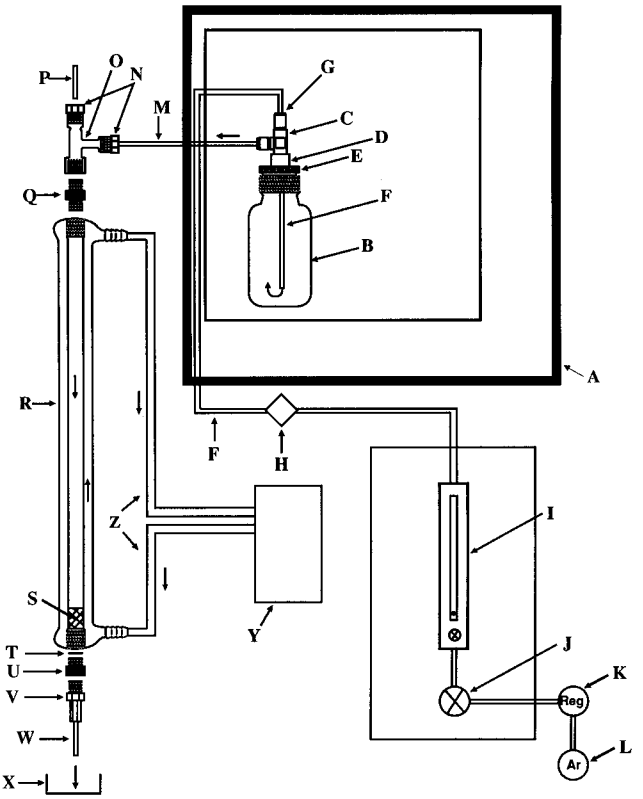

Figure 1. Microwave distillation−solid phase extraction (MD−SPE) apparatus.

cis-decahydro-1-naphthol (DHN, 98%), was purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received.

The internal standard for MIB, (±)-*endo*-norborneol acetate (NBA), was prepared through the reaction of (±)-*endo*-norborneol (100 mg, Aldrich) and acetic anhydride (2 mL, Aldrich). This mixture, together with 2 mL of triethylamine (Aldrich), was placed in a stoppered test tube in a 70 °C heater block for 1 h. The mixture was then removed from the water bath and to it was added 4 mL of deionized water. The resultant mixture was extracted three times with 1 mL aliquots of diethyl ether (J. T. Baker Chemical Co., Inc., Phillipsburg, NJ). The combined ether extracts were washed with 1 mL aliquots of 1 M hydrochloric acid (J. T. Baker), saturated sodium bicarbonate (J. T. Baker), and saturated brine (sodium chloride−water, J. T. Baker), sequentially. The ethereal layer was dried by passing it through a plug of anhydrous sodium sulfate (J. T. Baker), and the ether was removed under reduced pressure. The resultant product was determined by GC−FID to be approximately 96% pure.

The following reagents were purchased and used as received: ethyl acetate (J. T. Baker), methanol (J. T. Baker), and sea sand (Aldrich). The solid phase absorbents, C_{18} functionalized silica (Aldrich), Amberlite XAD-2 (Mallinckrodt, Inc., St. Louis, MO), and charcoal (Supelco, Inc., Bellefonte, PA) were purchased and activated as required for their use as a solid phase absorbent in the presence of water.

Instrumentation. A gas chromatograph ion trap mass spectrometer (Varian Saturn II, Walnut Creek, CA) equipped with an autosampler (Varian Model 8100) was utilized for component separation and detection. A DB5-MS fused silica capillary column (J&W Scientific, Folsom, CA) having dimensions of 30 m × 25 μm i.d. and with a stationary phase thickness of 0.25 μm was installed. The injector utilized was an SPI temperature programmable injector which, after injection, was held at 70 °C for 0.2 min and then heated at 180 °C/min to 250 °C, at which it was held for 20 min. The capillary column was initially held at 90 °C for 5 min, then heated at 10 °C/min to a final temperature of 250 °C, and held for 10 min.

Apparatus. The microwave distillation−solid phase extraction (MD−SPE) apparatus is illustrated in Figure 1. This apparatus was constructed on-site with commercially available materials. Table 1 gives a compilation of parts and their sources utilized to assemble the MD−SPE equipment.

Table 1. List of Components and Sources for the Construction of a Microwave Distillation Apparatus As Shown in Figure 1

label	description	source code[a]
A	700 W microwave oven	a
B	No. 8648-140 hydrogenation flask thick wall with No. 25 Ace-Thred	b
C	T-400-3-4TMT male run tee Teflon 1/4 tube 1/4 NPT with Teflon nuts and ferrules	c
D	1 in. × 3.5 in. Teflon rod drilled and tapped to receive 1/4 in. NPT(F) fitting	d
E	No. 7506-31 Teflon No. 25 Ace-Thred bushing with No. 7855-734 Teflon O-ring size 212	b
F	1/8 in. heavy wall Teflon tubing	e
G	No. RF-400/200-T 1/4 in. to 1/8 in. Teflon reducing ferrule	f
H	check valve	c
I	rotameter	g
J	toggle valve, nupro	c
K	Wilkerson regulator with pressure gauge	g
L	argon tank and two-stage regulator	
M	1/4 in. × 12−16 in. long heavy wall Teflon tubing	e or g
N	No. 5029-35 Teflon No. 7 Ace-Thred bushing with No. 7855-704 Teflon O-ring	b
O	No. 5828-20 adapter, feed tube, septum	b
P	1/4 in. Teflon rod	d
Q	No. 5841-46 No. 11 Ace-Thred coupling, Teflon	b
R	No. 5821-05 chromatographic column jacketed 300 mm long	d
S	solid phase adsorbent	h
T	No. 5848-07 support disk, polyethylene	d
U	No. 5838-72 No. 11 adapter, end fitting with 1/8 in. NPT(F)	d
V	No. H-06385-10 male pipe adapter 1/4 in. tube to 1/8 in. NPT(M) polypropylene or No. H-06374-02 in Teflon-PFA	e
W	1/4 in. × 4 in. heavy wall Teflon tubing	e or g
X	beaker	e
Y	refrigerated water chiller	e
Z	flexible tubing 3/8 in. i.d.	e

[a] a, Tappen, b, Ace Glass Inc., Vineland, NJ; c, Swagelok; d, Cope Plastics, Inc., Little Rock, AR; e, Cole Parmer; f, Alltech Associates, Inc., Deerfield, IL; g, industrial supply; h, Aldrich Chemical Co., Inc., Milwaukee, WI.

The nucleus of the MD−SPE apparatus is a standard 700 W microwave oven (A) modified as described below. The openings in the internal compartment of the microwave oven intended for a meat probe were uncovered and used for gas transfer ports. The larger opening was used for exit gases, and one of the smaller openings was used for introduction of argon sparge gas. A 1/4 in. opening was drilled in the outer case to allow for the straight passage of the effluent gases through the wall of the microwave oven via a 1/4 in. by 12 in. Teflon tube (M). The 1/8 in. by 36 in. Teflon sparge gas tube (F) was inserted through an opening in the bottom edge of the outer case and then through the small hole in the inner compartment. The sparge gas tube was fitted through a Teflon male run tee (C) and sealed in place with a Teflon reducing ferrule (G). C was connected to a 1 in. by 3.5 in. Teflon rod (D) drilled and tapped to received 1/4 in. NPT threads and sealed with Teflon tape. A 250 mL thick-wall hydrogenation flask with No. 25 Ace-Threds (B) was connected via a No. 25 Teflon nut E and a Teflon O-ring to seal D in place. F should be adjusted to within an inch of the bottom of B. A was elevated approximately 10 in. The remainder of the glassware was assembled as shown in Figure 1 and held in place with a clamp and ring stand. F was connected to a check valve (H) to prevent the reverse flow of liquids during and after a distillation. The sparge gas flow control was constructed on a panel as shown in Figure 1. The temperature of the condenser (R) was controlled by a bench-top recirculating

cooling unit (Y, Model RB2055AO, FTS Systems Inc., Stone Ridge, NY). The bottom of R is packed with 500 mg of C_{18} functionalized silica gel (S) held in place by screen (T) or a glass wool plug. Component P serves two purposes: (1) pressure relief and (2) inlet for elution solvent. For P to function as a pressure relief, nut N must not be overtightened. (A simple rupture disk could be used in place of P if connected via a $^1/_4$ in. o.d. tube for a more reliable pressure relief.) If P is removed for addition of elution solvent, it may be replaced, and the gas pressure of 7−10 psi and 40−60 mL/min will aid in pushing the elution solvent through W for collection in a test tube prior to analysis. Care must be taken to ensure all O-rings form a seal when equipment is assembled prior to collection of a sample.

The above-described equipment design originated from prototype configurations of the MD−SPE which occasionally experienced explosions. Components C, D and G, in Figure 1, were fabricated from a 1 in. glass bubbler tube (Ace No. 8762-03, Ace Glass Inc., Vineland, NJ) which was connected to B via E. M was connected to the bubbler tube with a $^1/_4$ in. Teflon Swagelok union. The head (components N, O, P, and Q) of the condenser was constructed with $^1/_4$ in. glass and a $ 14/20 male joint fashioned in the form of an elbow. This was connected to M with a $^1/_4$ in. Teflon Swagelok union. A laboratory condenser, 13 mm i.d., with $ 14/20 joints was connected to the $ 14/20 male joint of the elbow condenser head and sealed with Teflon tape. The absorbent, S, was held in place with glass wool. Other than these differences, the prototype apparatus was the same as shown in Figure 1. We have included explosion problems when they occurred with the prototype equipment. All of the explosions experienced with the prototype equipment resulted from high back pressure generated from the volatilization of water that resulted in the abrupt separation of the head assembly from B. **Note: All equipment exposed to microwave energy inside the oven should be Teflon, glass, or non-microwave-absorbing material.** The equipment described as shown in Figure 1 has never experienced an explosion.

Procedure. The C_{18} functionalized silica solid phase adsorbent contained in the condenser was pretreated by rinsing with 2 mL each of ethyl acetate, methanol, and deionized water in sequence. A 20 g subsample of homogenized catfish tissue, spiked prior to homogenization with DHN and NBA at 900 ppb and 5 g of sea sand, was placed in the hydrogenation vessel, B. The microwave was activated at 40% power for 10 min, while the sample container was purged with argon at 40 mL/min. Plug P was then removed, and W was rinsed with 2 mL of deionized water and then rinsed three times with 1 mL of ethyl acetate, under argon pressure. This extract was dried with sodium sulfate and then injected into the gas chromatograph ion trap mass spectrometer.

Calibration of the Detector. Calibration solutions were prepared by spiking the internal standards, DHN and NBA at 900 ppb, and MIB and GEO in a series of samples from 5 ppb to 10 ppm into the fish tissue, which was then ground. These samples were prepared according to the described procedure and then were injected to calibrate detector response. A calibration curve of the area ratio of the analytes (GEO and MIB) to the internal standards (DHN and NBA) vs the concentration of the analytes was produced.

RESULTS AND DISCUSSION

Apparatus Design. Initial designs of the apparatus did not include a thermostated condenser, rather a tube containing the solid phase adsorbent. A high flow rate of steam exited the tube during the microwave-on cycles. This resulted in very low recoveries of the analytes. Explosions of the sample container were experienced from the high back pressure created by the solid phase adsorbent as steam was released from the tissue sample. The thermostated condenser was utilized to circumvent these difficulties. The open volume of the condenser provided an area for condensation of the steam, which reduced the pressure and allowed water rather than steam to flow through the solid phase adsorbent. This eliminated the explosion hazards and improved recoveries.

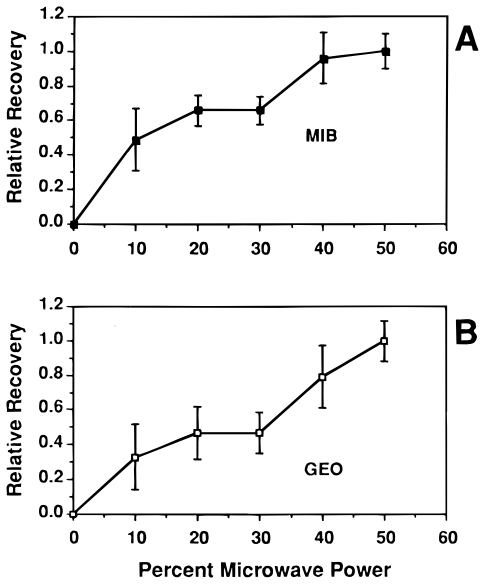

Figure 2. Relative recovery vs percent microwave power.

Optimization. Experimental parameters consisting of microwave power and time, solid phase adsorbent type and amount, argon flow, and sample pretreatment were investigated to optimize percent recoveries of the analytes from the fish tissue. Each optimization trial consisted of a 20 g ground fish tissue sample spiked at 1 ppm with MIB and GEO. Each optimization parameter consisted of three replicate trials for a given set of conditions.

The length of microwave time was investigated at 10% power from 0 to 50 min. Only modest increases in relative recovery for both analytes occurred at 30 min. To reduce the analysis time, recoveries were measured for increased microwave power levels and only a 10 min time period. As shown in Figure 2, from 10% to 30% power, the relative recovery is experimentally equal, while a significant increase occurs from 30% to 40% for MIB and from 30% to 50% for GEO. After the 50% trial, it was discovered that pyrolysis of the fish tissue was extensive and that a yellow oil distilled into the condenser. These two conditions resulted in a highly complex chromatogram, compared to the 40% power trial, as depicted in Figure 3. Also, a contaminated GC inlet sleeve resulted from the injection of the 50% power sample. The large number of peaks observed probably relates to distilled oils and/or pyrolysates. These added peaks only complicated the analysis of MIB and GEO. It was decided that 40% power and 10 min of analysis time would be an acceptable compromise.

Solid phase adsorbents consisting of C_{18} functionalized silica gel, Amberlite XAD-2, and charcoal were compared at 275 m^2 surface area. These adsorbents were chosen because of their affinity for organic molecules in an aqueous medium. The affinity of each adsorbent for MIB and GEO was examined as a function of adsorbing surface area, a material specification obtained from the suppliers.

As illustrated in Figure 4, the C_{18} functionalized silica gel was clearly superior for the recovery of MIB and GEO in the distillate. Amberlite XAD-2 gave a relative response approximately half that of the C_{18} functionalized silica gel, and the relative response from charcoal

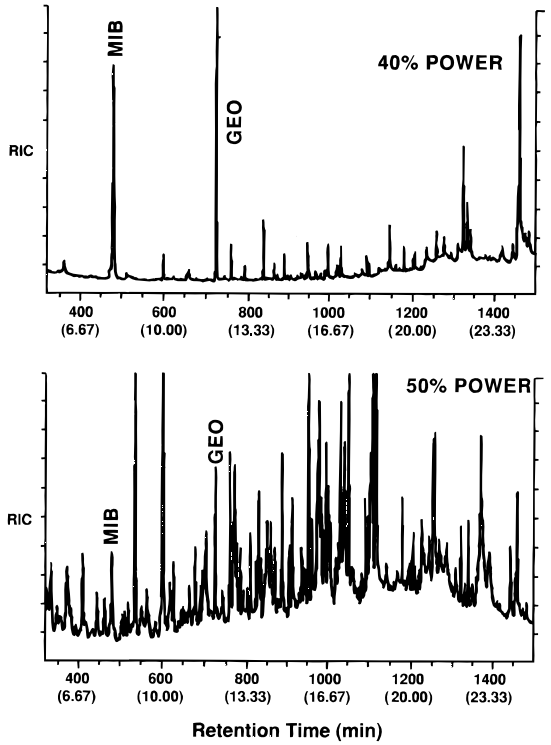

Figure 3. Chromatograms of fish distillate containing 1 ppm of MIB and 1 ppm of GEO at 40% and 50% microwave power.

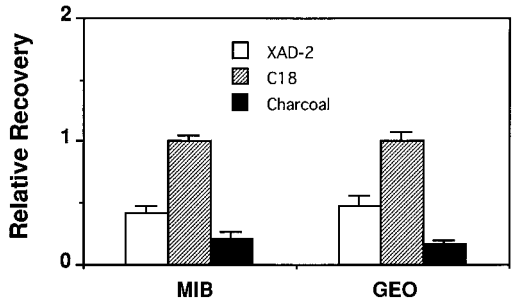

Figure 4. Relative recovery vs solid phase absorbent at 275 m^2 surface area.

was approximately half that of the Amberlite XAD-2. The C_{18} functionalized silica gel, S, was then optimized for recovery by varying the amount of absorbent from 100 mg to 2 g as shown in Figure 5. The MIB and GEO curves demonstrate nearly identical forms. The plateau observed at the 0.5 g level of absorbent indicates that the amount of absorbent used was sufficient at the 1 ppm level of MIB and GEO in fish tissue. The plateau observed at the relative recovery level of one, for 1 ppm of MIB and 1 ppm of GEO, as a function of the amount of absorbent is an indicator of 100% trapping efficiency. Since the trapping absorbent is placed in a 13 mm i.d. tube in each trial, this plateau may also be an indicator of a sufficient depth of absorbent to provide a back pressure to maintain good dynamic flow and allow for sufficient surface contact for efficient trapping. The C_{18} functionalized silica gel, being 60−200 mesh size at the lower levels, may not be trapping as efficiently as might be expected because of channeling and the 0.5 g level of absorbent is required for sufficient contact. The dip observed at the 1 and 2 g levels of absorbent in the GEO curve of Figure 4 may be an experimental artifact. At the 0.5 g level of absorbent it was determined that three 1 mL sequential volumes of ethyl acetate were sufficient to elute 99 ± 1% of the MIB and 100 ± 1% for GEO.

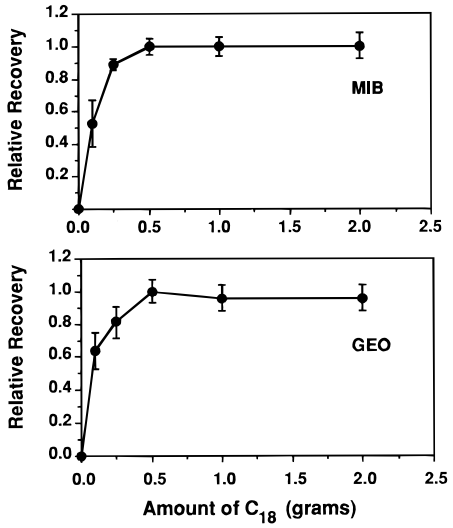

Figure 5. Relative recovery vs amount of C_{18} functionalized absorbent.

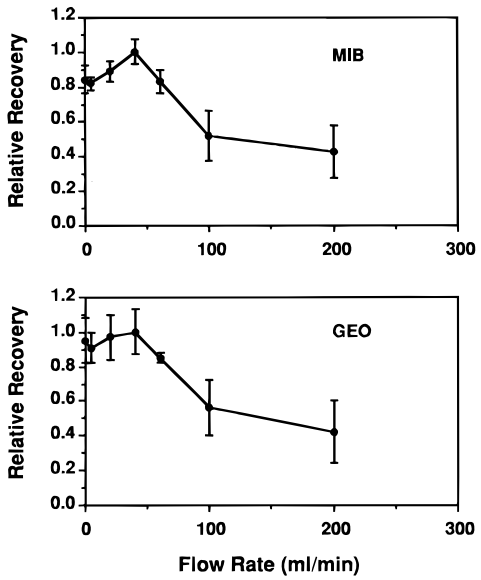

Figure 6. Relative recovery vs argon purge flow rate.

The temperature of the condensation tube, R, was also varied from 5 to 40 °C. The variation of temperature resulted in no difference in extraction efficiency within experimental error. It should be noted that the lower temperature can provide a margin of safety. When trials with no coolant flowing through the condenser were conducted, the equipment inside the microwave oven, head assembly and B, were abruptly separated. These occurrences confirm the importance of the condenser in condensing the steam and thus reducing the system pressure.

The flow of argon purge gas was also optimized at different rates from 0 to 200 mL/min as illustrated in Figure 6. A decreasing response, due to inefficient trapping on the solid phase adsorbent, can be observed for both analytes at argon flows in excess of 60 mL/min. The decrease in the relative recovery associated with increased sparge gas flow is probably due to channeling and the high rate at which the analyte passes through the absorbent. The optimal response for both analytes was achieved at 40 mL/min.

A temperature profile of a 10 min, 40% power microwave run before and after the condenser is shown in Figure 7. At about 1 min, the temperature upstream

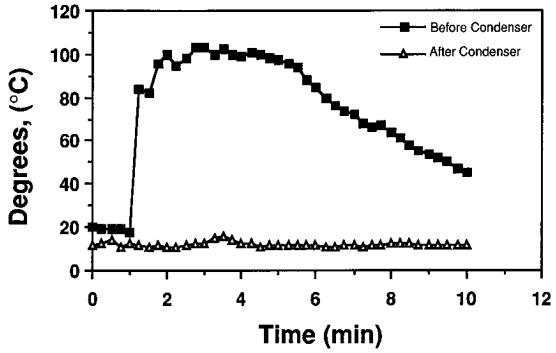

Figure 7. Temperature profile before and after condenser at 40% microwave power.

of the condenser increases rapidly due to the boiling water. After approximately 5 min, a decline in temperature occurs because most of the water from the sample has been distilled. During the entire run the temperature of the eluant exiting the condenser remains relatively constant due to the condenser.

The method percent recovery was measured after optimization of the above experimental parameters. At the 1 ppm spiked levels of MIB and GEO in catfish, the recoveries were determined to be 81 ± 2% for MIB and 71 ± 2% for GEO.

An experiment was performed to measure any analyte losses due to grinding. A 20 g sample spiked with the analytes after grinding resulted in no recovery difference within experimental error compared to the samples spiked before grinding.

Sample treatment by adding sodium chloride or sodium hydroxide was investigated to improve recoveries. The application of the "salting-out" phenomenon was tested by saturating the sample with sodium chloride, in an attempt to release the analytes from the matrix by increasing the ionic strength of the surrounding media. Alternatively, sodium hydroxide was added in an attempt to break down the lipid matrix. This resulted in the formation of sodium salts of fatty acids and resulted in foaming throughout the distillation system. Thus, for practical reasons, the addition of sodium hydroxide was abandoned. Neither of these techniques improved analyte recovery from the fish tissue matrix.

The physical state of the fish tissue was also examined with respect to recovery. Ground versus unground tissue samples were compared, and the results revealed an approximate 10% lower recovery and higher standard deviation for the unground samples. Frozen samples examined in the microwave generated bumping, which resulted in the abrupt separation of the head assembly and B inside the microwave oven. Therefore, the sample must be thawed prior to analysis.

To test the MD−SPE system recovery, another set of recovery experiments were conducted comparing the extraction efficiency of MIB and GEO from fish tissue to that from a less complex matrix, water. A 20 mL water sample containing 5 g of sea sand and spiked with the same levels of the analytes (1 ppm) was analyzed under the same conditions. The results were recoveries of 93 ± 2% for MIB and 98 ± 1% for GEO. The higher recoveries from water and sand suggest that, because of the highly lipophilic attraction between the tissue and MIB and GEO, total recovery from the tissue by any extraction method is unlikely. The 70−80% values obtained here for the recovery of the off-flavors from fish tissue is probably as high as can be expected.

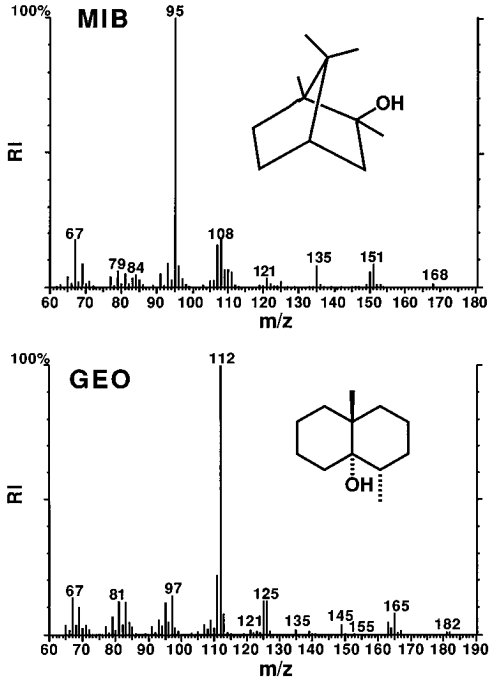

Figure 8. Mass spectra of MIB and GEO.

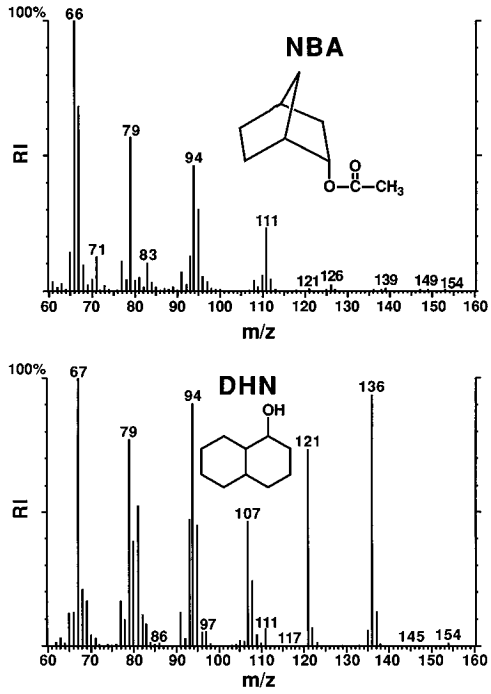

Figure 9. Mass spectra of the internal standards NBA and DHN.

Internal Standard. The internal standards, DHN and NBA, were chosen for their similarities in structure and probable extraction efficiencies to GEO and MIB respectively. Initially (±)-*endo*-norborneol was utilized as an internal standard, which eluted about 3.5 min before MIB. NBA was a less polar internal standard that eluted 1.5 min before MIB. The structures and mass spectra for MIB, GEO, and the internal standards (DHN and NBA) are shown in Figures 8 and 9, respectively.

Recoveries. The percent recoveries for MIB, GEO, and the internal standards are presented in Table 2. The recoveries for MIB and GEO were calculated at the 10 ppm and 50 ppb levels. The recoveries for the

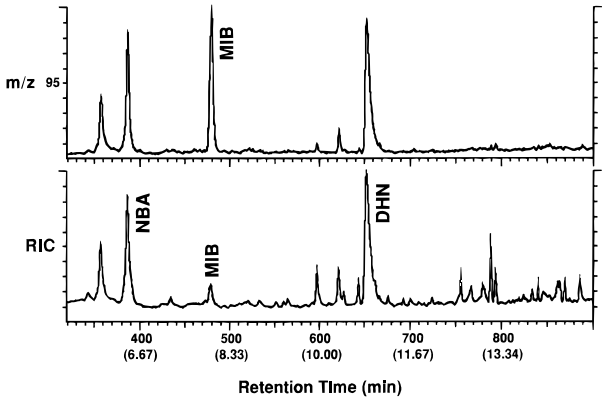

Figure 10. Chromatogram of fish extract found to contain 67 ppb of MIB.

Table 2. Percent Recoveries of GEO, MIB, and Their Respective Internal Standards, DHN and NBA, from Catfish Tissue ($n = 3$)

	GEO	DHN	MIB	NBA
50 ppb	58 ± 7%		73 ± 3%	
900 ppb		77 ± 8%		79 ± 4%
10 ppm	80 ± 3%		85 ± 5%	

Table 3. Detection Limits for GEO and MIB in the TIC and SIC

	detection limit (ppb)	
	GEO	MIB
TIC	8.0 ± 0.3	6.7 ± 0.6
SIC	1.1 ± 0.1 (m/z 112)	1.7 ± 0.1 (m/z 95)

internal standards were calculated at their spiked level of 900 ppb.

Detection Limits and Linearity. Table 3 depicts the detection limits of the method using total and selective ion chromatograms. The detection limit (DL) is obtained by

$$DL = 3s_B/S$$

where s_B is the standard deviation of the noise and S is the slope or sensitivity of response (Foley and Dorsey, 1984). s_B was approximated as $N_{p\text{-}p}/r$, where $N_{p\text{-}p}$ is the peak to peak noise and r is 5. This is a good approximation for random Gaussian noise. The detector calibration was determined from the linear regression analysis of total ion (TIC) and selective ion (SIC) chromatograms versus concentration. The slope, intercept, and correlation coefficients for GEO were 0.456, −0.642, and 0.9989 (TIC) and 0.0118, −0.0219, and 0.9992 (SIC, m/z 112) and for MIB were 0.0997, −0.1695, and 0.9998 (TIC) and 0.0292, −0.0634, and 0.9994 (SIC, m/z 95), respectively.

Application. A 1.5 lb channel catfish was placed in an aquarium that contained 18 gal of water spiked with MIB (10 ppb) for 6 h. The catfish was then removed from the spiked water and placed in an aquarium containing 18 gal of MIB-free water for 7 days. The water was continuously sparged with air and carbon filtered, and the catfish was fed a 10 g per day maintenance diet for the 7 day purge period. Figure 10 is a chromatogram of the channel catfish tissue microwave extract found to contain 67 ppb of MIB.

Figure 11 contains selected ion chromatograms of a channel catfish tissue microwave extract spiked at 5 ppb of MIB (m/z 95) and GEO (m/z 112).

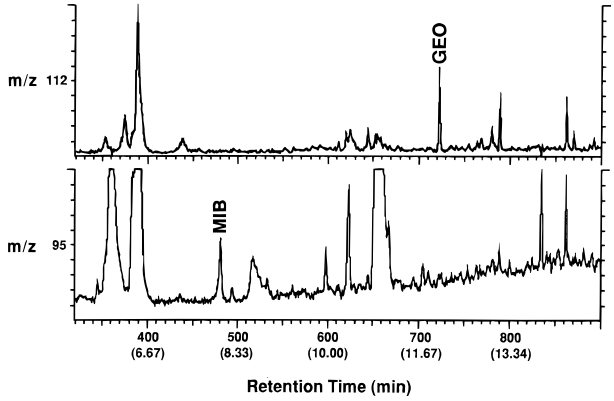

Figure 11. Selective ion chromatograms for 5 ppb spike of MIB and GEO.

CONCLUSIONS

The presented MD−SPE method offers several advantages over the vacuum distillation method of Lovell *et al.* (1973) and the microwave distillation method of Martin (1987). The vacuum distillation method requires the use of a cryogen, has a long sample collection time, and uses special glass traps. It requires thawing of the condensate prior to liquid−liquid extraction plus extracting the sample and washing down the traps to recover the sample analyte. The microwave distillation−cold trap method and the vacuum distillation method have similar disadvantages, although the microwave distillation−cold trap method offers a more rapid removal of the analyte from the fish tissue. The use of nitrogen as a sparge gas during sample collection may introduce oxygen-related artifacts due to the presence of low concentrations of oxygen in laboratory grade nitrogen.

The MD−SPE method allows for rapid analyte removal from the tissue in an inert environment and provides the analyte in a sample volume small enough to produce low detectability. In the MD−SPE method the need for a wash-down step using large volumes of organic solvent is avoided. In addition, this method does not require a concentration step. The schemes involving liquid−liquid extraction steps require concentration to provide reasonable method sensitivities. The described technique offers improved recovery for MIB at 81 ± 2% (1 ppm) compared to 62 ± 6% for microwave−cold trap collection. The calculated detection limits for this method were 1.7 and 1.1 ppb for MIB and GEO, respectively. The lowest concentration of MIB reported by the microwave−cold trap technique is 5 ppb.

Other practical advantages for MD−SPE may include the limited reuse of the solid phase absorbent. This potential may result from the absorbent not being abused with quantities of solid particles, strong acid or bases, and large organic compounds. Inherent in this method for MIB and GEO is the elimination of the potential for the formation of ice plugs commonly observed during cryogenic trapping of aqueous samples. All of the MD−SPE equipment is either purchasable or can be easily made with hand tools.

In general, the MD−SPE method may provide utility for sample preparation in which steam distillation has been previously used. Future possible applications of MD−SPE may include the extraction and analysis of volatile and semivolatile compounds in other foods, beverages, and food-packaging material.

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