Survey of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Fish from the Great Lakes and Selected Michigan Rivers

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Fish from the Great Lakes region and selected Michigan rivers were analyzed for residues of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by using combinations and modifications of published methods. Portions of homogenates of skinless fish fillets were digested in ethanolic KOH and TCDD was extracted with hexane. TCDD was separated from coextractives by passing through a silica gel supported sulfuric acid column followed by collecting the TCDD fraction of the eluate from three high-performance liquid chromatographic systems. Capillary gas chromatography (HRGC) with electron capture (EC) detection was used for residue screening. Residues found by HRGC-EC were confirmed by HRGC low-resolution mass spectrometry by using a 12-ion monitoring scheme. Fish from Saginaw Bay (Lake Huron), the Tittabawassee River in Michigan, and Lake Ontario contained the highest levels of contamination. No TCDD residues at or above the minimum confirmable level of 10 ppt were found in fish from Michigan rivers other than the Tittabawassee.

INTRODUCTION

The 2,3,7,8-tetrachloro isomer (TCDD) is considered to be the most toxic of the 75 chlorinated congeners of dibenzo-*p*-dioxin as reported by Schwetz et al. (1973), Kende et al. (1974), and Gray et al. (1976). Early evidence of bioaccumulation of the 2,3,7,8-tetra isomer by fish in preference to other tetrachlorodioxins was presented by Stalling et al. (1983) and conclusively shown in a study by Kuehl et al. (1984).

Fish from various areas of the Great Lakes and from selected Michigan rivers have been collected over the past several years by several regulatory agencies as part of their individual programs for monitoring the condition of these bodies of water. By previous agreement, these samples were submitted to the authors' laboratory to be analyzed for TCDD. The method of analysis consisted of combinations and modifications of the procedures of Firestone (1977), Lamparski et al. (1979), and Niemann et al. (1983). The results of these analyses and the validation for the procedural modifications are presented in this report.

The species selected consisted mainly of lake trout, coho salmon, and whitefish from the lakes, and channel catfish and common carp from the rivers and Saginaw Bay, since they are high on the food chain and/or relatively long lived. Surveys originated in 1979 with the collection of fish from the Saginaw Bay region of Lake Huron by Food and Drug Administration (FDA) investigators. Additional samples were collected by FDA from this area in 1981 and 1983 and from eastern Lake Erie in 1981. The New York State Department of Environmental Conservation (NYSDEC) also collected samples from eastern Lake Erie in 1981. In 1983, the Environmental Protection Agency (EPA) collected samples of lake trout and coho salmon from the Great Lakes and various rivers as part of their Great Lakes National Program for TCDD analysis to be done in our laboratory. Sample collections from Michigan rivers resulted from an article in the Detroit Free Press by Rohan (1983), which publicized the analytical data reported by a Michigan State University (MSU) graduate student in his doctoral thesis (Kaczmar and Zabik, 1983). Those data indicated that carp and suckers from 10 rivers in Michigan contained residues of TCDD averaging 200 ppt and ranging from 17 to 586 ppt. Since portions of these samples were unavailable for our analysis, the Michigan Department of Natural Resources (DNR) collected carp samples as near as possible to the same sites as those reported in the MSU study.

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EXPERIMENTAL SECTION

Apparatus. The equipment and operating parameters used were essentially as described in the referenced methodology. Notable exceptions were the use of Waters Associates Model U6-K injectors for high-performance liquid chromatography (HPLC) cleanups and J&W 60 m × 0.25 mm i.d. DB-1 columns for high-resolution gas chromatography with electron capture detector (HRGC-EC) determinations throughout the study. Three HPLC systems were assembled to facilitate sample throughput.

Sample Preparation. Samples collected by FDA consisted of whole fish which were subsequently skinned and filleted in the Detroit laboratory. Samples collected by EPA and DNR were received as skinless, boneless fillets. All fillets were triple ground through a meat grinder using a plate with 2 mm holes and were thoroughly mixed between grindings. Twenty-gram portions of the resulting homogenate were weighed for analysis.

Methodology. The method of Firestone (1977) was initially used to analyze several of the samples collected in 1979. This procedure included a C-18 HPLC cleanup step prior to packed column gas chromatography with electron capture detection (GC-EC), followed by confirmation with packed column gas chromatography–low-resolution mass spectrometry (GC–MS). The minimum level of GC-EC quantitation of TCDD in fish was approximately 100 ppt by this procedure. The use of HRGC lowered the EC detection limit to 10–15 ppt, depending on fish species and amounts of other organochlorine compounds present. However, sample extracts so prepared were not clean enough for MS confirmation at this level, even when coupled with HRGC.

In 1981, the multidimensional cleanup procedure of Niemann et al. (1983) was issued in draft form and was substituted for the Firestone procedure. The more rigorous cleanup provided by this methodology allowed for preliminary screening of the samples by HRGC-EC, followed by confirmation and quantitation by HRGC–MS for any sample showing a TCDD residue greater than ca. 10 ppt. The HRGC–MS confirmation/quantitation by multiple ion detection of 12 ions is described in Niemann et al. (1983).

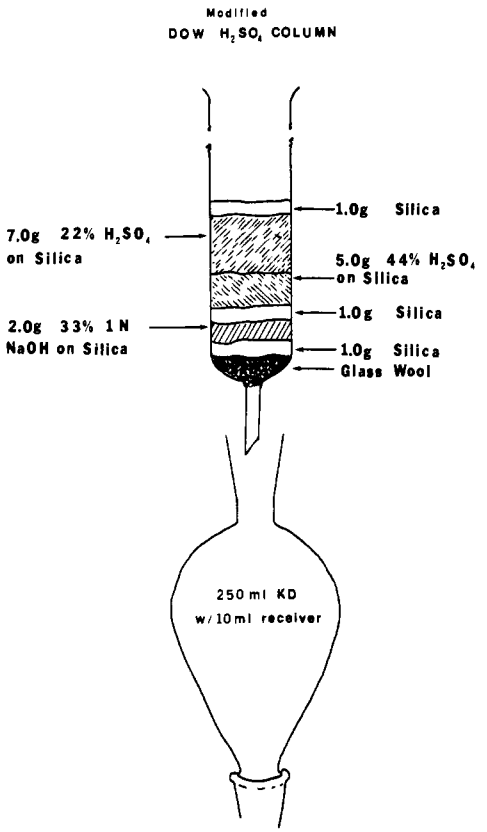

Figure 1. Modified silica-supported sulfuric acid column.

Two significant modifications of the Niemann et al. cleanup were made which improved the TCDD recovery, shortened analysis time and elimination of the hazard of separatory funnel extractions with concentrated sulfuric acid.

Figure 1 shows the modified silica-supported acid cleanup column used. This is a modification of the column first described by Lamparski et al. (1979) and later modified by Langhorst and Shadoff (1980). This column was used in place of the separatory funnel washings with 4 × 40 mL of concentrated sulfuric acid specified in the methods of Firestone (1977) and Niemann et al. (1983). The combined hexane extracts of the ethanolic KOH sample digestate were eluted through the column. The column was then washed with 50 mL of additional hexane, and the combined eluates were evaporated to a small volume (>2 mL) in a Kuderna-Danish concentrator. After evaporation to dryness with purified nitrogen, the resultant residue was completely soluble in 20–30 μL of the mobile solvent used by Niemann et al. for the subsequent size exclusion HPLC step. The average weight of residual material from a 20-g portion of fish after the modified silica acid cleanup was 1.3 mg (n = 16, range 0.4–2.1 mg) for carp and 5.0 mg (n = 24, range 2.5–6.4 mg) for catfish, which were particularly difficult samples to clean-up. Average residue weights from 20-g portions of these samples after separatory funnel washings per Niemann et al. were 9.3 mg (n = 2, range 9.2–9.4 mg) and 7.6 mg (n = 7, range 5.4–8.9 mg), respectively. Thus, the entire extract from the modified cleanup could be easily quantitatively introduced onto the high-performance size exclusion (HPSEC) column in a total maximum allowable volume of 100 μL (≤50 μL to dissolve the residue + 50 μL for rinsing the container and syringe) of HPSEC solvent (95 + 3 + 2, hexane + methanol + methylene chloride). The residue from the Niemann et al. separatory funnel washing required a minimum of 100 μL of solvent just to dissolve; therefore, a 75-μL aliquot was withdrawn for HPSEC cleanup since the extract could not be completely transferred (i.e., rinse could not be made) in the 100-μL volume. The 25% gain in the quantity of sample which can subsequently be carried through the method by using the silica-supported acid cleanup column increases the probability of a positive confirmation at a low (10 ppt or less) TCDD residue level by HRGC–MS.

Table I. Effect of 75:25 Acetonitrile–Water Injection Volume on Elution of TCDD through C-18 Column

vol inj	t_R, s	increase in t_R, s	increase in t_R/mL inj	peak width at base, s
50 μL	752			49
1.0 mL	802	50	50.0	45
1.75 mL	841	89	50.9	49
2.0 mL	855	103	51.5	51
3.0 mL	904	152	50.7	57
4.0 mL	954	202	50.5	67
5.0 mL	1002	250	50.0	75

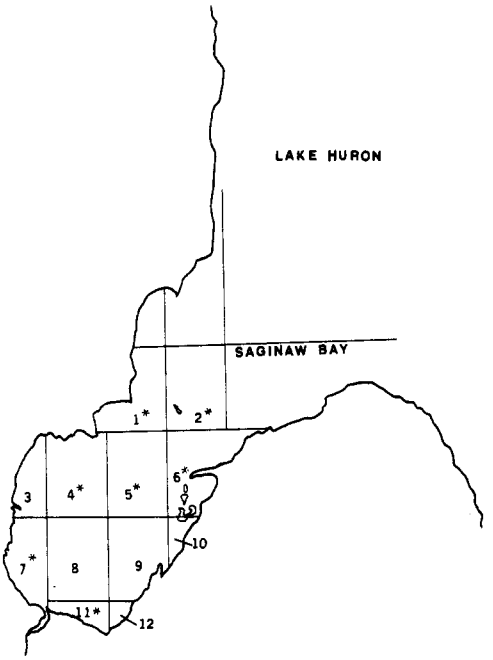

Figure 2. Sample collection sites in Saginaw Bay of Lake Huron.

The extraction step between the C-8 and C-18 HPLC column steps of the Niemann et al. procedure requires diluting the TCDD fraction collected from the C-8 column (in 75:25 acetonitrile–water) with 2% aqueous sodium bicarbonate solution, shaking with hexane, and allowing to stand overnight for complete phase separation. A second extraction is performed on the following day and the combined hexane is evaporated and redissolved in acetonitrile in preparation for introduction into the C-18 system. This entire step was replaced by a direct injection of the C-8 TCDD fraction in 75:25 acetonitrile–water into the C-18 system. In order to do so, the injector for the C-18 system was equipped with a 6-mL loop (minimum volume must be 3.5 mL). The TCDD fraction from the C-8 column (approximately 2.5–3.0 mL) was withdrawn in a 5-mL gas-tight syringe (Hamilton Cat. No. 1005 RN or SGE Cat. No. 5 MA-RN-GT) equipped with a needle compatible with the HPLC injector. The sample tube was rinsed with several small portions of 75:25 acetonitrile–water to make a total volume of 3–3.5 mL in the syringe. The final volume must be kept constant for all standard and sample injections, since the volume injected has a direct relationship to the elution time of the TCDD. The information presented in Table I demonstrates the effect on in-

Table II. Results of Analyses for TCDD

map no.	location	species	no. of samples	year	ppt of TCDD found HRGC–EC	HRGC–MS
1	Saginaw Bay	catfish	1	1981	20	28
		whitefish	1	1983	ND	
2	Saginaw Bay	carp	1	1981	ND	
		carp	1	1981	46	52
3	Saginaw Bay	sucker	2	1979	ND	
4	Saginaw Bay	carp	1	1979	15	19
		carp	2	1979	ND	
		catfish	1	1979	102	*a*
		catfish	1	1981	44	62
		bullhead	2	1979	ND	
		crappie	1	1979	ND	
		y. perch	1	1979	ND	
		suckers	3	1979	ND	
		bowfin	1	1979	ND	
		rock bass	1	1979	ND	
5	Saginaw Bay	carp	2	1983	ND	
		catfish	1	1983	18	18
		catfish	1	1983	ND	
6	Saginaw Bay	y. perch	6	1979	ND	
		sucker	4	1979	ND	
		sucker	1	1983	ND	
		whitefish	1	1979	ND	
		buffalo	1	1979	ND	
		carp	5	1979	ND	
		carp	1	1981	35	31
		carp	1	1983	20	24
		carp	2	1983	ND	
		catfish	1	1979	29	32
		catfish	1	1979	35	20
		catfish	3	1979	ND	
		catfish	1	1981	28	34
		catfish	1	1981	69	67
		catfish	2	1983	ND	
		whitefish	1	1983	ND	
		walleye	1	1983	ND	
7	Saginaw Bay	catfish	1	1979	35	34
9	Saginaw Bay	catfish	1	1979	14	14
11	Saginaw Bay	carp	1	1983	16	13
		carp	1	1983	30	20
		catfish	1	1983	ND	
13	Huron R. (MI)	carp	1	1983	ND	
14	Grand R. (MI)	carp	1	1983	ND	
15	Clinton R. (MI)	walleye	1	1983	ND	
		carp	1	1983	ND	
16	Kalamazoo R. (MI)	carp	1	1983	ND	
17	St. Joseph R. (MI)	carp	1	1983	ND	
18	Raisin R. (MI)	carp	1	1983	ND	
19	Pine R. (MI)	carp	1	1983	ND	
20	Muskegon L. (MI)	carp	1	1983	9	11
21	Muskegon R. (MI)	carp	1	1983	ND	
22	St. Clair R. (MI)	carp	1	1983	ND	
23	Tittabawassee R. (MI)	carp	1	1983	93	66
24	Cass R. (MI)	carp	1	1983	ND	
25	Flint R. (MI)	carp	1	1983	ND	
26	Shiawassee R. (MI)	carp	1	1983	ND	
27	Au Sable R. (MI)	carp	1	1983	ND	
28	L. Michigan (Empire, MI)	whitefish	1	1983	ND	
29	L. Michigan (Muskegon, MI)	sucker	1	1983	ND	
		whitefish	1	1983	ND	
30	L. Michigan (Garden Island)	whitefish	1	1983	ND	
31	L. Michigan (Green Bay)	whitefish	1	1983	ND	
32	L. Michigan	lake trout	1	1983	ND	
33	L. Huron (Thunder Bay)	whitefish	1	1983	ND	
34	L. Huron	lake trout	1	1983	6	6
35	L. Superior (Whitefish Bay)	whitefish	1	1983	ND	
36	L. Superior	lake trout	1	1983	ND	
37	L. Ontario (Wilson, N.Y.)	sucker	2	1981	ND	
		brown trout	1	1981	8	14
		rainbow trout	1	1981	21	13
		lake trout	1	1981	46	34
38	L. Ontario (Chaumont Bay)	y. perch	1	1981	ND	
		w. perch	1	1981	25	20
39	L. Erie (Luna Pier, MI)	carp	1	1981	ND	
40	L. Huron at Tawas R. (MI)	coho salmon	1	1983	ND	
41	L. Michigan at Trail R. (IN)	coho salmon	1	1983	ND	

Table II (Continued)

map no.	location	species	no. of samples	year	ppt of TCDD found HRGC-EC	ppt of TCDD found HRGC-MS
42	L. Erie at Trout Run (PA)	coho salmon	1	1983	ND	
43	L. Mich. at Kellogg R. (IL)	coho salmon	1	1983	ND	
44	L. Ontario at Salmon R. (NY)	coho salmon	1	1983	ND	
		coho salmon	1	1983	35	33
45	L. Erie at Huron R. (OH)	coho salmon	1	1982	ND	
		coho salmon	1	1983	ND	
46	L. Erie at Chagrin R. (OH)	coho salmon	1	1983	ND	
47	Detroit R. (MI)	coho salmon	1	1983	ND	
48	L. Mich. at Platte R. (MI)	coho salmon	1	1983	ND	
49	L. Erie at Cattaraugus Cr. (NY)	sucker	1	1981	ND	
	L. Erie at Eighteen Mile Cr. (NY)	sucker	1	1981	ND	
	L. Erie at Dunkirk Harbor (NY)	catfish	1	1981	ND	
		white bass	1	1981	ND	
		sheepshead	1	1981	ND	
		y. perch	1	1981	ND	
		chinook salmon	1	1981	ND	
		brown trout	1	1981	ND	

[a] HRGC–MS analysis of this sample was done in another laboratory and a quantitative result was not provided.

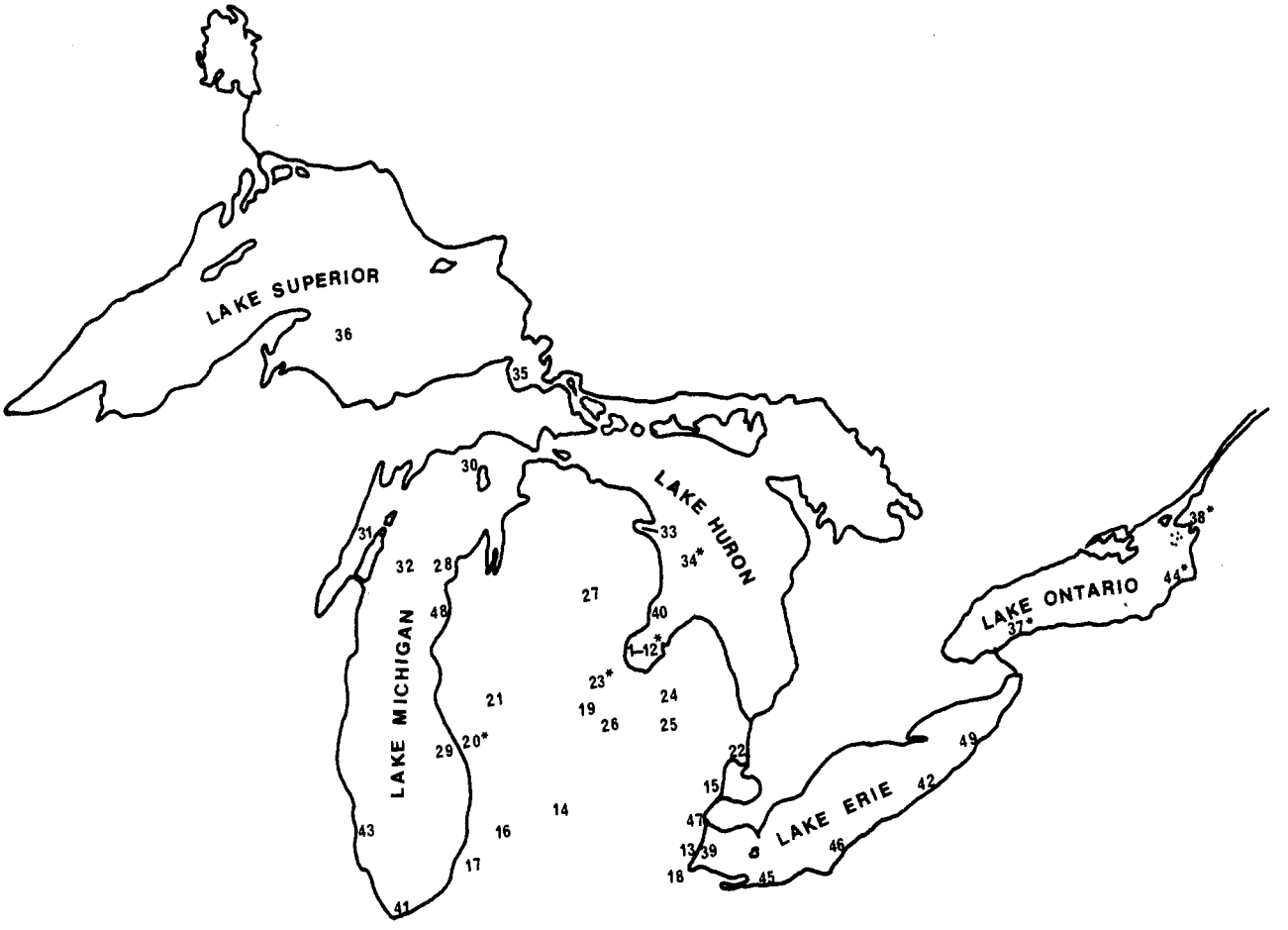

Figure 3. Collection sites for Michigan river and Great Lakes region samples.

jection volume of elution time. The uniform increase in elution time of 50–51 s per milliliter of solution indicates that the TCDD is highly retained from the 75:25 acetonitrile–water at the head of the C-18 column, regardless of injection volume. The peak width at base indicates minimal band spreading if the injection is limited to 3 mL or less. The sum of the volume of the collected TCDD fraction from the C-8 column plus the volume of tube rinsings is the determining factor for the final volume to be injected into the C-18 system. Although this volume usually exceeds 3 mL, a small amount of band spreading does not adversely affect the cleanup or quantitation at this point, providing the entire band is collected.

Recovery of standard TCDD from the C-8 and C-18 HPLC systems by using the direct injection technique was 100 ± 5% for five determinations at the 50–100 ppt level as determined by HRGC-EC with splitless injection. Results of replicate analyses of a carp sample containing bioincurred TCDD averaged 46 ppt after this technique was incorporated into the method, as compared with 44 ppt obtained previously. These results were corrected for the percent recovery of standard TCDD from fortified sample portions analyzed concurrently. Standard recoveries from fortified samples generally ranged between 65 and 85% with the use of the direct injection technique, as compared with a range of 50–70% obtained previously.

A logical next step in method modification would be to develop a system for direct, on-line transfer of eluate from

C-8 to C-18 columns by using switching valves. This approach is currently under evaluation.

RESULTS AND DISCUSSION

Figure 2 shows the Saginaw Bay area of Lake Huron overlaid with grid markings to identify the general collection locations of samples listed in Table II. Figure 3 is a map of the Great Lakes region, again with numbers indicating the collection sites of samples listed in Table II. The numbers 1–12 and 28–49 represent the locations from which samples were collected by FDA, EPA, and NYSDEC. Numbers 13–27 indicate locations of Michigan river sample collections by DNR. The asterisks designate collection sites for samples found to contain TCDD.

The analytical results presented in Table II indicate the major TCDD contamination in the Great Lakes region is in the Saginaw Bay, the Tittabawassee River, and Lake Ontario. Stalling et al. (1983) also reported that fish collected in 1981 from these areas contained the highest levels of TCDD. A comparison of the results of these two studies indicates a decrease in residue levels with time. With the exception of the low level found in one sample each from Lake Huron and Muskegon Lake, none of the other locations sampled showed detectable TCDD contamination. Detectable levels of TCDD were found only in carp (8 of 34 samples), catfish (11 of 18), salmon (1 of 11), white perch (1 of 1), and trout (4 of 7). No residues at or above the 10 ppt level of detection were found in sucker (14 samples), whitefish (9), yellow perch (9), walleye (2), bullhead (2), crappie (1), bowfin (1), rockbass (1), buffalo (1), white bass (1), and sheepshead (1).

The MSU report of high levels of TCDD in carp and suckers from Michigan rivers triggered studies by DNR/EPA and DNR/FDA. Fish for both studies were collected by DNR in the spring of 1983 at the same locations from 13 rivers. Analyses of skinless fillets in the DNR/FDA study show no confirmable levels of TCDD above the 10 ppt limit of GC/MS confirmation, except for the Tittabawassee River sample (93 ppt). See Table II numbers 13–27. The Michigan Division of Environmental Services recently reported the results of the DNR/EPA study (Duling, 1984). Levels of TCDD, based on whole fish analysis, ranged from none detected (limit of detection 0.2–2.5 ppt) to 8.6 ppt, except for the Tittabawassee River sample (190 ppt). The findings of these two studies disagree with those reported in the MSU study except for the high TCDD residue levels found in the Tittabawassee River. Confirmation of the TCDD findings reported for the samples analyzed in the MSU study (reportedly collected in 1979) is impossible since neither portions of those samples nor comparable samples collected at that time were available for the present study.

The HRGC-EC and HRGC–MS results are generally in good agreement in view of the low levels determined. The sample extracts had to be concentrated to a small volume (10 μL) for HRGC–MS confirmation at these concentrations. This severely limited the number of injections which could be made. In most cases, the HRGC–MS results are based on the analysis of a single 3-μL injection of extract. HRGC-EC injections were made from 100-μL volumes and two or more injections (1 or 2 μL) of each extract were routinely made to test the precision of peak responses. Only those HRGC-EC responses which agreed within ±5% were used in calculations and the results were averaged. The use of the HRGC–MS multiple-ion scheme complicates the quantitation and raises the minimum detection level,but the specificity of the analysis is enhanced providing greater confidence in the results.

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