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Gas chromatographic elution patterns of chlorinated dioxins *versus* column polarity

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ABSTRACT

A model has been developed which successfully predicts the order of elution and relative retentions of tetra-, penta- and hexachlorodibenzo-*p*-dioxins for gas chromatography (GC) columns of different polarity. These congeners include the most toxic 2,3,7,8-substituted isomers, and contain numerous difficult-to-separate isomers. This model allows the correlation of GC retention time to dioxin substitution pattern. The model also allows the prediction of dioxin elution order and relative retention time spacing for GC columns of different polarity.

INTRODUCTION

Environmental monitoring for chlorinated dibenzo-*p*-dioxins (PCDDs) is considered important, since many toxicological studies have demonstrated their potential toxicity, especially that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1,2]. Isomer-specific analyses of the 75 PCDDs by gas chromatography–mass spectrometry (GC–MS) have traditionally employed both very slightly polar GC columns (J&W DB-5, Restek RT_x-5, etc.) and highly polar columns (Supelco SP-2330, SP-2340, Restek RT_x-2330, etc.) [3–6]. The choice of GC column is important because the electron impact mass spectra of PCDDs are specific for level of halogenation but are not isomer specific. Therefore, isomer specificity results from the use of a GC column which maximizes the separation of the PCDD isomers within each congener group. The slightly polar GC column type allows elution of all congeners within a short analysis time while giving good separation of most isomers from each other. Highly polar GC columns are applied primarily to maximize the separation of 2,3,7,8-TCDD from the other 21 TCDDs, at the expense of longer analysis times and less column durability. It has been known for some time that the elution order of the PCDDs vary between the two column types, but these elution order changes were not quantified, so that a column having optimum polarity for separating other isomers could be developed efficiently. Recently it has been agreed internationally that those congeners having 2,3,7,8-substitution should be separately quantified for risk assessment [1]. These individual congeners [1 of 22 TCDDs; 1 of 14 pentachlorodibenzo-*p*-dioxins (PeCDDs); 3 of 10 hexachlorodibenzo-*p*-dioxins (HxCDDs); 1 of 2 heptachlorodibenzo-*p*-dioxins (HpCDDs), and octachlorodibenzo-*p*-dioxin (OCDD)] were assigned Toxicity Equivalency Factors (TEF) of 1, 0.5, 0.1, 0.01 and 0.001 for the TCDD, PeCDD, HxCDDs, HpCDD and OCDD, respectively [2]. Because of this development, it is worthwhile to develop a model for predicting elution profiles on new GC

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TABLE I

SCALED RELATIVE RETENTION INDICES (SRRI) FOR PCDDs AND MOLECULAR SHIFT INDICES (SI) FOR CHANGES IN GC COLUMN POLARITY

M = Compound selected to develop the SI model.

Congener	SRRI		SI		ΔSI exptl. − calcd.
	DB-5	SP-2330/ SP-2340	exptl.	calcd.	
1,3,6,8	0	0	0	M (1,3)	
1,3,7,9	10	12	2	6	−4
1,3,6,9	18	31	13	14	−1
1,2,4,7	36	31	−5	M (2)	
1,2,4,8	36	31	−5	−5	0
1,3,7,8	36	23	−13	−12	−1
1,4,6,9	37	66	29	M (1,4)	
1,2,4,6	41	48	7	5	2
1,2,4,9	41	48	7	5	2
1,2,6,8	43	37	−6	−2	−4
1,4,7,8	46	43	−3	−4	1
1,2,7,9	54	52	−2	4	−6
1,2,3,4	65	47	−18	M (unsub.)	
1,2,3,6	64	52	−12	M (1)	
1,2,6,9	64	73	9	12	−3
1,2,3,7	67	48	−19	−16	−3
1,2,3,8	67	48	−19	−16	−3
2,3,7,8	70	45	−25	M (2,3)	
1,2,3,9	74	68	−6	M (1,9)	
1,2,7,8	79	65	−14	−14	0
1,2,6,7	82	78	−4	M (1,2)	
1,2,8,9	100	100	0		
1,2,4,6,8	0	0	0		
1,2,4,7,9	0	0	0		
1,2,4,6,9	19	47	28	21	7
1,2,3,6,8	31	12	−19	−10	−9
1,2,4,7,8	36	25	−11	M (1,2,4)	
1,2,3,7,9	44	33	−11	−4	−7
1,2,3,6,9	51	60	9	4	5
1,2,4,6,7	56	63	7	5	2
1,2,4,8,9	56	63	7	5	2
1,2,3,4,7	64	48	−16	−21	5
1,2,3,4,6	70	72	2	−11	13
1,2,3,7,8	78	56	−22	M (1,2,3)	
1,2,3,6,7	85	75	−10	−12	2
1,2,3,8,9	100	100	0		
1,2,4,6,7,9	0	0	0		
1,2,4,6,8,9	0	0	0		
1,2,3,4,6,8	29	0	−29	−9	−20
1,2,3,6,7,9	43	23	−20	−3	−17
1,2,3,6,8,9	43	23	−20	−3	−17
1,2,3,4,6,9	52	66	14	5	9
1,2,3,4,7,8	69	42	−27	M (1,2,3,4)	
1,2,3,6,7,8	76	46	−30	−20	−10
1,2,3,7,8,9	82	81	−1	−14	13
1,2,3,4,6,7	100	100	0		

column types, so that optimum separations of those congeners having assigned TEF values could be achieved with minimum column development and testing. Additionally, for quality assurance, data obtained on one type of column could be more readily compared with data from another type, even if a limited number of standards were employed in the analyses.

EXPERIMENTAL

Data were selected from available literature [3–7] for the Cl_4, Cl_5 and Cl_6 congener groups and the reported chromatograms were labelled so that retention times were expressed on a 0–100 scale, with the first eluting isomer set at 0 and the last eluting isomer of that congener group set at 100. DB-5 is a relatively non-polar GC column phase. SP-2330 and the very similar SP-2331, SP-2340 are highly polar phases. Compounds chosen for the shift index (SI) model are indicated in Table I. Where appropriate, 2,3,7,8-substituted congeners were selected; these congeners are those of greatest interest, and for which the most reliable standards are available. Elution of TCDDs from DB-1701 and DB-225 columns were also modelled in the same way. TCDD isomer assignments (1,2,6,8-, 1,2,7,8- and 1,2,7,9-TCDD) were corrected where necessary [7,8].

DISCUSSION

The DB-5 and SP-2330 types of phases are frequently employed for dioxin analysis, and data are readily available in the literature. However, a model correlating dioxin substitution pattern to GC retention index (I) has been reported only for non-polar columns [7–9]. Because such a model is useful for isomer identifications, it was deemed worthwhile to extend this structure–I modelling concept to the popular SP-2330 polar types of GC columns. Both non-polar and polar phases were chosen in this study to model the relative retention time (RRT) shifts with change in column polarity. Susceptibility to RRT shifts was modelled for the TCDDs, PeCDDs and HxCDDs. These congener groups include the congeners having the highest assigned TEFs, and these congener groups have sufficiently large numbers of isomers to make modelling valuable. Because the normally employed GC column temperature programs have varied widely among column types and individual analyses, the RRTs were normalized, for each congener group addressed in this study, to a range of 0–100. In this way, a standardized scale for the isomers' RRTs was achieved for the various temperature programs utilized, and isomer shifts relative to each other could be quantified, independent of the GC temperature program. Since the first and last eluting isomers were found to remain the same regardless of GC column polarity, this strategy provided a means of determining the magnitudes of isomer RRT shifts within the congener elution profile, as column polarity was varied.

Shifts in scaled relative retention indices (SRRIs)

TABLE II

SINGLE RING SHIFT INDEX (SRSI) VALUES TO CONVERT SRRI FOR DB-5 TO SRRI FOR SP-2330 COLUMN PHASES

Single ring substitution pattern	Calculation process: [SRRI]/2 or [SRRI] − [SRSI]	SRSI
1−	[1,2,3,6] − [1,2,3−] = −12 − (−10)	−2
2−	[1,2,4,7] − [1,2,4−] = −5 − 1	−6
1,2−	[1,2,6,7]/2 = −4/2	−2
1,3−	[1,3,6,8]/2 = 0/2	0
1,4−	([1,4,6,9]-2RRI)/2 = (29 − 12)/2	+8
2,3−	[2,3,7,8]/2 = −25/2	−12
1,2,4−	[1,2,4,7,8] − [2,3−] = −1 − (−12)	+1
1,2,3−	[1,2,3,7,8] − [2,3−] = −22 − (−12)	−10
1,2,3,4−	[1,2,3,4,7,8] − [2,3−] = −27 − (−12)	−15
Unsubstituted	[1,2,3,4] − [1,2,3,4−] = −18 − (−15)	−3
1,9-Ring–ring interaction (RRI)	[1,2,3,9] − [1,2,3,6] = −6 − (−12)	+6

TABLE III

SCALED RELATIVE RETENTION INDICES FOR DIFFERENT POLARITY COLUMN PHASES

PCDD Isomer	SRRI			
	DB-5	DB-1701	DB-225	SP-2330/SP-2331/ SP-2340
1,3,6,8	0	0	0	0
1,3,7,9	10	10	10	12
1,3,6,9	18	23	28	31
1,2,4,7	36	34	32	31
1,2,4,8	36	34	32	31
1,3,7,8	36	30	23	23
1,4,6,9	37	49	59	66
1,2,4,6	41	44	48	48
1,2,4,9	41	44	48	48
1,2,6,8	43	41	39	37
1,4,7,8	46	44	43	43
1,2,7,9	54	53	52	52
1,2,3,4	65	57	51	47
1,2,3,6	64	59	54	52
1,2,6,9	64	67	71	73
1,2,3,7	67	57	49	48
1,2,3,8	67	59	51	48
2,3,7,8	70	57	45	45
1,2,3,9	74	72	68	68
1,2,7,8	79	72	66	65
1,2,6,7	82	82	81	78
1,2,8,9	100	100	100	100
1,2,4,6,8	0		0	0
1,2,4,7,9	0		0	0
1,2,4,6,9	19		41	47
1,2,3,6,8	31		15	11
1,2,4,7,8	38		26	23
1,2,3,7,9	44		32	30
1,2,3,6,9	52		56	59
1,2,4,6,7	56		62	62
1,2,4,8,9	56		62	64
1,2,3,4,7	65		50	47
1,2,3,4,6	69		71	71
1,2,3,7,8	78		56	53
1,2,3,6,7	86		77	74
1,2,3,8,9	100		100	100
1,2,4,6,7,9	0		0	0
1,2,4,6,8,9	0		0	0
1,2,3,4,6,8	29		6	0
1,2,3,6,7,9	43		26	24
1,2,3,6,8,9	43		26	25
1,2,3,4,6,9	50		60	68
1,2,3,4,7,8	78		47	43
1,2,3,6,7,8	83		53	47
1,2,3,7,8,9	100		81	80
1,2,3,4,6,7	100		100	100

for the TCDDs, PeCDDs and HxCDDs were described with reference to the substitution patterns of the single rings, as has been reported for calculating retention indices on non-polar and very slightly polar GC columns for the PCDDs [7]. As shown in Tables I and II, shifts to higher RRTs occurred, as column polarity increased, with the 1,4 and 1,2,4 single rings, and with the presence of 1,9-dichloro ring–ring interactions (RRI). The unsubstituted and the 1-, 2-, 1,2-, 2,3-, 1,2,3- and 1,2,3,4-chlorine-substituted single rings shortened RRTs as column polarity increased. For the compounds not used in developing this model, RRT shifts were calculated using the model; in general, very good agreements were obtained between predicted and observed values, with variations of the same magnitude as that observed between different data sets using these GC column phases. The fits of calculated to experimental SRRI were best for the TCDDs; RRT predictions for the TCDDs are perhaps the most useful, because the number of isomers is greatest. Fewer HxCDD data were available for comparison and validation of published isomer assignments *vs.* GC retention times. Additionally, some temperature programs used for measuring those data terminated before elution of all HxCDDs; retention time–index modelling is more accurate for isomers eluting during the linear temperature program ramp. These situations may have caused the fits of predicted to observed SI values to be somewhat poorer for the HxCDDs. Quantitative single ring shift index (SRSI) values for the single rings and for the 1,9-dichloro ring–ring interaction are shown in Table II. The SI value for a dioxin molecule in SRRI units which is observed for a PCDD upon changing GC column phase may thus be expressed as the sum of contributions from the SRSI of the single rings plus that of the 1,9-ring–ring intereaction effect, if applicable.

Values of SRRI were calculated for the TCDD isomers on intermediate polarity columns, DB-1701 and DB-225 (see Table III), using reported retention time data [6]. These columns have intermediate levels (14% and 50%, respectively) of cyanopropylphenyl substituents in their liquid phases to increase column polarity, as compared to 0% and 68% for DB-5 and SP-2330, respectively. Elution profiles of the TCDDs on DB-1701 and DB-225 were also intermediate, as would be expected, between the

DB-5 and SP-2330 elution profiles. The SRRI shifts from one column type to another agreed well with these increases in phase polarity. The results obtained with these two columns will facilitate the estimation of SI values for other GC columns. The SRRI values were also calculated for DB-5, SP-2331, and DB-225 phases for PeCDDs and HxCDDs (see Table III) from recently reported data [8]. These data for SP-2331 agreed well with the data for the very similar SP-2330 and SP-2340 columns. One retention data were scaled into SRRI units, the various data sets were found to be in close agreement for a given column type (entries generally + 2 SRRI units).

CONCLUSIONS

The 1,4 and 1,2,4 single rings and 1,9 ring/ring interactions tended to shift the relative retention times to higher normalized values with increasing GC column polarity. The 1-, 2-, 1,2-, 2,3-, 1,2,3- and 1,2,3,4-substituted (chlorinated) single rings tended to shift the isomers to lower normalized relative retention times with increased GC column polarity. These effects were quantifiable with good precision across the different analyses, temperature programs, and GC columns utilized and reported by various investigators, and applied to this study. This SI model allows the extension of a structure–*I* model for chlorinated dioxins from the DB-5 type GC column to other types of GC columns. With this SI model, retention indices of the 2,3,7,8-substituted congeners can be calculated. Knowledge of these retention indices facilitates environmental monitoring by GC–MS for the congeners having assigned TEF values. Elution order and spacing of dioxin isomers within a congener series (TCDDs, PeCDDs or HxCDDs) can be predicted for GC columns of different polarity by the application of this modelling technique.

NOTICE

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