ApplicationNOTE

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ULTRA TRACE ANALYSIS OF DIOXINS AND FURANS IN HUMAN ADIPOSE TISSUE USING SFE-LC EXTRACTION/CLEANUP AND THE WATERS AUTOSPEC ULTIMA NT

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Introduction

Legislative limits for dioxins and furans are ever decreasing[1], providing a greater challenge for the dioxin analyst. The extraction and clean-up processes used by the analyst can be imperative when attempting ultra trace analysis, and will have a direct effect upon the final analytical method limit of detection.

Biological matrices often form the most difficult matrix to obtain efficient extraction and clean-up, while maintaining high recoveries of internal standards. The method described here provides an efficient extraction and clean-up of human adipose tissue, which contains extremely low levels of dioxins and furans, followed by high resolution gas chromatography—high resolution mass spectrometry (HR/GC-HR/MS) analytical determination of the extracts, using the Waters® Micromass® AutoSpec Ultima™ NT.

Methods

SFE-LC extraction/clean-up of Human Adipose Tissue[2].

Human adipose tissue was spiked with $^{13}C_{12}$ labelled internal standards at a level of between 180 and 360 pg/sample/congener. The tissue was then blended with Na_2SO_4 (ratio of 1g tissue to 4 g Na_2SO_4).

Extraction of the human adipose tissue was performed on an Agilent 7680T extractor. Carbon dioxide (N48 grade, Air Liquideor 4.8 grade, AGA Gas AB) was used as the supercritical fluid. Standard 7 mL extraction thimbles were used. The extraction temperature and pressure were set at 40 °C and 281 bar, corresponding to a density of 0.90 g/mL. A volume of carbon dioxide corresponding to 10 thimble volumes was pumped through the sample at 2 mL/min. During the 34 minute dynamic extraction step, the nozzle and trap temperatures were kept at 45 °C and 40 °C, respectively, in order to avoid plugging as the pressure dropped to atmospheric pressure. The target analytes were trapped on a solid phase trap consisting of a 54 mg portion of PX-21 active carbon, 10-20µm, which was mixed with 1.0 g of 40µm octadecyl silica The mixture was ultrasonicated for 15 min at 35 kHz. From the homogeneous mixture, a dilution was made containing 5.4 mg of PX-21per gram of ODS. A standard 1.02 mL solid phase trap was filled with 0.5 g of the solid phase material.

The nonplanar fraction was eluted from the trap with 6 mL of hexane/methylene chloride and the planar fraction with 9 mL of xylene.

Between extractions, the column was washed with 10 mL of xylene and 10 mL of hexane/methylene chloride, respectively, to avoid cross contamination and to regenerate the trap. Excellent results were achieved in a study where a series of human adipose tissue samples were extracted and cleaned up using SFE-LC3.

The extracts were then spiked with $^{13}C_{12}$-labelled recovery standards, with the final extract volumes adjusted to 30µL in tetradecane.

Analysis

The extracts were analyzed using a Waters Micromass AutoSpec Ultima NT mass spectrometer, operating in the selected ion recording mode, at a resolution of >10,000 (5% height definition). The mass spectrometer, GC oven and autosampler were all controlled using MassLynx™ software, version 4.0, with all acquired data processed and reported using QuanLynx™ and TargetLynx™ application managers.

The selected ion recording acquisition parameters were taken directly from Section 23, Table 8 of US EPA Method 1613, revision B[4]. The mass spectrometer was directly interfaced to an Agilent 6890N gas chromatograph, fitted with a CTC analytics GC-PAL autosampler.

All injections were of a 1µL volume, in splitless mode onto a 30 m DB5-ms 250 mm ID 0.25 µm df GC column, with a He flow rate of 1ml/min.

The temperature ramp 200 °C for 2 minutes, 6 °C/minute ramp to 310 °C, hold 10 minutes, was used for all injections. The splitless injector was maintained at 280 °C, with a purge time of 2.1 minutes and a purge flow of 30 ml/minute.

A series of six human adipose tissue extracts were acquired, followed by a single solvent blank injection and US EPA 1613 calibration standards CS1 and CS2 (Cambridge Isotope Laboratories).

The responses from the two standard injections were compared with the response from a previously acquired five point calibration curve (US EPA 1613 standards CS1 to CS5), and the percentage deviations in response for the 17 2,3,7,8-chlorinated dioxins and furans are presented in Table 1.

Name	CS1 Daily check % deviation	CS2 Daily check % deviation
2378-TCDF	3.8	-3.8
12378-PeCDF	0.5	-0.5
23478-PeCDF	-1.3	1.3
123478-HxCDF	-0.8	0.8
123678-HxCDF	0.8	-0.8
234678-HxCDF	-0.3	0.3
123789-HxCDF	0.4	-0.4
1234678-HpCDF	-1.6	1.6
1234789-HpCDF	-0.3	0.3
OCDF	-3.4	3.4
2378-TCDD	-2.7	2.7
12378-PeCDD	2.8	-2.8
123478-HxCDD	-0.9	0.9
123678-HxCDD	-0.7	0.7
123789-HxCDD	-0.2	0.2
1234678-HpCDD	-0.7	0.7
OCDD	0.5	-0.5

Table 1: Concentration deviations for the two calibration standards acquired as part of the analysis sequence.

Results

One of the most important aspects of confirming the presence of trace levels of dioxins and furans in complex matrices is the accuracy of the measured ion ratios, coupled with the signal-to-noise (S/N) ratio for the peak in question.

The ion ratios and S/N ratios for all of the detected peaks for 2,3,7,8-chlorinated dioxins and furans were reviewed from within QuanLynx and found to be acceptable in all cases. A minimal number of peaks required manual re-integration. Figure 1 presents the primary ion chromatogram for 1,2,3,7,8,-Pentachloro dibenzo-p-dioxin (1,2,3,7,8-PeCDD) for one of the human adipose tissue sample extracts, showing good signal to noise, showing good S/N for an on-column mass of 11 pg.

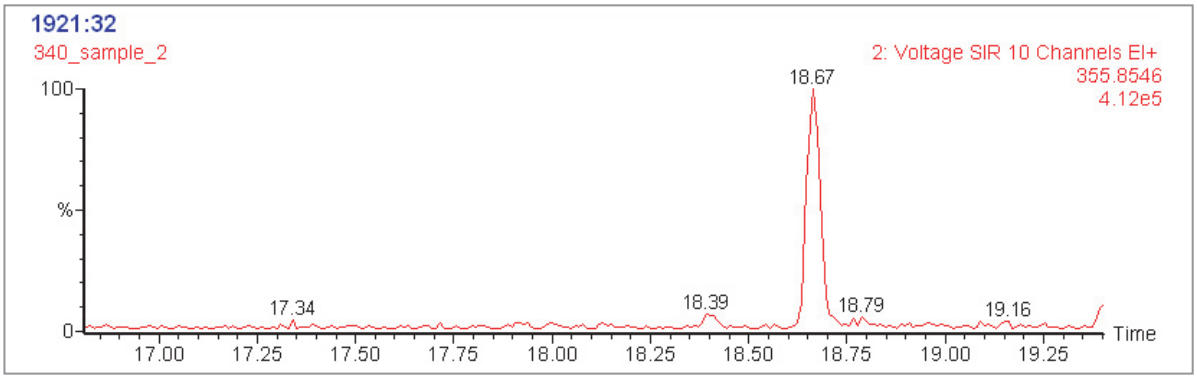

Figure 1: Primary ion chromatogram for 1,2,3,7,8-PeCDD, showing excellent S/N for an on-column mass of 11pg, in a complex matrix extract.

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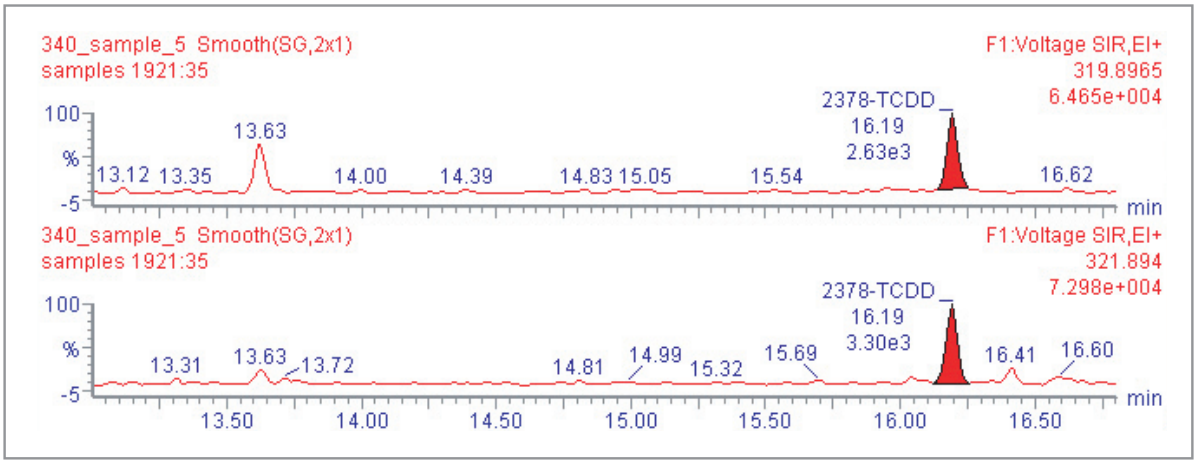

Figure 2: TargetLynx browser display for 2,3,7,8-TCDD at a level of 1.5 pg on column in a human adipose tissue extract.

Figure 2 presents the TargetLynx browser display for 2,3,7,8-Tetrachloro dibenzo-p-dioxin (2,3,7,8-TCDD), showing the primary and secondary ions for a mass of 1.5 pg on column. The ion ratio for 2,3,7,8-TCDD is 0.80 compared with a theoretical value of 0.77. This small deviation falls well within the specified +/- 15% tolerance allowing confirmation of the presence of this congener.

The two congeners displayed above both have high toxic equivalent factors (TEF), being the two most toxic dioxin or furan congeners. As a result, they are often found at higher levels in biological extracts. 2,3,7,8-tetrachloro dibenzofuran (2,3,7,8-TCDF) has a much lower TEF, being assigned by the WHO to have 10% of the toxicity of 2,3,7,8-TCDD. In the human adipose tissue extracts, 2,3,7,8-TCDF was detected at much lower levels, while still giving accurate ion ratios. Figure 3 shows the TargetLynx browser view for 2,3,7,8-TCDF in a human adipose tissue extract at a level of 0.1pg on column, with a reported ion ratio of 0.73, compared with the theoretical ion ratio of 0.77. This ion ratio is within the +/- 15% tolerance specified.

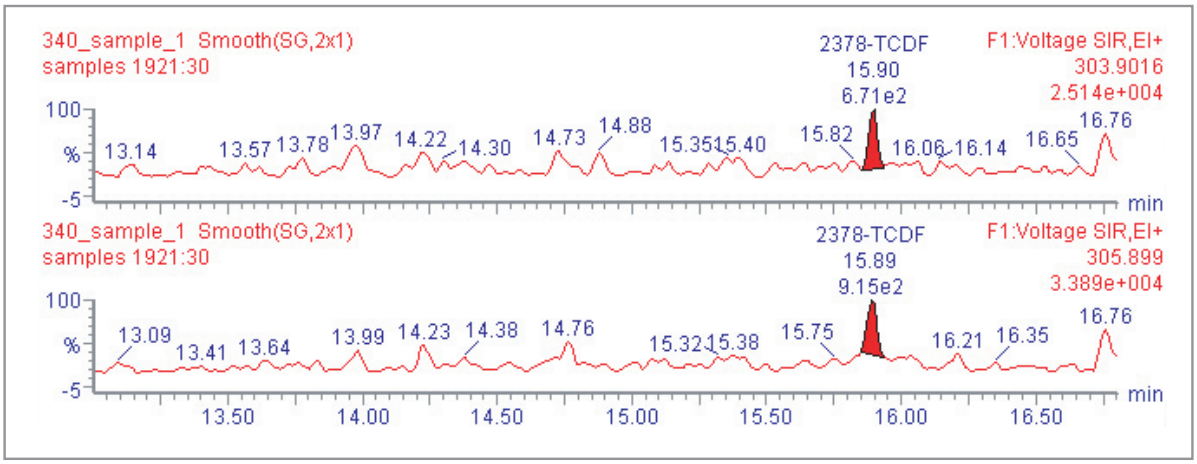

Figure 3: TargetLynx browser display for 2,3,7,8-TCDF at a level of 0.1 pg on column in a human adipose tissue extract.

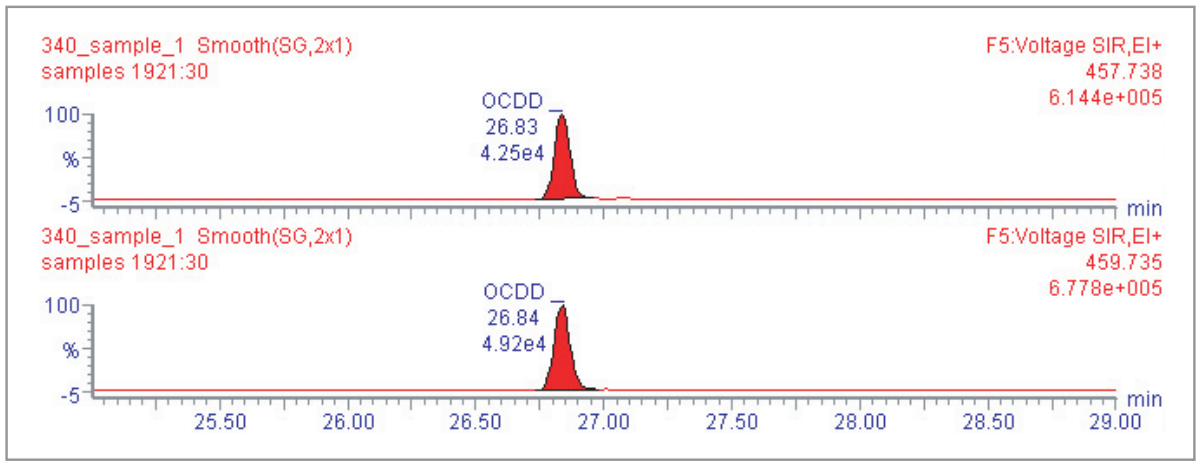

Figure 4: TargetLynx browser display for OCDD at a level of 43 pg on column in a human adipose tissue extract.

Octachloro dibenzo-p-dioxin (OCDD) has the lowest of the TEF's, being 0.0001 times less toxic than 2,3,7,8-TCDD. It is often found at much higher levels in sample extracts, as is the case with these samples. In the same sample that 2,3,7,8-TCDF was detected at a level of 0.1pg on column, OCDD is present at a level of 43pg on column. Figure 4 presents the TargetLynx browser report for OCDD in human adipose tissue extract at a level of 43 pg on column, maintaining a correct ion ratio with no requirement for any manual re-integration.

Conclusions

The absolute sensitivity and resolving power of the Waters Micromass AutoSpec Ultima NT allows the analyst to detect decreasing levels of dioxins and furans in complex matrix extracts. The ability of the instrument to consistently report accurate ion ratios, requiring minimal manual re-integrations, reduces the amount of time spent processing each sample, and gives the analyst greater confidence in the results obtained.

References

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4. Method 1613, Tetra- through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, US EPA, November 1994.

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