Analytical Artifacts during Enantioselective Determination of Chiral Organochlorines with GC/ECNI-MS

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Introduction

The determination of enantioratios (ERs) of chiral organochlorines has become subject of an increasing number of studies [1]. Several detection techniques in combination with enantioselective gas chromatography have been developed for this purpose. One of the most promising detection technique seemed to be GC/MS in the selected ion monitoring (SIM) mode. High selectivity is obtained in the SIM mode and, therefore, it is widely applied for quantitation of organochlorines in environmental samples. In the SIM mode, GC/MS was also predestined for determination of ERs in biological samples. Due to the many compounds in environmental samples and the ambivalency of enantioselective gas chromatography (e. g. the lower the elution temperature the higher the enantiomeric resolution but the higher the elution temperature the sharper the peaks), a selective detection method seems to be superior to non-selective methods such as GC/ECD.
In this presentation we provide evidence that interfering compounds may cause analytical artifacts, at least in the case of GC/ECNI-MS.

Experimental

GC/ECNI-MS was performed with an HP 5989B mass spectrometer coupled to an HP 5890 gas chromatograph (Hewlett-Packard). The chiral stationary phase consisted of 25% *tert.*-butyldimethylsilylated ß-cyclodextrin in PS086 (BGB Analytik, Adliswil, Switzerland).
All measuring parameters were recently described in detail [2].
The compounds studies in this investigation were 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (B9-1679 or Parlar #50) and 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (B7-515 or Parlar #32).

Results and Discussion

In a recent publication coelution of the second eluted B7-515 enantiomer with the first eluted B9-1679 enantiomer was reported on ß-BSCD [2]. While the latter is a major CTT in environmental samples, the B7-515 is a major CTT in technical mixtures but easily metabolized in biota. E. g., B7-515 was neither detected in fish [3][4] nor in in the blubber of marine mammals [5].

Both compounds have different molecular weights and the typical m/z-values in the selected ion monitoring mode of B9-1679 (m/z 411 and m/z 413) are absent in the ECNI mass spectrum of B7-515 (see Figure 1). Consequently, B7-515 gives no ECNI response if m/z 411 and m/z 413 are monitored in the SIM mode.

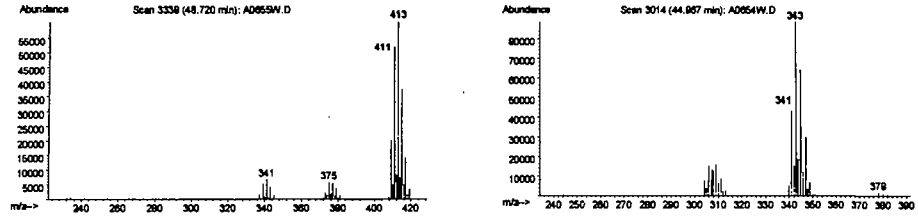

Figure 1: GC/ECNI-MS spectra of left: B9-1679 (Parlar #50), right: B7-515 (Parlar #32)

Enantioseparation of mixtures of B9-1679 and B7-515 resulted in a strange phenomenon: while the pure B9-1679 standard resulted in the expected racemic mixture (see Figure 2, left), injection of mixtures of B9-1679 and B7-515 and monitoring of m/z 411 and m/z 413 resulted in a significantly higher peak at the retention time of the first eluted B9-1679 enantiomer. This effect is obviously caused by the second B7-515 enantiomer which interfers with the first eluted B9-1679 enantiomer. Note that B7-515 is not monitored with this technique. This is clearly observable from the lack of a signal at the retention time of the first eluted B7-515 enantiomer.

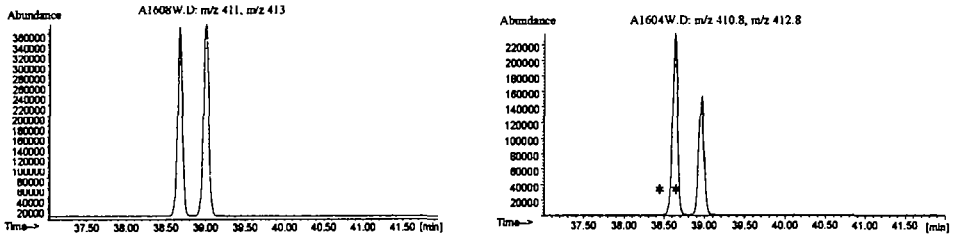

Figure 2: Enantioseparation on ß-BSCD
left: B9-1679 (TIC: m/z 411, m/z 413)
right: mixture of B9-1679 and B7-515 (SIM: m/z 411; m/z 413)
visible: enantiomers of B9-1679; invisible: B7-515 enantiomers (asterisks mark retention times of the B7-515 enantiomers)

For an extensive study of the effect several solutions of B9-1679 alone and mixtures of B9-1679 and B7-515 were injected (see Table 1). The data clearly demonstrates that only the pure non-interfered B9-1679 (Table 1, #2,#5,#9,#10,#12) solutions resulted in the racemic composition while the spiked solutions (Table 1, #1,#3,#4,#6,#7,#11) deviated from the expected result of 1.0. Furthermore, the artifact was the more pronounced the higher the injected amount and the higher the amount of the interferent B7-515 relative to B9-1679.

Table 1: GC/ECNI-MS Enantioseparation of B9-1679 (Parlar #50): Effect of interference with B7-515 (Parlar #32)

Run no.	Sample		Level [pg] B9-1679 / B7-515	$ER_{B9-1679}$
#1 (A1599W)	B9-1679 + B7-515	spiked	100 / 100	1.2
#2 (A1600W)	B9-1679	unspiked	100	1.0
#3 (A1601W)	B9-1679 + B7-515	spiked	100 / 100	1.2
#4 (A1602W)	B9-1679 + B7-515	spiked	100 / 100	1.3
#5 (A1603W)	B9-1679	unspiked	100	1.0
#6 (A1604W)	B9-1679 + B7-515	spiked	500 / 500	1.5
#7 (A1605W)	B9-1679 + B7-515	spiked	100 / 200	1.6
#8 (A1606W)	blank	-	-	-
#9 (A1607W)	B9-1679	unspiked	100	1.1
#10 (A1608W)	B9-1679	unspiked	100	1.0
#11 (A1609W)	B9-1679+ B7-515	spiked	100 / 100	1.5
#12 (A1610W)	B9-1679	unspiked	100	1.0

We also studied this effect with biological samples (Figure 3).

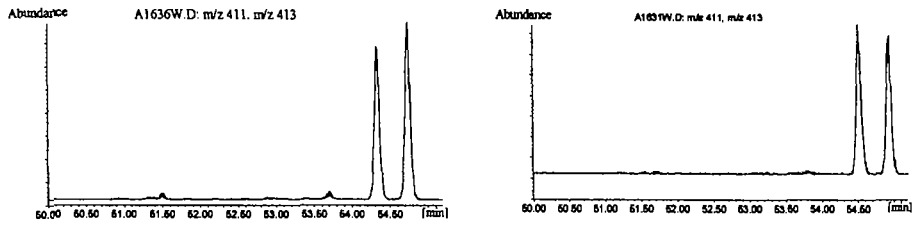

Figure 3: left: Enantioseparation of B9-1679 in the blubber of a harbor porpoise (*Phocoena phocoena*), monitored ions: m/z 411 and m/z 413
right: reinjected solution of blubber of a harbor porpoise spiked with B7-515

While the unspiked harbour propoise sample resulted in an ER < 1, the spiking with B7-515 reversed the peak ratio resulting now in an ER >1 (artifact). In this worst scenario, the interpretation of the result would lead to reversed conclusions (higher abundance of the first eluted enantiomer versus higher abundance of the second eluted enantiomer).
There are several questions left:

- Is this a problem only with the present method/instrument?
- Is this problem limited to GC/ECNI-MS?
- Is this problem limited to ß-BSCD?
- Is this problem limited to toxaphene compounds?
- Is this effect also valid for other compounds?

The phenomenon might either be caused by effects in the column or by association effects in the mass spectrometer. At the moment we have no solution to avoid this effect but to check the peak purity of the enantiomers at the respective retention times by either isolation of the compound or recording of full scan mass specta.
We have applied GC/ECNI-MS in several publications to establish the enantioratios of CTTs [2][6][7][8]. Fortunately, the results presented in the respective publications were confirmed with additional data (see Table 2).

Table 2: Reconsideration and confirmation of ER obtained with GC/ECNI-MS

CTT	sample	ER	confirmation of the result
B7-1453	isolate from Melipax	1.26 [7]	identical result on ß-TBDM with GC/ECD [7]
B8-1413	seal blubber	1.1 - 1.3 [2][6]	comparable value in an isolate of the compound from seal blubber [9]
B8-2229	seal blubber	2.6 - 3.4 [6]	identical value in an isolate of the compound determined on ß-TBDM with GC/ECD [10]
B9-1679	seal blubber	1.2-1.4 [2][6]	comparable value in an isolate of the compound from seal blubber [9]

Work is ongoing to study the effect on other CSPs. A major problem is the identification of presumable interferences which allow for a study of the phenomenon.
During the last months we have determined ER of CTTs in several matrices. After recognition of the artifact, these results were withhold until an unequivocal poof of their correctness is achieved by confirmation of the results with as many as possible of the following strategies:

- recording of ECNI-MS full scan mass spectra
- confirmation of the results with another CSP
- exclusion of coelutions by GC/ECD analysis
- isolation of the compound

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