ADVANTAGES OF TRITIUM-LABELED TCDD AND CARBON -LABELED OCDD FOR THE DEVELOPMENT OF ANALYTICAL METHODS

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ABSTRACT

Tritium-labeled TCDD and Carbon 14-labeled OCDD can be determined by scintillation counters easily and simultaneously, even in pale fatty raw extracts. With the use of these two radioisotope labeled dioxin standards, quick preliminary tests for the development of analytical methods are possible. Both congeners cover the range of extraction conditions and elution profiles for PCDD and PCDF with 2,3,7,8-structure very closely.

The use of these radioisotopes instead of the application of native and labeled PCCD/F has two important advantages: First, every analytical step can be tested individually without the necessity of eliminating all remaining disturbing coextractives in a cumbersome clean up-procedure. This is demonstrated by the development of a procedure to separate dioxins from candle wax (for the determination of dioxins and furans in dyed candle wax). Second, scintillation counters are less difficult to run in the daily routine than a GC/MS-system.

INTRODUCTION

Usually, the development of analytical methods for the detection of PCDD and PCDF requires the detection of analytes by low or high resolution MS, which generally demands the injection of highly purified extracts. This can become a cumbersome method.

In comparison to MS detection, scintillation counters allow the determination of radioisotope labeled compounds even in fatty raw extracts. The basic requirement for a correct detection is simply the removal of an intense colour. Otherwise, the light absorbing effect will result in losses of light puls counting and this will cause wrong results.

Tritium and carbon 14-labeled dioxins ("3H-TCDD"; "14C-OCDD") are usually applied for toxicological studies. The application for the development of analytical methods has not been described as far as we know.

EXPERIMENTAL

1. Radioisotopes:

a) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (1,5-$^{3}H_{2}$, 40 Ci/mmol; Promochem Catalog No. ED-941). The content of 1 ampoule was solved in 10 ml toluene. Concentration and activity of this stock solution: 4,1 µg/ml respectively 18,5 MBq/ml.

b) 1,2,3,4,6,7,8,9-Octachloro[U-14C]-dibenzodioxin (120 mCi/mmol; Promochem, without Catalog No.). The content of 1 ampoule was solved in 10 ml toluene. Concentration and activity of this stock solution: 38 µg/ml respectively 0,37 MBq/ml.

2. Spiked amounts:

For a reliable evaluation, a sample (e.g. 0,5 - 3 g fat) is spiked with 4,1 pg 3H-TCDD (18,5 Bq) and 760 pg 14C-OCDD (7,4 Bq). This reflects roughly the range for contamination of human fat samples with 2,3,7,8-TCDD and OCDD.

3. Scintillation counter:

LKB 1220 "Quantulus" (low level scintillation counter with active shielding). Acquisition parameters: auto window, dpm-mode, SQP(E) [spectral quench parameter of external standard], dual label (H-3/C-14), counting time 15 min

4. Preparation for scintillation counting

Any extract is concentrated to a final volume of about 1-2 ml and transferred into a sampler vial. The glass vessel with possible residues of the extract is purged with roughly the same volume of the same solvent (as sum, maximal up to 5 ml solvent in the sampler vial). Then, 10 ml of the high flash point liquid scintillation cocktail "Ultima Gold XR" (Packard) are added. After shaking, the sampler vial should stand under protection from light until scintillation counting (wait at least about 30 min).

5. Quantification

To get the real activity in "disintegrations per minute (dpm)", the number of "counts per minute (cpm)" registered by the LSC has to be corrected for counting losses caused by the sample. This is done by an automated quench correction procedure for each sample using an external Ra 226-standard.

RESULTS AND DISCUSSION

In our laboratory, all basic steps for dioxin analysis have been tested preliminarily using 3H-TCDD and 14C-OCDD: extraction, liquid/liquid-purification, gelchromatography, adsorption chromatography on florisil and carbon and a sulfuric acid/silica gel column. With this procedure, elution profiles or reagent amounts could be determined quickly and easily. Afterwards, the steps were tested with native and 13C-labeled dioxins and furans and GC/MS-detection. As a result, the preliminary tests were confirmed. Occasionally, minor changes had to be made to improve the reliability in the daily routine. For all 17 congeners with 2,3,7,8-structure, no discrimination in comparison to the profiles determined with 3H-TCDD and 14C-OCDD were seen. With the usual care in residue analysis, the blank problems could be solved.

The development of a procedure for the determination of dioxins and furans in dyed candle wax demonstrates an important advantage of spiking with radioisotopes. The basic problem is the separation of wax and dioxins. The efficiency of different approaches can be determined by scintillation counting directly, even with relatively high amounts of remaining wax in the final solution to be detected. With colourless wax as matrix for spiking tests, the only requirement is the solubility of the extract in the final solvent.

So, with radioisotope spiked wax a florisil column could be optimized directly, without the influence of any additional clean up step and without the necessity to have such a procedure available. Then, after optimization of this basic separation, the additional steps could be adjusted according to the specific demands (removal of remaining coextractives).

As a result, wax can be separated from dioxins and furans as follows: 3 g wax are liquefied by warming, solved in 20 ml hexane (held at 50 °C) and transferred onto a Florisil column (20 g, deactivated with 3 % water). Wax is removed by elution with 80 ml hexane. Then dioxins and furans are eluted with 150 ml toluene. The column and solvents are kept at about 40 - 50 °C (chromatographic column with jacket for warm water flow). Recoveries for 3H-TCCD and 14C-OCDD were about 90 %. In general, less than 10 mg wax was eluted in the dioxin fraction.

The separation of dioxins from wax on the florisil column could be optimized without any further clean up of the eluted fractions. On the contrary to scintillation counting, the GC/MS-detection requires highly purified extracts. Thus, reasons for losses of dioxins are more difficult to trace. The direct detection of dioxins in fatty extracts is impossible. Sometimes it

remains unclear whether losses of dioxins are caused by absorption on the chromatographic column or by co-elution in the removed fat.

Another important advantage of the application of radioisotopes is the fact that scintillation counters are less difficult to run in the daily routine than mass spectrometers: GC/MS requires the constant cleaning of the injection system and control of acceptable chromatographic conditions, calibration and maintenance of a complex machine. If a laboratory is set up for the analysis of dioxins in food samples and for this purpose equipped solely with a high resolution MS, it will appreciate the low susceptibility to faults of a scintillation counter. Thus, in institutes with radioisotope laboratories which are already equipped with low level scintillation counters it is recommendable for economic reasons to consider the use of radioisotopes.

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