

# Dioxin '97, Indianapolis, Indiana, USA

## Study of Chromatographic Behavior of Polyhalogenated Dioxins in High Performance Reverse Phase Liquid Chromatography with C-18 Phase in Water-Dioxane Systems

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### Abstract

A method for separation of preparative quantities of polyhalogenated dibenzo-*p*-dioxins (dioxins) by high-performance liquid chromatography (HPLC) was described. Mixtures of dioxane-water were suggested for increasing of dioxin's solubility in chromatographic separation. Dependencies of retention times from type of halogens-substitutes (Cl, Br or F), their quantities and locations in molecule of dioxin were investigated.

### Introduction

Polyhalogenated dioxins comprise a group of potentially hazard compounds for biological objects because of their high toxicity and accumulation in environment caused by many industrial processes. Elaboration of methods for detection of dioxins in the objects of environment is an actual analytical problem. Today only methods for determination of polychlorinated dioxins are developed<sup>(1) - 5)</sup>. Others polyhalogenated dioxins ( firstly with Br-substitutes) were demonstrated to have a high toxicity also<sup>(6) - 8)</sup>.

The present investigation has two main objectives: 1 - to find universal system for purification of preparative quantities of dioxins with different halogens-substitutes; 2 - to study chromatographic behavior of some individual dioxins in this system for their purification in synthesis process of radioactive labelled congeners.

### Experimental Methods

Chemicals. All reagents and solvents were of analytical grade.

Polyhalogenated dioxins were synthesized by heating of dipotassium salt of catechol with various polyhalobenzenes in dimethyl sulfoxide<sup>9)</sup>. Products were characterized by using data of HPLC, IR-, NMR- and UV-spectroscopy, temperature of melting, element analyses.

Solubility of dioxins in dioxan-water mixtures. 1,2,3,4-Tetrachlordibenzo-*p*-dioxin was used as model compound for determination of its solubility in water-dioxane mixtures at different ratio

of water and dioxane. The determination of concentration of the dioxin was conducted by analyzing of optical absorption at 320 nm ( $\epsilon$  5020 in pure dioxane). 5 mg of the dioxins were added to one ml of dioxane-water mixtures and mixtures were shaken overnight. Then mixtures were centrifuged at 10000 xg and optical absorptions of supernatants were measured.

**HPLC apparatus.** The HPLC system consisted of two Waters Model 590 pumps, Waters Lambda-max Model 481 detector, Waters Automated Gradient Controller and BBC Goerz Metrawatt SE-120 recorder. Samples were injected manually using a Rheodyne 7125 with 100 mkl sample loop.

**Chromatographic conditions.** The HPLC apparatus was connected to Vydac Protein & Peptide C18 column (4.6 mm x 25 cm). All chromatographic procedures were carried out at room temperature (about 25°C). A mixtures of water:dioxane (25:75 v/v) as solution A and methanol:water:dioxane (20:5:75) as solution B were used in all chromatographic procedures as mobile phase. Samples were dissolved in 100 mkl of solution A and injected into the column. Gradient elution was used: 10 min - 100% of solution A; 60 min - from 100% of solution A to 100% of solution B. Flow rate was 0.5 ml/min. UV detection was carried out at characteristic wave length for analyzing compounds.

## Results and Discussion

Reverse phase HPLC of dioxins base on using of acetonitrile-water and acetonitrile-alcohols mixtures usually <sup>3), 10) - 11)</sup>. But in this systems solution of dioxins is very small and not exceed some mkg/ml as a rule. In some cases it is necessary to increase concentration of dioxins to level of some hundreds mkg/ml for purification of dioxins in their derivatives. Dioxane is known to be a fine nonpolar solvent for many dioxins. We attempted to use dioxane-water and dioxane-methanol solutions as mobile phases for reverse phase HPLC.

**Solubility of dioxins in dioxane-water mixtures.** For studying solubility of dioxins in dioxane-water mixtures we used 1,2,3,4,-tetrachlordibenzo-*p*-dioxin. Obtained results presented in table 1.

Table 1. Solubility of 1,2,3,4-tetrachlordibenzo-*p*-dioxin in dioxane-water mixtures.

Concentration of dioxane in water (% , v/v)	Saturated concentration of the dioxin (mg/ml)
25	< 0.01
50	0.05
75	0.21
100	4.26

We found that solubility of the dioxin in mixtures of dioxane-methanol surpassed such in the dioxane-water with same ratio of their concentrations. Mixture of the dioxane-water with dioxane concentration 75% was fitted for our preparative purification's purposes. Solubility of others tested dioxin in this mixture surpassed the level of 100 mg/ml. We used this mixture as mobile phase for reverse phase HPLC in conditions described in experimental methods.

**Behavior of some dioxins in used HPLC system.** Retention times of polyhalogenated dioxins interesting for us in this chromatographic system are presented in table 2.

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Table 2. Retention times of some dioxins in used chromatographic condition.

Number	Dioxin	Retention time (min)
1	dibenzo- <i>p</i> -dioxin (dioxin)	19.2
2	1,2,3,4-tetrachloro-dioxin	35.3
3	2,3,7,8-tetrachloro-dioxin	34.8
4	1,2,3,4,7-pentachloro-dioxin	40.9
5	1,2,3,4,7,8-hexachloro-dioxin	45.6
6	1,2,3,4-tetrachloro-7,8-dibromo-dioxin	45.6
7	1,2,3,4-tetrafluoro-dioxin	35.3

Analyzing the data from table 2 we conclude that:

1 - Retention time increases with increasing of quantity of halogen-substitute in molecule of dioxin (rows from 1 to 5). The same results was obtained earlier for acetonitrile system for C 18 phase<sup>1)</sup>;

2 - Retention time doesn't depend of the type of halogen-substitute (row 2 and 7; 5 and 6);

3 - Retention time practically doesn't depend of the location of halogen-substitute in the molecule of dioxin. But we believe that this question requires more detailed investigation.

We used this chromatographic system for purification of some dioxins at preparation their derivatives labelled by tritium. Obtained compounds were chemically and radiochemically homogeneous according to the data of analytical HPLC and thin-layer chromatography after one stage HPLC purification in this system.

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