Journal of Chromatography A, 1217 (2010) 1106–1113

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

New asymmetrical per-substituted cyclodextrins (2-*O*-methyl-3-*O*-ethyl- and 2-*O*-ethyl-3-*O*-methyl-6-*O*-*t*-butyldimethylsilyl-β-derivatives) as chiral selectors for enantioselective gas chromatography in the flavour and fragrance field

Carlo Bicchi*, Cecilia Cagliero, Erica Liberto, Barbara Sgorbini, Katia Martina, Giancarlo Cravotto, Patrizia Rubiolo

Dipartimento di Scienza e Tecnologia del Farmaco, Facoltà di Farmacia, Università degli Studi di Torino, Via Pietro Giuria 9, Turin 10125, Italy

ARTICLE INFO

Article history:
Available online 4 October 2009

Keywords:
Enantiomer separation
Capillary GC
Cyclodextrin derivatives
2/3 asymmetrical substitution pattern
Flavour and fragrance field
Essential oils

ABSTRACT

Asymmetrically substituted $6^{I\text{-}VII}$-*O*-t-butyldimethylsilyl(TBDMS)-$3^{I\text{-}VII}$-*O*-ethyl-$2^{I\text{-}VII}$-*O*-methyl-β-cyclodextrin (MeEt-CD) and $6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$-*O*-ethyl-$3^{I\text{-}VII}$-*O*-methyl-β-cyclodextrin (EtMe-CD) were synthesised to evaluate the role of the substitution pattern in positions 2 and 3 on the enantioselectivity, in particular in view of their application to routine analysis in fast enantioselective gas chromatography (Es-GC). The chromatographic properties and enantioselectivities of the new derivatives were tested by separating the enantiomers of a series of medium-to-high volatility racemates in the flavour and fragrance field, and compared to those of the corresponding symmetrically substituted $6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$,$3^{I\text{-}VII}$-*O*-methyl-β-CD (MeMe-CD) and $6^{I\text{-}VII}$-*O*-*TBDMS*-$2^{I\text{-}VII}$,$3^{I\text{-}VII}$-*O*-ethyl-β-CD (EtEt-CD), and were then applied to analysis of real-world essential oil (e.o.) samples. A new synthetic process including the sonochemical approach to obtain synthetic reproducibility and significant yields of the per-substituted derivatives with acceptable reaction times was developed. The results show that asymmetrically substituted methyl/ethyl CDs compared to the methyl or ethyl symmetrical derivatives in general provide better enantioselectivity in terms of both enantiomer resolution and number of separated chiral compounds, and show how the substitution pattern in positions 2 and 3 of the CD ring can influence the separation. Moreover, these new CD derivatives with better enantioselectivity are also shown to be very useful in routine analysis for the exhaustive control of samples containing several chiral characterizing markers in a single run.

1. Introduction

The demand for enantiomer recognition is constantly increasing in particular in the flavours and fragrances field, where enantiomeric excess (ee) and/or ratio (er) determination is mandatory (i) for quality control and to detect fraud or adulteration of "natural" samples; (ii) to correlate chemical composition to organoleptic properties; (iii) to study the biosynthesis of a compound or classify a vegetable sample; and (iv) as an aid to define the geographic origin of a vegetable matrix [1].

Cyclodextrin derivatives (CDs) are widely used as chiral stationary phases (CSPs) for GC because of their wide enantioselectivity and ability to separate underivatized enantiomers of different volatilities. When used in enantioselective gas chromatography (Es-GC), CDs are in general diluted in apolar or moderately polar polysiloxanes to obtain highly efficient capillary GC columns [2–4].

The Es-GC separation of enantiomers by CD derivatives is known to be based on fast kinetics and entirely governed by thermodynamics [5,6] and, as a consequence, it is closely dependent on temperature. The discrimination of two enantiomers depends on a small difference in the energy of association between each enantiomer and the CD selector, thus requiring very high chromatographic efficiency [7,8]. This mechanism of recognition results in long analysis times, which severely limit the use of Es-GC in routine quality control. This limitation can be overcome by applying the approaches developed for fast-GC analysis to Es-GC mainly acting on column length, inner diameter and/or flow-rates, since only rather low temperature rates can be applied. Short CDs columns were already used in Es-GC since the early 1990s, enabling enantiomer separations even in a few seconds [9–13]. Recently, Bicchi et al. [14] discussed the use of short conventional inner diameter and narrow bore columns in Es-GC in combination with MS, applying them successfully to routine analysis of essential oils. This study also emphasized the need to develop new highly enantioselective CD derivatives, not only to increase the number of optically active compounds separated but also to improve their resolution and therefore enable further speeding-up of Es-GC-(MS) analysis.

* Corresponding author. Tel.: +39 011 6707662; fax: +39 011 6707687.
E-mail address: carlo.bicchi@unito.it (C. Bicchi).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.09.079

The ring size and substituents at C-2, C-3 and C-6 positions of the sugar units strongly influence the CD's chemical and physical properties and enantioselectivity. In general, CD derivatives with the same "small" substituents (mainly acetyl, methyl or ethyl groups) on the ring secondary side, and bulky groups (e.g. *t*-butyldimethylsilyl-(TBDMS) and *t*-hexyldimethylsilyl-(THDMS)) on the primary side provide good enantioselectivity and chromatographic properties for Es-GC. The influence of the substituents in positions 2, 3, and 6 of the CD ring has been discussed extensively [15–17]. 6-TBDMS-β-CDs substituted in both positions 2 and 3 with methyl, ethyl or acetyl groups, are among the most effective derivatives used as CSP for Es-GC [18,19], and their enantioselectivities are very often complementary. One possible strategy to develop CDs with increased enantioselectivity and separation capability and at the same time good chromatographic properties, is either to combine two chiral selectors in a single phase [20–24] or to exploit the specific advantages of the above mentioned stationary phases by synthesising one asymmetrical hybrid derivative. Bicchi et al. introduced $6^{I\text{-}VII}$-*O*-THDMS-$3^{I\text{-}VII}$*O*-acetyl-$2^{I\text{-}VII}$-*O*-methyl-γ-CD and $6^{I\text{-}VII}$-*O*-THDMS-$2^{I\text{-}VII}$*O*-acetyl-$3^{I\text{-}VII}$-*O*-methyl-γ-CD and tested them with a set of racemates in the flavour and fragrance and pesticide fields, with not univocal results [25]. For short, from here onwards, CD derivatives substituted with the same groups in positions 2 and 3 of the secondary side of the ring will be indicated as "symmetrical", whereas those substituted in the same positions but with different groups will be called "asymmetrical".

This article reports the synthesis of asymmetrically substituted CDs in positions 2 and 3 with methyl and ethyl groups (i.e. $6^{I\text{-}VII}$-*O*-TBDMS-$3^{I\text{-}VII}$*O*-ethyl-$2^{I\text{-}VII}$-*O*-methyl-β-CD (MeEt-CD, **4**) and $6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$*O*-ethyl-$3^{I\text{-}VII}$-*O*-methyl-β-CD (EtMe-CD, **3**)). The study aimed to evaluate the influence of the substitution pattern on enantioselectivity, in particular in view of the application of the new derivatives to routine analysis in fast Es-GC. Their performances were compared to those of the corresponding symmetrically substituted $6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$, $3^{I\text{-}VII}$-*O*-methyl-β-CD (MeMe-CD) and $6^{I\text{-}VII}$-*O-TBDMS*-$2^{I\text{-}VII}$, $3^{I\text{-}VII}$-*O*-ethyl-β-CD (EtEt-CD) by analysing a series of medium-to-high volatility racemates in the flavour and fragrance field. Lastly, the four CD derivatives were used in the recognition of the markers of a set of essential oils.

2. Experimental

2.1. Synthesis of $6^{I\text{-}VII}$-O-TBDMS-$3^{I\text{-}VII}$O-ethyl-, $2^{I\text{-}VII}$-O-methyl-β-CD and $6^{I\text{-}VII}$-O-TBDMS-$2^{I\text{-}VII}$O-ethyl-, $3^{I\text{-}VII}$-O-methyl-β-CD

2.1.1. Chemicals and equipment

Reactions were monitored by TLC on Merck 60 F254 (0.25 mm) plates, which were visualized by UV inspection and/or by heating after spraying with 5% H_2SO_4 in ethanol. Merck silica gel was used for column chromatography (CC). IR spectra were recorded with a Shimadzu FT-IR 8001 spectrophotometer. NMR spectra were recorded with a Bruker 300 Avance (300 and 75 MHz for ^{1}H and ^{13}C, respectively) at 25 °C; chemical shifts were calibrated to the residual proton and carbon resonances of the solvent: $CDCl_3$ (δ H = 7.26, δ C = 77.0). Chemical shifts (δ) are given in ppm, coupling constants (*J*) in Hz. ESI-mass spectra were recorded on a Waters Micromass ZQ equipped with ESI source. The sonochemical apparatus was developed in the authors' laboratory [26]. Commercially available reagents and solvents were used without further purification unless otherwise stated. Native CDs were kindly provided by Wacker Chemie.

2.1.2. Synthesis of cyclodextrin derivatives

A diagram of the whole synthetic process is reported in Fig. 1.

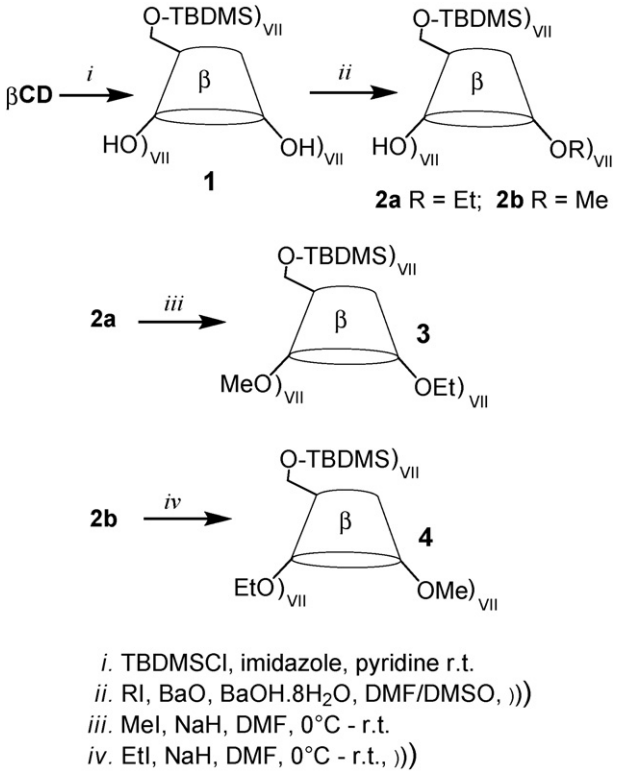

Fig. 1. Synthesis of CD derivatives 3 and 4.

2.1.2.1. $6^{I\text{-}VII}$-O-t-butyldimethylsylil-β-CD (1). $6^{I\text{-}VII}$-*O*-*t*-butyldimethylsylil-β-CD (1) was prepared as described in the literature [32]. Analytical data were in accordance with reported values.

2.1.2.2. $6^{I\text{-}VII}$-O-t-butyldimethylsylil-$2^{I\text{-}VII}$-O-ethyl-β-CD (2a). BaO (500 g, 3.25 mmol) and $Ba(OH)_2{\cdot}8H_2O$(500 g, 1.58 mmol) were added to a solution of $6^{I\text{-}VII}$-*O*-*t*-butyldimethylsylil-β-CD **1** (200 mg, 0.103 mmol) in 10 mL of a 1:1 mixture of DMF/DMSO. The suspension was transferred into the sonochemical reactor [26] and sonicated (80 W) for 45 min. Ethyl iodine (975 μL, 6.25 mmol) was added in two portions during 45 min and sonicated for 2 h, then the solution was filtered through a sintered glass funnel. The filtrate was concentrated under vacuum, EtOAc was added and the organic layer was washed three times with brine and dried over Na_2SO_4. The crude residue was purified by silica-gel column chromatography (petrolether/EtOAc 8:2) yielding 76 mg (35%) of **2a**.

2a: white powder; R_f = 0.38 (PE/EtOAc 7:3); ^{1}H NMR (300 MHz; $CDCl_3$) δ = 4.91 (d, 7H, 1-H, *J* = 3.6 Hz), 4.07 (t, 14H, CH_2CH_3, *J* = 6.9 Hz) 3.96–3.90 (m, 14H, H-6^A, H-3), 3.8–3.64 (m, 14H, H-6^B, CH_2CH_3), 3.51–3.45 (m, 14H, H-4, H-5) 3.27–3.23 (dd, 7H, H-2, *J* = 3.6, 9.6 Hz,) 1.25 (t, 21H, CH_2CH_3, *J* = 6.9 Hz,) 0.87 (s, 63H, OSiCMe_3), 0.04 (s, 42H, OSiCMe_3); ^{13}C NMR($CDCl_3$): δ = 101.65 (C1), 82.1 (C4), 80.93 (C2), 73.42 (C3), 71.78 (C5), 68.85 (CH_2CH_3), 61.85 (C-6) 26.25 (OSiCMe_3), 18.69 (OSiCMe_3), 15.78 (CH_2CH_3), −4.7, −4.6 (OSiCH_3) ppm; *m*/*z* (ESI-MS) calcd. for $C_{98}H_{196}O_{35}Si_7$ $[M+2Na]^{2+}$ 1087.75; found 1087.5.

2.1.2.3. $6^{I\text{-}VII}$-O-t-butyldimethylsylil-$2^{I\text{-}VII}$-O-methyl-β-CD (2b). The same procedure was used for **2b**; the crude residue was purified by silica-gel column chromatography (petrolether/EtOAc 7:3) yielding 106 mg of pure compound (yield 50%).

2b: white amorphous powder; R_f = 0.38 ($CHCl_3$/MeOH 98:2); ^{1}H NMR (300 MHz; $CDCl_3$) δ = 5.1 (s, 3H, 1-H), 4.96 (s, 4H, 1-H) 3.95(m, 14H, H-6^A, H-3), 3.68-3.49 (m, 42H, H-4, H-5, H-6^B, CH_3), 3.2–3.1

Table 1
Resolutions (R_S) of the racemates separated with resolution above 1.5 at least in one of the columns tested. Bold: highest resolution; italic: lowest resolution.

Compound	R_S			
	MeMe-CD	MeEt-CD	EtMe-CD	EtEt-CD
Hydrocarbons				
1. Camphene	*2.2*	5.4	4.7	**6.8**
2. β-Citronellene	*NS*	**1.6**	*NS*	1.2
3. Limonene	*5.0*	6.8	**8.4**	7.4
4. β-Phellandrene	*3.8*	**6.1**	4.6	**6.1**
5. α-Pinene	2.9	**4.5**	4.0	*NS*
6. β-Pinene	*3.4*	3.7	**5.8**	3.6
7. Sabinene	6.5	8.1	**8.4**	*6.3*
Heterocycles				
8. *cis*-Rose oxide	**4.2**	2.9	3.5	*2.0*
9. *trans*-Rose oxide	*NS*	1.3	*NS*	**1.9**
Esters				
10. Butyl butyryllactate	1.6	*1.5*	**2.6**	1.7
11. Dimethyl methylsuccinate	2.2	**2.9**	2.2	*1.1*
12. Ethyl 2-phenylbutyrate	*1.0*	2.4	1.7	**2.7**
13. Ethyl 3-hydroxybutyrate	**3.2**	1.6	2.4	*NS*
14. Ethyl 3-hydroxyhexanoate	3.0	**4.1**	3.3	*1.5*
15. Ethyl 2-methylbutyrate	*2.9*	4.6	**5.5**	5.1
16. Ethyl 3-methyl-3-phenylglycidate	*NS*	1.6	2.3	**2.5**
17. Ethyl 3-methyl-3-phenylglycidate	*2.0*	**5.2**	3.8	4.7
18. Isobornyl isobutyrate	*1.0*	1.7	**1.9**	1.4
19. Lavandulyl acetate	*1.6*	**3.1**	2.3	2.3
20. Linalyl acetate	0.7	*NS*	2.6	**3.7**
21. Menthyl acetate	*14.0*	**23.5**	19.7	17.0
22. *cis*-2-Methyl-(3Z)-hexenyl butyrate	*1.8*	2.5	**2.8**	2.3
23. Methyl 3-hydroxyhexanoate	*5.7*	**7.1**	6.9	6.8
24. Propyleneglycol butyrate	2.3	**4.5**	2.6	*1.4*
25. Stirallyl acetate	*15.6*	42.8	30.7	**56.3**
Lactones				
26. Aerangis lactone	*2.2*	**3.2**	2.6	2.8
27. δ-Hexalactone	*NS*	0.9	*NS*	**1.5**
28. δ-Heptalactone	**3.2**	1.5	2.5	*1.4*
29. δ-Octalactone	*1.8*	*1.8*	**4.4**	3.4
30. δ-Nonalactone	1.5	1.5	**2.1**	*1.0*
31. δ-Decalactone	*1.0*	*1.0*	**1.6**	*1.0*
32. δ-Undecalactone	1.4	*1.2*	**2.0**	*1.2*
33. δ-Dodecalactone	*1.1*	*1.1*	**1.6**	*1.1*
34. γ-Pentalactone	7.6	*2.7*	16.2	**20.5**
35. γ-Hexalactone	6.0	*2.7*	10.1	**13.6**
36. γ-Heptalactone	8.9	*5.1*	11.4	**13.9**
37. γ-Octalactone	6.4	*4.5*	9.2	**11.6**
38. γ-Nonalactone	5.7	*4.5*	7.7	**9.8**
39. γ-Decalactone	*4.0*	5.5	6.8	**7.2**
40. 3-Methyl-γ-decalactone	*6.4*	8.6	**9.1**	8.0
41. γ-Undecalactone	*3.4*	3.5	4.5	**6.1**
42. γ-Dodecalactone	*2.9*	3.3	3.8	**4.8**
43. γ-Tetradecalactone	*2.0*	2.9	2.7	**3.2**
44. γ-Pentadecalactone	*1.6*	**2.5**	2.3	2.4
45. ε-Decalactone	*5.0*	6.9	**8.3**	8.0
46. ε-Dodecalactone	*4.3*	5.8	**7.1**	5.4
47. Massoia dodecalactone	1.2	*NS*	**1.5**	*NS*
48. Whiskey lactone A	*11.2*	11.4	21.5	**27.1**
49. Whiskey lactone B	2.8	1.8	**4.8**	*1.4*
Alcohols				
50. Borneol	4.3	5.7	**6.6**	*3.8*
51. Fenchyl alcohol	*2.7*	3.6	5.1	**8.6**
52. Geosmin	**1.7**	**1.7**	1.5	*1.3*
53. Isoborneol	*3.6*	3.8	**4.6**	3.7
54. Isomenthol	*4.4*	6.1	4.5	**8.6**
55. Isopinocampheol	**5.6**	4.9	5.2	*1.8*
56. Lavandulol	*8.6*	9.3	**15.2**	13.7
57. Linalool	*3.9*	4.5	**7.7**	7.4
58. *trans*-Linalool oxide	9.6	**10.1**	8.0	*2.0*
59. *cis*-Linalool oxide	*4.8*	**10.7**	6.9	5.8
60. Menthol	1.3	**2.7**	*NS*	1.3
61. 2-Methylbutanol	*1.2*	1.5	1.4	**2.4**
62. 6-Methyl-5-hepten-2-ol	*6.3*	**7.5**	7.3	6.7
63. 4-Methyl-1-phenylpentanol	3.5	**3.8**	*NS*	2.3
64. Neoisomenthol	*11.0*	13.4	17.2	**17.4**

Table 1 (*Continued*).

Compound	R_S			
	MeMe-CD	MeEt-CD	EtMe-CD	EtEt-CD
65. Neomenthol	6.9	7.5	**8.0**	*6.4*
66. *cis*-Nerolidol	*2.2*	3.0	4.2	**4.3**
67. *trans*-Nerolidol	*2.7*	4.0	4.4	**4.5**
68. 1-Phenylethanol	*6.0*	**9.4**	6.4	6.5
69. 1-Phenyl-2 pentanol	4.1	**4.8**	1.5	*1.1*
70. 1-Phenil-1-propanol	2.0	**4.8**	*1.2*	3.9
71. 2-Phenil-1-propanol	3.2	**4.3**	3.8	*3.1*
72. Terpinen-4-ol	2.4	**3.6**	3.2	*1.7*
73. α-Terpineol	5.1	*5.0*	**7.7**	6.9
74. Tetrahydrolinalool	*4.2*	5.8	**7.0**	**7.0**
Ketones				
75. Camphorquinone	2.2	*1.4*	**4.1**	3.8
76. Camphor	*2.6*	3.6	3.3	**3.7**
77. Carvone	1.2	1.5	**1.6**	*NS*
78. 3,6-Dimethylocta-2-en-6-one	*1.7*	3.3	4.1	**5.0**
79. 1,8-Epoxy-p-menthan-3-one	*12.5*	15.1	**16.9**	13.8
80. α-Ionone	5.1	**7.5**	6.2	*4.9*
81. Iso-menthone	10.4	**15.5**	13.0	*9.0*
82. Menthone	1.5	**3.2**	*NS*	2.2
83. 3-Methylcyclohexanone	1.7	*1.1*	3.6	**5.3**
84. 3-Oxocineole	*17.0*	19.6	26.3	**28.9**
85. Piperitone	*6.0*	**9.9**	8.7	8.8
86. Pulegone	4.6	**6.4**	4.6	*3.8*
87. Verbenone	2.9	1.8	*NS*	**3.5**
Aldehydes				
88. Perillyl aldehyde	*6.4*	6.5	7.8	**8.2**
Acids				
89. Citronellic acid	1.7	**1.8**	*1.0*	1.1
90. Chrysanthemic acid	8.4	**14.3**	8.0	*7.5*
91. 2-Phenylpropionic acid	*NS*	**2.3**	1.1	2.0
92. 2-Methylbutyric acid	2.1	**3.4**	2.6	*1.5*

(m, 7H, H-2), 0.88 (s, 63H, t-Bu), 0.04 (s, 42H, Si–CH_3) ppm; *m*/*z* (ESI-MS) calcd. for $C_{91}H_{182}O_{35}Si_7$ $[M+2Na]^{2+}$ 1038.44; found 1038.2.

2.1.2.4. 6$^{I\text{-}VII}$-O-t-butyldimethylsylil-2$^{I\text{-}VII}$-O-ethyl-3$^{I\text{-}VII}$-O-methyl-β-CD (3). Sodium hydride (62 mg, 1.61 mmol, 66% suspension) was added to 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsylil-2-*O*-ethyl-β-CD (100 mg, 0.046 mmol) (**2a**) dissolved in 3 mL of dry DMF. The reaction was cooled at 0 °C and stirred for 30 min. Methyl iodide (61 μL, 1.61 mmol) was added to the resulting mixture and stirred for 4 h at room temperature. The reaction mixture was then treated with MeOH to decompose the NaH excess and extracted with diethyl ether; the organic layer was washed three times with water and dried over Na_2SO_4. The crude residue was purified by silica-gel column chromatography (petrolether/EtOAc 8:2) yielding 82% (82 mg) of **3**.

3: white amorphous powder; MP = 106 °C, R_f = 0.67 (PE/EtOAc 8:2); IR ν_{max}(KBr) = 2956, 1473, 1362, 1253, 1159, 972 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ = 5.19 (d, 7H, 1-H, *J* = 3.6 Hz), 4.16–4.15 (m, 7H, H-6^A), 3.8–3.72 (m, 14H, H-4, CH_2CH_3), 3.68 (s, 21H, CH_3), 3.66–3.53 (m, 21H, H-3, H-5, H-6^B), 3.14 (dd, 7H, H-2, *J* = 3.4, 9.9), 1.25 (t, 21H, CH_2CH_3, *J* = 7.2 Hz), 0.87 (s, 63H, OSiCMe_3), 0.02 (s, 42H, OSiCMe_3) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ = 98.2 (C-1), 82.1 (C-3), 80.47 (C-2), 78.0 (C-4), 72.2 (C-5), 66.2 (CH_2CH_3), 62.4 (C-6), 61.6 (CH_3), 26.05 (OSiCMe_3), 18.43 (OSiCMe_3), 15.91 (CH_3CH_2), −5.03 (OSiCH_3) ppm; *m*/*z* (ESI-MS) calcd. for $[M+2Na]^{2+}$ 1136.55, found 1135.55.

2.1.2.5. 6$^{I\text{-}VII}$-O-t-butyldimethylsylil-3$^{I\text{-}VII}$-O-ethyl-2$^{I\text{-}VII}$-O-methyl-β-CD (4). Sodium hydride (62 mg, 1.61 mmol, 66% suspension) was added to 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsylil-2-*O*-methyl-β-CD **2b** (100 mg, 0.046 mmol) dissolved in 3 mL of dry DMF. The reaction was cooled at 0 °C and stirred for 30 min and ethyl iodine (80 μL, 1 mmol) was then added. The suspension was transferred into the sonochemical bath and sonicated (80 W) for 2 h. The crude product was purified by silica-gel column chromatography (petrolether/EtOAc 8:2) yielding 63 mg of **4** (yield 62%).

4: white amorphous powder; MP = 105 °C, R_f = 0.69 (PE/EtOAc 8:2); IR ν_{max}(KBr) = 2956, 1471, 1404, 1252, 1159, 972 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ = 5.3(m, 7H, 1-H), 4.25–4.21 (m, 7H, H-6^A), 4.18–4.16 (m, 7H, H-4,), 4.06–3.46 (m, 56H, CH_3, H-3, H-5, H-6^B, CH_2CH_3), 3.19 (dd, 7H, H-2, *J* = 3.4 Hz, 9.9 Hz), 1.23 (t, 21H, CH_2CH_3, *J* = 7.1 Hz), 0.87 (s, 63H, OSiCMe_3), 0.06 (s, 42H, OSiCMe_3) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ = 98.1 (C-1), 82.1 (C-2), 81.2 (C-3), 78.0 (C-4), 73 (C-5), 69.5 (CH_2CH_3), 63.4 (C-6), 59.3 (CH_3), 26.1 (OSiCMe_3), 18.43 (OSiCMe_3), 15.51 (CH_3CH_2), −5.03 (OSiCH_3) ppm; *m*/*z* (ESI-MS) calcd. for $[M+2Na]^{2+}$ 1136.55, found 1136.95.

2.2. Column preparation and testing

Fused silica columns (25 m × 0.25 mm i.d.) were prepared by static coating. Columns coated with a 0.15 μm film of the synthesised CDs diluted at 30% in PS086 (polymethylphenylpolysiloxane, 15% phenyl) were from MEGA (Legnano, Italy). Deactivation was with Carbowax 20 M. The procedures have been described in detail elsewhere [27–29]. The columns were conditioned starting from 40 °C and gradually increasing the maximum operative temperature to 220 °C at 1 °C/min over a few days. All columns were operative from room temperature.

Column performances were evaluated by the Grob test and through a chiral test developed in the authors' laboratory, consisting of ten compounds with different structures and polarities [30]: limonene (3), 2-octanol (93), camphor (76), isobornyl acetate

Table 2
Comparison between the enantioselective performances of the four columns investigated.

Investigated compounds	104	
Chiral compounds separated on EtEt-CD	74	
Chiral compounds separated on MeMe-CD	77	
Chiral compounds separated on EtMe-CD	82	
Chiral compounds separated on MeEt-CD	83	
Chiral compounds separated on at least one of the four columns	92	
Chiral compounds separated on all columns investigated	62	
Comparisons between the four investigated CD derivatives	EtMe-CD	MeEt-CD
Chiral compounds separated with R_S > EtEt-CD	49/82 (59.8%)	48/83 (57.8%)
Chiral compounds separated with R_S > MeMe-CD	72/82 (87.8%)	67/83 (80.7%)
Chiral compounds separated with R_S > EtEt-CD and MeMe-CD	39/82 (47.6%)	41/83 (49.4%)
Chiral compounds separated with R_S < EtEt-CD	32/82 (39.0%)	34/83 (41.0%)
Chiral compounds separated with R_S < MeMe-CD	9/82 (11.0%)	13/83 (15.7%)
Chiral compounds separated with R_S < EtEt-CD and MeMe-CD	1/82 (1.2%)	8/83 (9.6%)

(94), linalyl acetate (20), *cis*-2-methyl-(3Z)-hexenyl butyrate (22), menthol (60), hydroxycitronellal (95), γ-decalactone (39) and δ-decalactone (31). In addition, each column was tested with 104 medium-to-high volatility racemates that 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 solubilised in cyclohexane at a concentration of 100 ppm each. A set of different essential oils (bergamot, lemon, orange, bitter orange, lavender, peppermint, rosemary and sage essential oils) were also analysed; the essential oils, obtained by hydrodistillation following the method described in the European Pharmacopoeia (6th edition) [31], were diluted 1:200 in cyclohexane before analysis. The performance of the columns were periodically tested through the above Grob and chiral tests. Up to now, each column was submitted to several hundredth of injections without loosing in enantioselectivity and chromatographic performance.

2.3. Capillary GC conditions

Es-GC analyses were carried out on a Shimadzu 2010 GC-FID system and a Shimadzu QP2010 GC-MS system, both provided with an AOC-20i automatic injector, and with Shimadzu GC Solution 2.53SU1 software and Shimadzu GCMS Solution 2.51 software, respectively (Shimadzu, Milan, Italy).

GC-MS conditions: injection mode: split; split ratio: 1:20; injection volume: 1 μL. Temperatures: injector: 220 °C, transfer line: 230 °C; ion source: 200 °C; carrier gas: He, flow rate 1.0 mL/min. The MS operated in electron impact ionization mode (EI) at 70 eV, at a scan rate of 666 u/s and a mass range of 35–350 *m*/*z*, suitable to cover the full fragmentation pattern of all analytes investigated. All samples were analysed with the following temperature programme: from 50 to 220 °C (2 min) at 2 °C/min. Resolution was calculated with the following equation: $R_S = 1.18\,(t_{R(2)} - t_{R(1)})/(w_{0.5(1)} + w_{0.5(2)})$.

3. Results and discussion

This study consisted of four main steps: (a) development of a synthetic procedure affording an acceptable yield of pure CD derivatives in a reasonable time, to be used as chiral stationary phase; (b) evaluation of chromatographic properties and enantioselectivities of the new asymmetrical CDs; (c) comparison of their performances to those of the corresponding symmetrical CDs; (d) application of the new CDs to the chiral recognition of real-world samples.

3.1. Synthesis of asymmetrical CD derivatives

Two selectively per-substituted CDs with inverse substitution pattern in positions 2 and 3 were synthesised (Fig. 1). Primary hydroxyl groups of β-CD were silylated by reacting with *t*-

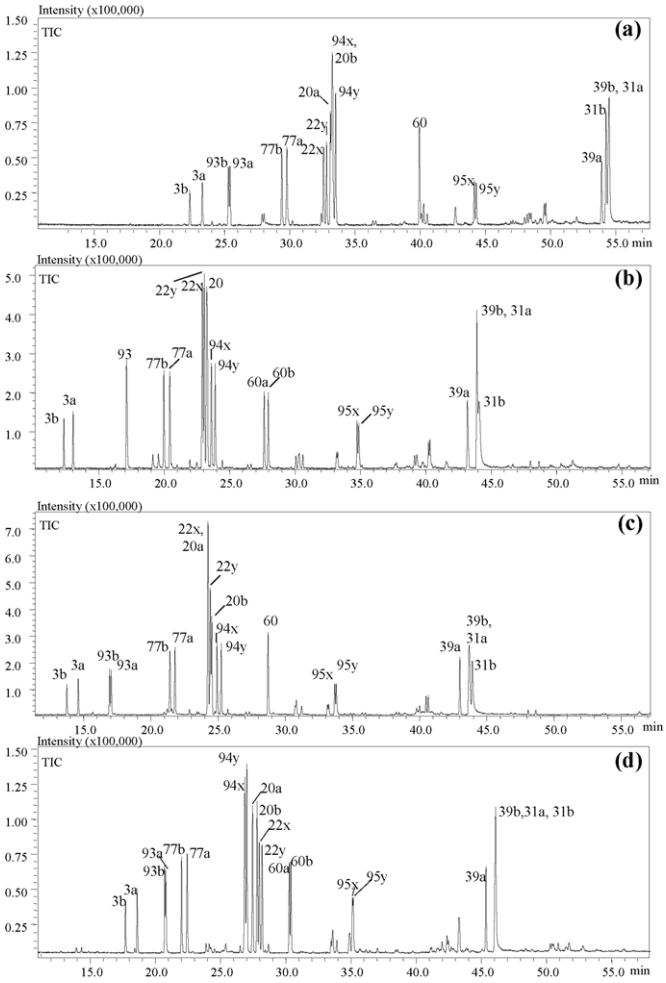

Fig. 2. Chiral test profiles carried out on the four columns investigated. (3) limonene, (93) 2-octanol, (76) camphor, (94) isobornyl acetate, (20) linalyl acetate, (22) *cis*-2-methyl-(3Z)-hexenyl butyrate, (60) menthol, (95) hydroxycitronellal, (39) γ-decalactone, (31) δ-decalactone; a: (*R*) enantiomer, b: (*S*) enantiomer, *x* and *y*: enantiomer configuration not assigned. Columns: MeMe-CD (a), MeEt-CD (b), EtMe-CD (c) and EtEt-CD (d).

butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in dry pyridine [32,33]. Pure 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsilyl-β-CD was crystallized in good yield by a sequence of solvent mixtures (CH_3Cl/MeOH, acetone/MeOH) without chromatographic purification [33]. Selective *O*-alkylation in the C-2 position was carried out following the method of Takeo et al. and Icheln et al. [34,35] with a mixture of barium oxide and barium hydroxide. Because the original method required several days to achieve substantial conversion, the reaction with ethyl or methyl iodide (**2a** and **2b** respectively) was carried out under power ultrasound (US). The success of this reaction depends on both the DMSO/DMF optimal solvent ratio and the percentage of water either in the hydrated barium hydroxide itself or directly added. After chromatographic purification, 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsilyl-2$^{I\text{-}VII}$-*O*-ethyl-β-CD (**2a**) and 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsilyl-2$^{I\text{-}VII}$-*O*-methyl-β-CD (**2b**) were obtained in 35% and 50% yields, respectively.

The following *O*-alkylation in the C-3 position, with methyl- or ethyl-iodide in DMF with sodium hydride, gave the 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsilyl-2$^{I\text{-}VII}$-*O*-ethyl-3$^{I\text{-}VII}$-*O*-methyl-β-CD (**3**, EtMe-CD) and 6$^{I\text{-}VII}$-*O*-*t*-butyldimethylsilyl-2$^{I\text{-}VII}$-*O*-ethyl-3$^{I\text{-}VII}$-*O*-methyl-β-CD (**4**, MeEt-CD) in yields of 62% and 82%, respectively. While *O*-methylation in C3 with methyl iodide was complete in a few hours under magnetic stirring, *O*-ethylation with ethyl iodide was more troublesome, and required ultrasound (US) and 2 h. Both CD derivatives were fully characterized by ^{1}H-, ^{13}C NMR, IR and ESI mass spectrometry (see Section 2).

3.2. Evaluation of chromatographic properties and enantioselectivities of the new asymmetrical CD derivatives

A new CD derivative for routine chiral recognition must possess both good chromatographic properties and high enantioselectivity. Several CDs reported in the literature show high enantioselectivity but poor chromatographic properties, making them almost useless for everyday work. All columns in this study were therefore first submitted to the Grob test, to evaluate their chromatographic properties, and then to the chiral test usually used in the authors' laboratory to evaluate enantioselectivity (see Section 2.2) [30].

The Grob test showed that the columns prepared with the new asymmetrical CDs were highly effective in chromatographic terms since both showed $E_{10\text{-}11}$ and $E_{11\text{-}12}$ Trennzhal (TZ) [36,37] above 35, and even a little higher than those of the corresponding symmetrical MeM-CDe and EtEt-CD derivatives. Fig. 2 reports the Es-GC-MS profiles of the chiral test with the columns prepared with the four symmetrical and asymmetrical CD derivatives investigated. The GC pattern shows that the new asymmetrical CDs present enantioselectivity comparable to that of the symmetrical derivatives.

3.3. Comparison of the asymmetrical CD performances with those of the corresponding symmetrical derivatives

The performance of each asymmetrical CD as a chiral selector was evaluated by comparing its enantioselectivity to that of the corresponding symmetrical derivative, in separating the enantiomers of 104 standard racemates in the flavour and fragrance field. Table 1 reports resolutions (R_S) of the racemates separated with resolution above 1.5 at least by one of the columns tested. Resolutions below 1.5 are only reported for comparison. This resolution limit was chosen in view of the simultaneous determination of enantiomeric ratios (er) or excesses (ee) by GC-MS of several components in a real-world sample [14] (see also Section 3.4). Table 2 reports the performance of the four CD columns showing the improvement in performance of the asymmetrical methyl/ethyl derivatives.

The reported data show that 92 chiral compounds were separated with resolution above 1.5 (~90%) with at least one of the CDs investigated and that 62 (65%) of them were separated on the four columns, meaning that a specific column is not required for their chiral recognition. The enantiomers of eleven racemates (3-hexanol, 2-octanol, hydroxycitronellal, 3-octanol, isobornyl acetate, 1-octen-3-ol, linalyl propionate, phenylethyl methyl ethyl carbinol acetate, massoia decalactone, α terpinyl acetate, 1,3-octanediol) were not separated

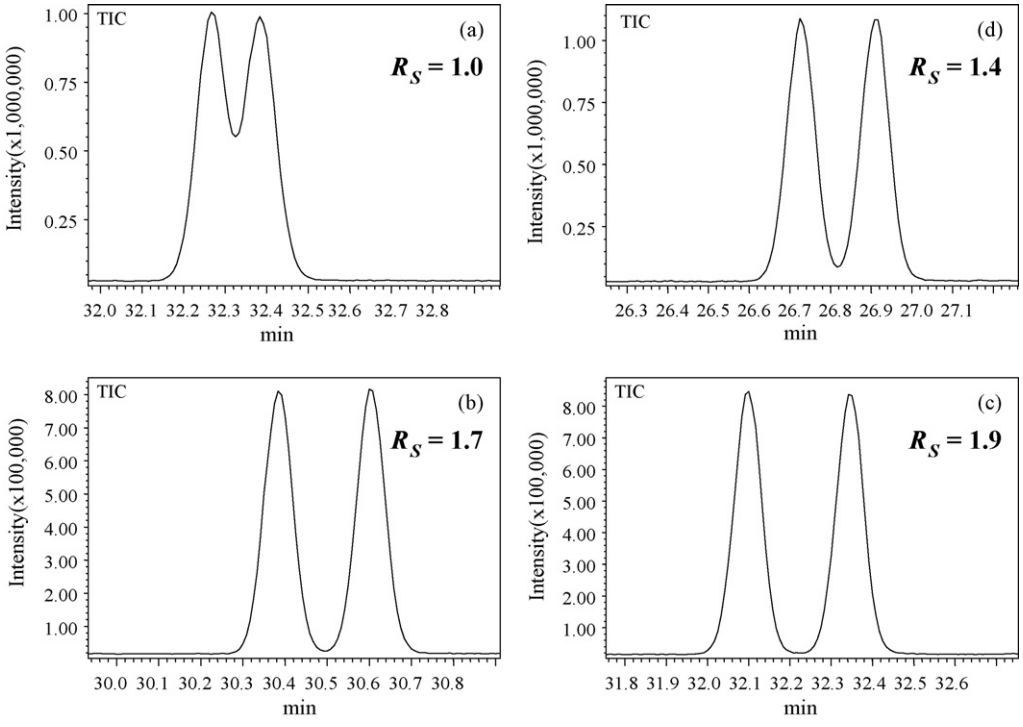

Fig. 3. Es-GC profiles of isobornyl isobutyrate analysed on columns coated with the four CD derivatives under investigation (see caption of Fig. 2 for columns).

by any of the four CD derivatives with resolution of at least 1.5.

These results show that the asymmetrical CDs have a wider and (in most cases) better enantioselectivity than the symmetrical derivatives. For most compounds (62 out of 93) the asymmetrical CD shows enantioselectivity similar to or higher than the symmetrical counterpart, most probably because of the combined effect of methyl and ethyl groups as substituents in positions 2 and/or 3 of the sugar unit. Fig. 3 reports the Es-GC-MS profiles of isobornyl isobutyrate analysed with the four columns, as an example of the need for the asymmetrical substitution to obtain a base-line separation. Some racemates show resolutions of their enantiomers more than 50% higher with the asymmetrical than with the symmetrical derivative, among others β-pinene (6), propyleneglycol butyrate (24), whiskey lactone B (49), *cis*-linalool oxide (59), menthol (60) α-ionone (80), iso-menthone (81), menthone (82), pulegone (86), chrysanthemic acid (90), and 2-methylbutyric acid (92).

The comparison between the performances of symmetrical and asymmetrical CD derivatives is also very useful to clarify how a substituent and its position in the ring can influence the separation of the enantiomers of racemates that are not separated with all columns. For instance, α-pinene (5) and carvone (77) require at least a methyl group in the CD for their separation, just as ethyl 3-methyl-3-phenylglycidate (16) and 2-phenylpropionic acid (91) require an ethyl group. Other chiral compounds requires a methyl or an ethyl group in position 2 or 3 of the sugar unit of the CD ring fort their separation: for instance β-citronellene (2), *trans*-rose oxide (9), and δ-hexalactone (27) require at least an ethyl group in position 3, linalyl acetate (20) an ethyl group in position 2 while massoia dodecalactone (47) needs a methyl group in position 3.

The role played by the substituents in positions 2 and 3 is shown by the fact that some racemates are base-line separated with the methyl and ethyl groups in positions 2 and 3 of the sugar units, but not vice versa. Menthol (60), 4-methyl-1-phenylpentanol (63), verbenone (87), menthone (82) are separated with resolution well above 1.5 with the CD with a methyl group in position 2 and an ethyl group in position 3, but not when the substituents are inverted; the opposite occurs for linalyl acetate (20). These results are further evidence of how specific and/or critical the host-guest interactions leading to the enantiomer separation with CD as chiral selector can be.

3.4. Analysis of real-world samples

One of the main characteristics required to new CD derivatives is an extended and better enantioselectivity to enable the separation of as many chiral compounds as possible in a single run to enable an exhaustive control, in particular in routine analysis. New CD derivatives must tend to the enantiomer separation of all chiral markers characteristic of a sample with a single column with the goal of moving the thus far most popular "one column for one compound" approach with the most exhaustive "one column for one problem" approach [38]. This need is especially important in the flavour and fragrance fields, not only to detect adulterations or frauds more effectively, but also because many samples consist of several ingredients containing many chiral components whose enantiomers can have different odours [39]. MeEt-CD (**4**) and EtMe-CD (**3**) were also synthesised in this light, and gave interesting results. A set of different essential oils (bergamot, lemon, orange, bitter orange, lavender, peppermint, rosemary and sage essential oils) containing several characteristic chiral components were analysed with columns coated with the four CD derivatives investigated. The analysis of bergamot essential oil is a clear example of the effectiveness of the new asymmetrical derivatives. The composition of this essential oil and its indices of genuineness have already been investigated in depth [40]. Bergamot essential oil contains

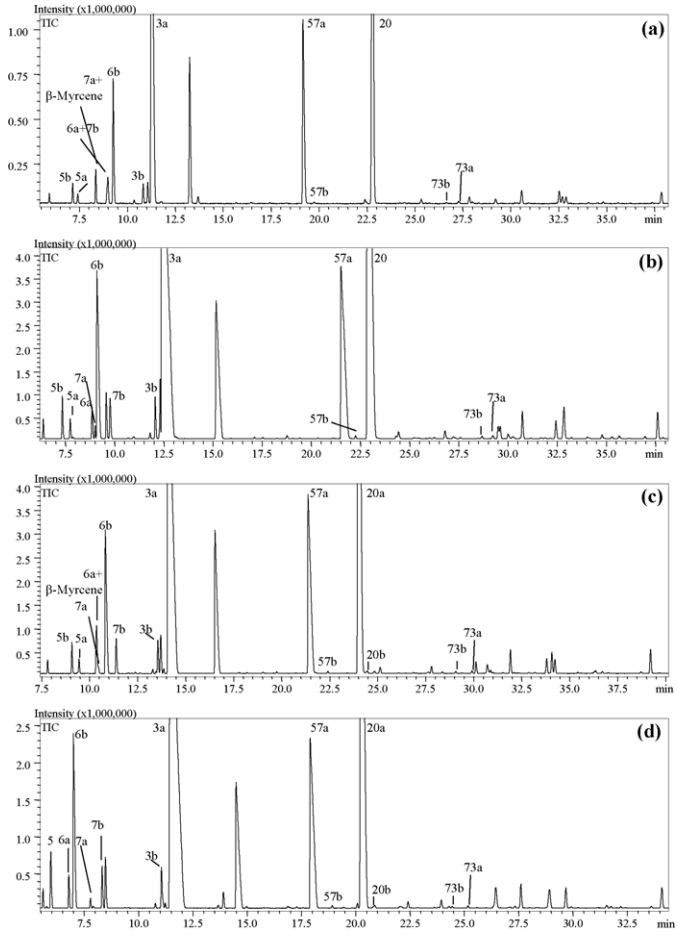

Fig. 4. Es-GC-MS profile of bergamot essential oil (5) α-pinene, (6) β-pinene, (7) sabinene, (3) limonene, (57) linalool, (20) linalyl acetate, (73) α-terpineol; a: (*R*) enantiomer, b: (*S*) enantiomer (see caption of Fig. 2 for columns).

seven chiral components: α-pinene (5), β-pinene (6), sabinene (7), limonene (3), linalool (57), linalyl acetate (20), and α-terpineol (73). Several samples of this essential oil were analysed with the four CD columns investigated. Fig. 4 reports the enantioselective GC-MS profiles analysed with columns coated respectively with MeMe-CD (a), MeEt-CD (b), EtMe-CD (c), EtEt-CD (d), all dissolved in PS-086. These results show that only EtMe-CD separates the enantiomers of all seven chiral components simultaneously and with resolutions above 1.5, as required for a correct ee or er determination. The other CD derivatives separate only six of them: MeMe-CD gives insufficient separation of linalyl acetate enantiomers (20) plus co-elution of (*R*)-sabinene (7a) and β-myrcene; MeEt-CD does not separate linalyl acetate (20) while EtEt-CD fails with α-pinene (5).

4. Conclusions

The results show that asymmetrically substituted methyl/ethyl CDs can extend enantioselectivity in comparison to that of the corresponding methyl or ethyl symmetrical derivatives, in terms of both enantiomer resolution and number of chiral compounds separated. Their synthesis is more complex than that of the symmetrical derivatives, and thus more sophisticated methods, such as the sonochemical approach, must be used. This synthetic procedure gave regioselective per-substituted derivatives in good yields, with high reproducibility and relatively short reaction times.

These results also show that there is still a need for new CD derivatives with better enantioselectivity to increase the number of chiral compounds separated with a single chiral selector, and/or

improve their resolution, thus affording to apply analysis conditions (i.e. column length and inner diameters and temperature rates) suitable to speed-up Es-GC-(MS) routine analysis of real-world samples. New CD derivatives with better performances can actively contribute to increasing the adoption of the "one column for one problem" approach and, as a consequence, can extend the use of Es-GC-(MS) in routine analysis.

Acknowledgments

This research was carried out within the project entitled: "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari" (FIRB Cod.: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).

References

[1] A. Mosandl, Food Rev. Int. 11 (1995) 597.
[2] V. Schurig, H.P. Nowotny, J. Chromatogr. 441 (1988) 155.
[3] H.P. Nowotny, D. Schmalzing, D. Wistuba, V. Schurig, J. High Resolut. Chromatogr. 12 (1989) 383.
[4] V. Schurig, M. Jung, D. Schmalzing, M. Schleimner, J. Duvekot, J.C. Buyten, J.A. Peene, P. Mussche, J. High Resolut. Chromatogr. 13 (1990) 470.
[5] V. Schurig, J. Chromatogr. A 906 (2001) 275, and reference cited therein.
[6] P.A. Levkin, V. Schurig, J. Chromatogr. A 1184 (2008) 309, and reference cited therein.
[7] M. Jung, D. Schamalzing, V. Schurig, J. Chromatogr. 552 (1991) 43.
[8] V. Schurig, H.P. Nowotny, Angew. Chem. Int. Ed. Engl. 29 (1990) 939.
[9] M. Lindstroem, J. High Resolut. Chromatogr. 14 (1991) 765.
[10] C. Bicchi, G. Artuffo, A. D'Amato, G.M. Nano, A. Galli, M. Galli, 2nd International Symposium on Chiral Discrimination 27-31/05/1991, Rome (Italy), 1991.
[11] C. Bicchi, G. Artuffo, A. D'Amato, A. Galli, M. Galli, Chirality 4 (1992) 125.
[12] H. Grosenick, V. Schurig, J. Constante, A. Collet, Tetrahedron Asymmetr. 6 (1995) 87.
[13] V. Schurig, H. Czesla, Enantiomer 6 (2001) 107.
[14] C. Bicchi, E. Liberto, C. Cagliero, C. Cordero, B. Sgorbini, P. Rubiolo, J. Chromatogr. A 1212 (2008) 114.
[15] A. Shitangkoon, G. Vigh, J. Chromatogr. A 738 (1996) 31.
[16] T. Beck, J.-M. Liepe, J. Nandzik, S. Rohn, A. Mosandl, J. High Resolut. Chromatogr. 23 (2000) 569.
[17] C. Bicchi, G. Cravotto, A. D'Amato, P. Rubiolo, A. Galli, M. Galli, J. Microcolumn Sep. 11 (1999) 487.
[18] B. Maas, A. Dietrich, D. Bartschat, A. Mosandl, J. Chromatogr. Sci. 33 (1995) 223.
[19] C. Bicchi, A. D'Amato, V. Manzin, A. Galli, M. Galli, J. Chromatogr. A 742 (1996) 161.
[20] A. Ruderisch, J. Pfeiffer, V. Schurig, J. Chromatogr. A 994 (2003) 127.
[21] P.A. Levkin, A. Levkina, V. Schurig, Anal. Chem. 78 (2006) 5143.
[22] M.Y. Nie, L.M. Zhou, Q.H. Wang, D.Q. Zhu, Chromatographia 51 (2000) 736.
[23] D.-Q. Xiao, Y. Ling, Y.-X. Wen, R.-N. Fu, J.-L. Gu, R.-J. Dai, A.-Q. Luo, Chromatographia 46 (1997) 177.
[24] O. Stephany, F. Dron, S. Tisse, A. Martinez, J.-M. Nuzillard, V. Peulon-Agasse, P. Cardinael, J.-P. Bouillon, J. Chromatogr. A 216 (2009) 4051.
[25] C. Bicchi, C. Brunelli, G. Cravotto, P. Rubiolo, M. Galli, J. Sep. Sci. 25 (2002) 125.
[26] G. Cravotto, G. Omiccioli, L. Stevanato, Ultrason. Sonochem. 12 (2005) 213.
[27] V. Schurig, D. Schmalzing, U. Muehleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot, J.C. Buyten, J. High Resolut. Chromatogr. 13 (1990) 713.
[28] L. Blomberg, T. Wannmann, J. Chromatogr. 148 (1978) 379.
[29] C. Bicchi, A. D'Amato, G. Artuffo, G.M. Nano, A. Galli, M. Galli, J. High Resolut. Chromatogr. 14 (1991) 301.
[30] C. Bicchi, G. Artuffo, A. D'Amato, V. Manzin, J. High Resolut. Chromatogr. 15 (1992) 710.
[31] European Pharmacopoeia, 6th Ed., 2008.
[32] P. Fugedi, Carbohydr. Res. 192 (1989) 366.
[33] T. Carofiglio, M. Cordioli, R. Fornasier, L. Jicsinszky, U. Tonellato, Carbohydr. Res. 339 (2004) 1361.
[34] K. Takeo, H. Mitoh, K. Uenura, Carbohydr. Res. 187 (1989) 203.
[35] D. Icheln, B. Gehrcke, Y. Piprek, P. Mischnick, W.A. König, M.A. Dessoy, A.F. Morel, Carbohydr. Res. 280 (1996) 237.
[36] K. Grob, G. Grob, K. Grob, J. Chromatogr. 156 (1978) 1.
[37] K. Grob, G. Grob, K. Grob, J. Chromatogr. 219 (1981) 13.
[38] C. Bicchi, A. D'Amato, V. Manzin, in: K. Swift (Ed.), Flavours and Fragrances, The Royal Society of Chemistry, Cambridge, UK, 1997, p. 57.
[39] B. Koppenhoefer, R. Behnisch, U. Epperlein, H. Holzshoh, A. Bernreuther, P. Piras, C. Roussel, Perfumer Flavorist 19 (1994) 1.
[40] L. Mondello, A. Verzera, P. Previti, F. Crispi, G. Dugo, J. Agric. Food Chem. 46 (1998) 4275.