QUADRUPOLE ION STORAGE TANDEM MASS SPECTROMETRY AND HIGH-RESOLUTION MASS SPECTROMETRY: COMPLEMENTARY APPLICATION IN THE DETERMINATION OF PCDDS AND PCDFS IN U.S. AND KAZAKHSTAN FOODS

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Introduction

The major source of non-occupational exposures to humans from PCDD/Fs is animal foods[1]. PCDD/F levels reported on the U.S. food often originate from limited sampling of a few foods[2] in a single region. Daily intakes are often based on a few measurements of pooled samples from a few regions[3]. Past FDA studies measured only 2,3,7,8-TCDD in fish and shellfish with relatively high levels, for example, fish from the Great Lakes[4]. US EPA has surveyed beef, pork and chicken and empirically validated limits of quantitation (LOQs) in beef back fat providing high confidence in the data set[5]. In support of these efforts, FDA has been collecting and analyzing a large number of dairy, fish and shellfish products throughout the U.S. The goal is to produce a data set on food levels that can be used to estimate human exposures. Methods used by FDA and EPA should demonstrate similar LOQs with the required precision and accuracy.

HRMS based methodology has become the standard determinative step in dioxin methods. The very high sensitivity and specificity achieved by these methods is unmatched by any other method including bioassays. Increasing demand for PCDD/F measurements in foods prompted FDA to investigate an alternative tandem mass spectrometry method using a relatively inexpensive quadrupole ion storage mass spectrometer[6].

The U.S. Food and Drug Administration has simultaneously utilized both high-resolution mass spectrometry (HRMS) and quadrupole ion storage tandem mass spectrometry (QISTMS) in the measurement of polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) in 239 food samples collected in the U.S. and 65 food samples collected in southern Kazakhstan. From the U. S., 42 catfish fillet composites and 67 egg composites were analyzed by QISTMS. In addition, 9 scallops, 6 blue crabs, 8 American lobsters, 10 pollack, 17 striped bass, 5 rockfish, 10 crawfish, 19 salmon, along with 18 cream samples and 17 mozzarella cheese samples were measured for PCDD/Fs. From Kazakhstan, 19 cows' and 2 camels' milk samples, 20 butter samples, 14 lamb fat samples, 8 cottonseed oil samples, 2 beef fat samples were measured by QISTMS. Quadrupole ion storage tandem mass spectrometry (QISTMS) and HRMS were used in a complementary manner to increase the throughput for these analyses. Sixty-six U.S. food samples were analyzed using both methods. QISTMS and HRMS results for 2,3,7,8-chlorine substituted dibenzo-p-dioxins and dibenzofurans are compared for replicate analyses of chicken eggs, several

species of fish and shellfish, cream and mozzarella cheese. Interference encountered using QISTMS and HRMS is discussed.

Sample Collection

In 1997, catfish and chicken eggs were collected in eight states in both the Southern U.S. and throughout selected parts of the remaining continental U.S. Forty-two catfish samples, 12 1-2-lb. fish per sample, were collected over a one month period as live whole fish from producing farms in Arkansas, California, Georgia, Louisiana, Maryland, Michigan, Mississippi, Missouri, Oklahoma, Texas and Wisconsin. FDA inspectors collected 31 chicken egg samples from 6 companies at a total of 11 locations with potentially contaminated products in Arkansas, Texas and Louisiana. They also collected 15 samples from 8 companies located in 8 states with no known source of contamination (California, Ohio, Georgia, New York, Pennsylvania, Oregon, Minnesota and Wisconsin)[7]. In 1998, a total of 144 samples of fish, shellfish, eggs and mozzarella cheese were taken from retail outlets in all regions of the continental U. S.

Kazakhstan foods were collected co-incident with the collection of human milks that were suspected to have elevated 2,3,7,8-TCDD. A sample of either butter, lamb fat, beef fat or cottonseed oil was collected from households in selected rural villages and state farms in southern Kazakhstan. Control samples of butter, lamb fat and beef fat were collected from neighboring cities where human milk TCDD levels were known to be lower[8].

Methods

Analytical methodology is described elsewhere[6]. Briefly, 25 g test portions were fortified with 15 $^{13}C_{12}$ PCDD/F standards before extraction and purified as described elsewhere[6]. PCDD/Fs were measured by either QISTMS or HRMS as described in detail elsewhere[6]. A two-fold increase in sensitivity was observed with QISTMS when deactivated "silchrom" electrodes were used. QISTMS instrumental limits of detection (LODs) for all congeners was 150 to 500 femtograms.

Results and Discussion

QISTMS methodology has achieved LOQs in foods of 0.2 pg/g for TCDD[6]. In 1997, FDA responded to an incident where chicken eggs and catfish were fed a diet containing PCDDs from a feed additive, ball clay[7]. QISTMS was used almost exclusively to measure the contamination and distinguish between contaminated and background TCDD levels. Table 1 provides a comparison of HRMS and QISTMS for 17 PCDD/Fs congeners in a ball clay affected egg and a catfish sample. Samples 97-35 and 97-37 were farm-raised catfish not exposed to ball clay. Results using both methods were comparable in either contaminated or background samples for TCDD. HRMS produced LOQs below 0.1 pg/g for hexachlorinated congeners. In some cases, interference prevented unambiguous identification of congeners by HRMS while no interference was observed by QISTMS. Table 2 provides the results for two ball clay affected catfish fillets split and analyzed by either HRMS or QISTMS at CFSAN or at ARL. Results between 0.2 and 2 pg/g agreed closely. Laboratory background can explain some of the differences in the heptachlorodibenzo-p-dioxin and octachlorodibenzo-p-dioxin results.

Table 1. Determinations for chicken eggs and catfish fillets by quadrupole ion storage tandem mass spectrometry (MS/MS) or by HRMS, duplicate analyses from extraction to final GC/MS measurement. All values are in pg/g wet wt. whole egg or skinned catfish fillet. 25 g test portions for catfish; 50 g for egg (50 g for 97-37 and 100 g for egg by MS/MS)

Method	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS
sample #	97-6	97-6	97-60	97-60	97-35	97-35	97-37	97-37
matrix	egg	egg	catfish	catfish	catfish	catfish	catfish	catfish
2,3,7,8-TCDD	0.56	0.6	0.28	0.3	0.11	0.2	0.07	0.1
1,2,3,7,8-PeCDD	0.34	0.2	0.59	0.6	0.07	0.1 I	0.08	0.08 I
1,2,3,4,7,8-HxCDD	0.13	0.05	0.74	0.8	0.3 *	0.04 i	0.2 *	0.05
1,2,3,6,7,8-HxCDD	0.34	0.2 i	0.68	1.2	0.3 *	0.1 i	0.2 *	0.2
1,2,3,7,8,9-HxCDD	0.4	0.45	0.2	1.1	0.3 *	ND	0.2 *	0.07
1,2,3,4,6,7,8-HpCDD	0.59	0.65	6.7	7.7	0.61	0.5	0.36	0.8
OCDD	4.4	4.4	54	126	3.4 L	4.8L	0.82 L	7.7 L
2,3,7,8-TCDF	0.02 *	ND	0.07 I	0.09	0.12	0.5	0.15 I	0.3
1,2,3,7,8-PeCDF	0.055	0.05 i	0.13 *	0.07	0.1 *	ND	0.055	ND
2,3,4,7,8-PeCDF	0.025 I	0.025 i	0.23	0.2 i	0.12	0.1 i	0.14	0.1 i
1,2,3,4,7,8-HxCDF	0.06 I	0.035	0.2 *	0.07	0.3 *	ND	0.2 *	0.03 i
1,2,3,6,7,8-HxCDF	0.07 I	0.03	0.2 *	0.07	0.3 *	ND	0.2 *	ND
1,2,3,7,8,9-HxCDF	0.09 *	ND	0.2 *	ND	0.3 *	ND	0.2 *	ND
2,3,4,6,7,8-HxCDF	ND	ND	ND	0.1	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	0.09 L	0.03 i	0.14	0.1	0.2 *	0.07 i	0.2 *	ND
1,2,3,4,7,8,9-HpCDF	0.01 *	ND	0.15 *	ND	0.2 *	ND	0.2 *	ND
OCDF	0.08 L	0.03 I	0.27	0.2	0.4 *	0.2 i	0.2 *	0.2 i

I = interference, product ion ratio incorrect or HRMS molecular ion cluster interference
L = upper limit; analyte amount is less than 3 the times blank
* analyte not detected; value is an estimate of LOD by quadrupole ion storage tandem MS
i = not confirmed (COCl loss not detected or ratio incorrect in HRMS)
ND = not detected by HRMS

The means, LODs and number of detects for 56 different U.S. food samples including striped bass (11), chicken eggs (7), salmon (16), crawfish (2), lobster (3), crab (5) rockfish (4), cream (5) and mozzarella cheese (3) analyzed by HRMS and QISTMS were compared. The number of detects were similar for the two methods as were the mean levels found for many of the congeners. HRMS produced significantly lower LODs for hexa-chlorinated congeners and OCDD. The means for the 17-2,3,7,8-substituted congeners using QISTMS were between 0.24 to 8.4 and 0.2 to 5.0 pg/g wet weight using HRMS. The ITEQs for the means in the 56 samples were 1.1 pg/g wet weight by QISTMS and 0.99 pg/g by HRMS.

Table 2. Results for catfish PCDD analysis by high-resolution mass spectrometry (HRMS) or by quadrupole ion storage tandem mass spectrometry (QISTMS). pg/g wet wt.

Laboratory	CFSAN	CFSAN	ARL	CFSAN
MS method	HRMS	QISTMS	HRMS	QISTMS
Sample ID	97-45	97-45	97-69	97-69
2,3,7,8-TCDD	0.8	0.8	1.3	1.2
1,2,3,7,8-PeCDD	0.5	0.6	0.9	0.9
1,2,3,4,7,8-HxCDD	1.6	0.4	0.3	0.3
1,2,3,6,7,8-HxCDD	0.5	0.6	0.6	0.6
1,2,3,7,8,9-HxCDD	0.7	0.9	0.8	0.8
1,2,3,4,6,7,8-HpCDD	1.7	2.4	1.7	4
OCDD	32	22	10	14

CFSAN = Center for Food Safety and Applied Nutrition; ARL = Arkansas Regional Lab.

In 1998, QISTMS was used to investigate TCDD levels in food from rural areas of southern Kazakhstan. TCDD concentrations in the state farms were as much as 100 times higher than urban control samples, while rural villages were 10 times higher. QISTMS provided lipid adjusted LODs low enough to measure elevated TCDD levels even though 95% of the animal fat samples were less than 15 g and 40 % were less than 10 g total weight wet. Either method can be used to resolve problems that arise during the analysis of a particular food. QISTMS ease of operation and lower downtime allowed it to screen quicker than HRMS. Using both instruments together improves the productivity of the laboratory and permits a wider surveillance of the U. S. food supply.

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