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Conventional and narrow bore short capillary columns with cyclodextrin derivatives as chiral selectors to speed-up enantioselective gas chromatography and enantioselective gas chromatography–mass spectrometry analyses

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ABSTRACT

The analysis of complex real-world samples of vegetable origin requires rapid and accurate routine methods, enabling laboratories to increase sample throughput and productivity while reducing analysis costs. This study examines shortening enantioselective-GC (ES-GC) analysis time following the approaches used in fast GC. ES-GC separations are due to a weak enantiomer-CD host–guest interaction and the separation is thermodynamically driven and strongly influenced by temperature. As a consequence, fast temperature rates can interfere with enantiomeric discrimination; thus the use of short and/or narrow bore columns is a possible approach to speeding-up ES-GC analyses. The performance of ES-GC with a conventional inner diameter (I.D.) column (25 m length × 0.25 mm I.D., 0.15 μm and 0.25 μm d_f) coated with 30% of 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin in PS-086 is compared to those of conventional I.D. short column (5 m length × 0.25 mm I.D., 0.15 μm d_f) and of different length narrow bore columns (1, 2, 5 and 10 m long × 0.10 mm I.D., 0.10 μm d_f) in analysing racemate standards of pesticides and in the flavour and fragrance field and real-world-samples. Short conventional I.D. columns gave shorter analysis time and comparable or lower resolutions with the racemate standards, depending mainly on analyte volatility. Narrow-bore columns were tested under different analysis conditions; they provided shorter analysis time and resolutions comparable to those of conventional I.D. ES columns. The narrow-bore columns offering the most effective compromise between separation efficiency and analysis time are the 5 and 2 m columns; in combination with mass spectrometry as detector, applied to lavender and bergamot essential oil analyses, these reduced analysis time by a factor of at least three while separation of chiral markers remained unaltered.

1. Introduction

Cyclodextrin derivatives (CDs) have gained an increasing acceptance as chiral stationary phases (CSPs) in GC separation of enantiomers because of their wide enantioselectivity and ability to separate underivatized enantiomers of different volatilities. When used in enantioselective gas chromatography (ES-GC), CDs are generally diluted in apolar or moderately polar polysiloxanes to obtain highly efficient capillary GC columns, as proposed by Schurig et al. [1–3]. The success of a separation technique depends on its suitability for use in the routine analysis of real-world samples, whose requirements include the ability to identify characteristic markers in a sample and short analysis time. In general, a correct enantiomeric excess (EE) or enantiomeric ratio (ER) determination of optically active markers in complex real-world samples requires a two-dimensional approach, since enantioselective analysis can make the resulting chromatographic profile more complex, because enantiomer separation may cause peak doubling, increasing the risk of peak overlap. Two complementary but distinct approaches are those most commonly adopted: the first and most popular one is based on a second dimension *in separation* with heart-cut GC–GC [4–7] or comprehensive two-dimensional GC (GC × GC) [8–11]; the second approach includes a second dimension *in identification* using mass spectrometry (MS) (or very rarely FT-IR) as ES-GC detector. MS is known not to be a selective chiral probe making enantiomer MS spectra indistinguishable. These spectra can therefore successfully be exploited to determine EE or ER correctly in both the total and the extract ion modes although they can only be

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Table 1
Columns and analysis conditions adopted for the present study.

Conventional (internal diameter 0.25 mm)				Narrow bore (internal diameter 0.10 mm)			
Columns		Analysis conditions		Columns		Analysis conditions	
Length (m)	Film thickness (μm)	Flow rate (mL/min)	Temperature rate (°C/min)	Length (m)	Film thickness (μm)	Flow rate (mL/min)	Temperature rate (°C/min)
25	0.25	1	2	10	0.10	0.4	2
25	0.15			5	0.10	0.7	3.5
5	0.15			2	0.10	1	5
				1	0.10		10

used to locate the enantiomers in the chromatogram since their linear retention indices (I^T) [12] are necessary to identify them unequivocally [13].

One of the approaches used to satisfy the increasing demand for analytical controls is to adopt fast chromatographic techniques, which enable high sample throughput and laboratory productivity, while at the same time reducing analysis costs. Fast GC and fast GC–MS have long been established for routine analysis [14–19]; these methods involve contemporary contributions of several parameters including column length and inner diameter (I.D.), stationary phase film thickness (d_f), temperature programme and linear carrier gas velocity. A limit of ES-GC in routine quality control is the long analysis time since the separation depends on a small difference in the energy of the association between each enantiomer and the CD chiral selector and requires a very high chromatographic efficiency. The ES-GC separation of enantiomers by CD derivatives is known to be based on fast kinetics and entirely governed by thermodynamics [20,21], and therefore strongly controlled by temperature. In contrast to what happens with fast conventional GC, the temperature rates that can be used to speed up an ES-GC separation with CDs as CSPs are therefore rather low. Fast ES-GC can thus mainly be achieved by operating on column length, inner diameter and/or flow-rate and in particular with short narrow bore (NB) columns. These columns not only increase analysis speed and analyte detectability due to peak sharpening [22], but also reduce enantiomer elution temperature, resulting in a gain of enantioselectivity that compensates (in full or in part) for the loss of efficiency (N). Short columns were applied in ES-GC with CDs since the beginning of the 1990s enabling enantiomer separations even in a few seconds [23–26]. After an in-depth study of ES-GC, Schurig and Czesla [27] concluded that short conventional 0.25 mm I.D. columns should be used for fast ES-GC because of their good loadability, integration characteristic, use of conventional instrumentation and lower consumption of carrier gas.

However, conventional I.D. short columns can be applied in monodimensional ES-GC only when chiral compounds must be determined in low complexity samples and/or when a low number of enantiomers must be analysed simultaneously. With medium-to-high complexity samples, a highly efficient separation system combined with single- or multiple-ion monitoring-MS detection is necessary to determine EE and/or ER correctly. This article examines how to optimise the parameters conditioning the speeding up of ES-GC–MS analysis of medium-to-high- and medium-to-low-volatility analytes; it also looks at the application of fast ES-GC–MS to routine analysis of real world samples in the field of flavours and fragrances.

2. Experimental

2.1. Samples

Pure standards of racemic linalool, linalyl propionate, γ-lactones (C6, C7, C8, C11, C12, C14, C15), δ-octalactone, α-hexachlorohexane (HCH), trichlorfon, trans-chlordane, and heptachlor, together with the components of the laboratory-prepared chiral test mixture consisting of limonene, 2-octanol, camphor, isobornyl acetate, linalyl acetate, 2-methyl-(3Z)-hexenyl butyrate, menthol, hydroxycitronellal, γ-decalactone and δ-decalactone racemates [28], were from the collection of standards in the authors' laboratory or, if unavailable there, were obtained from Sigma–Aldrich (Milan, Italy). All standard compounds were dissolved in cyclohexane at a concentration of 100 ppm each. Solvents were all HPLC grade from Riedel-de Haen (Seelze, Germany). Lavender (*Lavandula angustifolia* P. Mill.) and bergamot (*Citrus bergamia* Risso et Poiteau) essen-

Table 2
Retention time (t_R) of the second enatiomer, elution temperature of both enantiomers (T_{el}1-2), resolution (R_s) and % analysis time reduction (ATR) calculated on the second eluting enantiomer of the chiral standard compounds investigated.

Compound	25 m × 0.25 μm d_f			25 m × 0.15 μm d_f			5 m × 0.15 μm d_f					
	t_R (min)	T_{el} (°C) 1–2	R_s	t_R (min)	T_{el} (°C) 1–2	R_s	t_R (min)	T_{el} (°C) 1–2	R_s	ATR 25 m 0.15 μm *vs.* 25 m 0.25 μm	ATR 5 m 0.15 μm *vs.* 25 m 0.15 μm	ATR 5 m 0.15 μm *vs.* 25 m 0.25 μm
Limonene	18.67	85–87	8.2	11.72	72–73	8.4	3.66	57–58	4.1	37.2%	68.7%	80.4%
Linalool	25.15	99–100	7.9	18.77	86–87	7.8	7.94	65–66	4.2	25.4%	57.8%	68.4%
Linalyl acetate	27.80	105–106	3.0	20.60	90–91	3.7	8.76	67–68	2.5	25.9%	57.5%	68.5%
Linalyl propionate	31.78	113–114	1.0	24.15	98–98	1.2	11.16	72–72	1.0	24.9%	53.8%	64.9%
γ-Hexalactone	28.30	103–106	11.7	21.99	90–93	13.6	8.97	66–69	5.2	22.3%	59.2%	68.3%
γ-Heptalactone	32.50	112–115	11.9	26.04	99–102	13.9	13.08	73–76	5.2	20.6%	49.8%	62.7%
γ-Octalactone	36.73	121–123	10.0	30.05	107–110	11.6	16.69	80–83	4.8	18.2%	44.4%	56.7%
γ-Decalactone	46.06	141–142	5.7	38.96	126–128	7.2	24.88	98–100	3.8	15.4%	36.1%	46.0%
γ-Undecalactone	50.85	151–152	4.9	43.52	136–137	6.1	29.12	106–108	3.4	14.4%	34.2%	42.7%
γ-Dodecalactone	55.60	160–161	4.0	48.05	145–146	4.8	33.34	115–117	3.0	13.6%	33.1%	40.0%
γ-Tetradecalactone	65.41	179–180	2.4	56.82	163–164	3.2	41.34	131–133	2.7	13.1%	27.2%	36.8%
γ-Pentadecalactone	69.45	188–189	1.9	60.65	171–172	2.5	45.01	139–140	1.9	12.7%	25.7%	35.2%
δ-Octalactone	36.98	123–124	3.7	30.22	109–110	3.4	16.84	83–84	2.2	18.3%	44.3%	54.4%
α-HCH	29.00	156–158	5.9	21.09	140–142	8.8	7.60	114–115	5.3	27.3%	64.0%	73.8%
Trichlorfon	26.27	149–152	14.4	20.15	138–140	12.2	8.56	116–117	5.8	23.3%	57.5%	67.4%
trans-Chlordane	46.19	192–192	0.7	36.56	172–173	1.0	18.70	137–137	1.2	20.8%	48.9%	69.5%
Heptachlor	36.48	173–173	0.6	26.83	153–154	1.0	10.96	122–122	0.9	26.5%	59.2%	70.0%