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**Development of Fast Enantioselective Gas-chromatographic Analysis Using GC Method Translation Software in routine essential oil analysis (Lavender essential oil)**

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(Article begins on next page)



## UNIVERSITÀ DEGLI STUDI DI TORINO

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1 **Development of Fast Enantioselective Gas-chromatographic Analysis Using GC Method**  
2 **Translation Software in routine essential oil analysis (Lavender essential oil)**

3  
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17

## 18 **Summary**

19 The study aimed to find the best trade-off between separation of the most critical peak pair  
20 and analysis time, in enantioselective GC-FID and GC-MS analysis of lavender essential oil, using  
21 the GC method translation approach. Analysis conditions were first optimized for conventional 25m  
22 x 0.25mm inner diameter ( $d_c$ ) column coated with 6<sup>I-VII</sup>-*O*-*tert*-butyldimethylsilyl-2<sup>I-VII</sup>-3<sup>I-VII</sup>-*O*-  
23 ethyl- $\beta$ -cyclodextrin (CD) as chiral stationary phase (CSP) diluted at 30 % in PS086  
24 (polymethylphenylpolysiloxane, 15% phenyl), starting from routine analysis. The optimal multi-rate  
25 temperature program for a pre-set column pressure was determined and then used to find the  
26 pressures producing the efficiency-optimized flow (EOF) and speed-optimized flow (SOF). This  
27 method was transferred to a shorter narrow-bore (NB) column (11 m x 0.10mm) using method-  
28 translation software, keeping peak elution order and separation.

29 Optimization of the enantioselective GC method with the translation approach markedly  
30 reduced the analysis time of the lavender essential oil, from about 87 minutes with the routine  
31 method to 40 minutes with an optimal multi-rate temperature program and initial flow with a  
32 conventional inner diameter column, and to 15 minutes with FID as detector or 13.5 minutes with  
33 MS with a corresponding narrow bore column, while keeping enantiomer separation and efficiency.  
34

## 35 **Keywords**

36 Enantioselective GC, fast enantioselective GC, cyclodextrin derivatives, method-translation  
37 software, efficiency-optimized flow (EOF), speed-optimized flow (SOF), lavender essential oil.  
38

## 39 **1. Introduction**

40 One of the approaches used to meet the increasing demand for routine chiral recognition of  
41 real-world samples is to speed up enantioselective GC (Es-GC), thus increasing sample throughput,  
42 laboratory productivity and, as a consequence, reducing analysis costs. Cyclodextrin derivatives  
43 (CDs) are the most widely-used chiral selectors in Es-GC in the flavour and fragrance field [1, 2].  
44 Chiral recognition with CDs is due to the small difference in the energy of the host/guest  
45 interactions between each enantiomer and the chiral selector, and is entirely governed by  
46 thermodynamics [3, 4]; in consequence it is closely controlled by temperature. The decisive  
47 contribution of temperature to chiral discrimination limits the heating rates that can be applied, and  
48 makes column length, inner diameter and/or carrier gas and flow rate the most important parameters  
49 on which to act to speed up an enantiomer GC separation. In a previous article, Bicchi et al. [5]  
50 successfully applied short conventional and narrow-bore CD columns to speeding up Es-GC  
51 analysis of real-world samples in the essential oil field, by applying temperature rates up to  
52 10°C/min and using mass spectrometry (MS) as a further dimension of discrimination, i.e. to  
53 overcome peak co-elution due to column shortening and increased heating rates. A resolution limit  
54 of 1.5 was assumed to enable correct enantiomeric excess (ee) and/or enantiomeric ratio (er)  
55 determination. The lower enantiomer elution temperatures due to short columns increased CD  
56 enantioselectivity and (at least partially) compensated for the loss of efficiency due to column  
57 shortening. The study aimed to achieve the best trade-off between analysis speed and loss of  
58 resolution of chiral compounds, without considering the effect on the total separation compensated  
59 by the MS action. It started from routine analysis conditions usually applied to conventional inner  
60 diameter (0.25 mm) and length (25 m) columns coated with 6<sup>I-VII</sup>-*O*-*tert*-  
61 butyldimethylsilyl(TBDMS)-2<sup>I-VII</sup>,3<sup>I-VII</sup>-*O*-ethyl- $\beta$ -cyclodextrin diluted at 30% in PS-086  
62 (polymethyl-phenylsiloxane, 15% phenyl) and with a helium flow rate of 1.0 mL/min.

63 In general, a laboratory routinely analysing numerous different samples in a single field (e.g.  
64 essential oils from different plants) tends to adopt the same standardized GC conditions for all of  
65 them, rather than optimizing the method for each matrix; this approach also enables automatic peak  
66 identification from chromatographic data (relative retention times, linear retention indices, etc.).  
67 Moreover, satisfactory separations are usually obtained because the chromatographic system  
68 provides an efficiency much higher than is required, although this excess is generally paid for in

69 terms of analysis times that are longer than they need be. Optimization of analysis conditions of a  
70 given sample can successfully and drastically speed up a routine GC analysis. The main question is  
71 how to optimize a method without losing separation and keeping the same order of analyte elution.  
72 This topic was investigated in depth by Blumberg and co-workers in a series of studies highlighting  
73 the most important theoretical concepts to optimize capillary GC methods and achieve the best  
74 speed/separation trade-off [6-10]. The result was the well-known GC method translation [11] – a  
75 method optimisation approach that preserves the peak elution order. In GC method translation, the  
76 parameters influencing the analysis are divided into two main groups: translatable and non-  
77 translatable. Stationary phase type and phase ratio are non-translatable column parameters. All other  
78 column and method parameters including column dimension ( $d_c$  and length), outlet pressure (1 atm  
79 for FID, vacuum for MS, etc.), carrier gas and flow rate ( $F$ ), are translatable [10]. This approach  
80 adopts the hold-up time as time unit to express all time-related parameters, including the duration of  
81 temperature plateau(s) and heating rate(s), which, in turn, leads to a normalized temperature  
82 programme for the analysis considered. As a result, two methods are translatable when they have  
83 identical non-translatable parameters and normalized temperature programmes. For a given  
84 temperature-programmed analysis, thanks to the method-translation principles, it is possible to  
85 optimize either the flow rate producing the highest efficiency (i.e. the plate number) of a given  
86 column (efficiency-optimized flow, EOF), or a combination of flow rate, column dimensions and  
87 carrier gas type that corresponds to the shorter analysis time for a given required plate number  
88 (speed-optimized flow, SOF [8]). Method translation software is available free of charge from the  
89 Internet [12].

90 This study sought the best trade-off between separation of the most critical peak pairs and  
91 total efficiency of the chromatographic system, while shortening analysis time, in the  
92 enantioselective analysis of the lavender essential oil in a conventional  $d_c$  column, and then to  
93 transfer the optimised method to a shorter narrow bore column. Suitable GC method translation was  
94 used in the method modifications to preserve the peak elution order during flow rate optimisation in  
95 the conventional bore column, and to keep that elution order in the shorter narrow bore column with  
96 FID and MS as detectors. Lavender oil was again used as model sample because it is characterized  
97 by a large number of chiral markers [13] and their enantiomeric composition in a genuine oil is  
98 reliably described in the literature [14].

99

## 100 **2. Experimental**

101

### 102 **2.1 Samples**

103 Pure standards of nonane, decane, undecane and racemic  $\alpha$  pinene, limonene and linalool,  
104 were from the collection of standards in the authors' laboratory. All standard compounds were  
105 solubilised in cyclohexane at a concentration of 100 mg/L each. Solvents were all HPLC grade from  
106 Riedel-de Haen (Seelze, Germany). Lavender (*Lavandula angustifolia* P. Mill.) essential oil (e.o.),  
107 obtained by hydrodistillation following the method described in the European Pharmacopoeia (6th  
108 edition) [13], was diluted 1:200 in cyclohexane before analysis.

109

### 110 **2.2 Instrumental set-up**

111 A Shimadzu GC 2010 system (Shimadzu, Milan, Italy) provided with Shimadzu GC  
112 Solution 2.53SU1 software and an Agilent 6890 GC system (Agilent, Little Falls, DE, USA) with  
113 Agilent – LC/MSD ChemStation (version A.08.03 - 847) software were used for the GC-FID  
114 analyses. Agilent 6890-5975 GC-MS with an Agilent – MSD ChemStation version D.02.00.275  
115 software was used for the GC-MS analyses.

116 Columns: GC analyses were carried out on two columns coated with 6<sup>I-VII</sup>-*O*-TBDMS-2<sup>I-VII</sup>-  
117 3<sup>I-VII</sup>-*O*-ethyl- $\beta$ -CD [15] as chiral stationary phase (CSP) diluted at 30 % in PS086: a 25 m  
118 conventional  $d_c$  (25 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) column and an (approximately) 11 m narrow bore  
119 (11.13 m  $\times$  0.10 mm  $\times$  0.10  $\mu$ m) column. Both columns were from MEGA (Legnano, Italy).

120 Shimadzu equipment was used in the method with conventional column. Agilent equipment was  
121 used in the method with narrow-bore column.

122 GC-FID conditions: temperatures: injector: 220°C, detector: 230°C, FID data acquisition  
123 rate: 50Hz. Practically, 10 or 20 Hz would be enough even for our fastest analysis using 11.13 m ×  
124 0.10 mm column. 50 Hz was selected prior to beginning of experiments to avoid any issues with  
125 peak broadening due to insufficiently high data rate. Injection mode: split; for conventional  $d_c$   
126 columns: split ratio: 1:50, injection volume: 1µl, for narrow-bore columns: split ratio 1:397,  
127 injection volume: 0.5µl. All analyses were carried out with helium as carrier gas in constant  
128 pressure mode. The initial flow rates resulting from the applied pressures, rather than the pressures  
129 themselves, are reported in text and tables. Temperature programs were from 50 to 220°C at the  
130 rates reported in the text.

131 GC-MS conditions: temperatures: injector: 220°C, transfer line: 230°C; ion source: 200°C;  
132 carrier gas: He, flow control mode: constant pressure. The MS operated in electron impact  
133 ionization mode (EI) at 70 eV, scan rate: 4.5 scan/sec, mass range: 35–350 m/z (suitable to cover  
134 the full fragmentation pattern of most e.o. components). For injection conditions see GC-FID.

135 All reported data are the means of three repetitions. The following lavender essential oil  
136 components were chosen to evaluate the influence of Es-GC conditions on separation:  $\alpha$ - and  $\beta$ -  
137 pinene (**1**) and (**3**), camphene (**2**),  $\beta$ -phellandrene (**4**), limonene (**5**), 1-octen-3-ol (**6**), camphor (**7**),  
138 linalool (**8**), borneol (**9**), linalyl acetate (**10**), terpinen-4-ol (**11**), lavandulyl acetate, (**12**), lavandulol  
139 (**13**) and  $\alpha$ -terpineol (**14**).

140

141

### 141 **3. Results and discussion**

142

143 The main aim of the study was to show that the approach and principles of method  
144 translation can successfully be applied to speed up Es-GC analysis of an essential oil with CDs as  
145 chiral selector, without interfering with the specific mechanism of enantiomer recognition. The  
146 following strategy was employed: a) optimization of the chromatographic conditions affording the  
147 best speed/separation trade-off with a conventional  $d_c$  column, and b) translation of the method to a  
148 narrow-bore column. Step (b) was first operated with FID as detectors and then with MS. As for the  
149 previous study [5], a limit of resolution of 1.5 for the enantiomers of each marker was fixed to  
150 afford correct ee or er determination. In the following, the term *analysis time* indicates the retention  
151 time of the last marker object of this investigation.

151

152

#### 152 *3.1 Optimization of Es-GC analysis conditions of lavender e.o. with a conventional 25 m × 0.25 mm column*

153

154 This part involved three main steps a) choice of initial conditions for the optimisation process  
155 (3.1.1), b) determination of optimal multi-rate temperature program for a predetermined fixed  
156 column pressure (3.1.2), and c) determination of optimal pressure for the normalized optimal multi-  
157 rate temperature program (3.1.3). In all cases, column pneumatic conditions are not expressed in  
158 terms of column pressure (which is fixed over the entire analysis), but in terms of initial flow rate  
159 (i.e. the flow rate at the beginning of the analysis), for easier comparison with flow conditions (such  
160 as 2 mL/min of He in 0.25 mm column of any length) recommended by the GC instrument  
161 manufacturer and justified in the literature [8]. Once the optimal initial flow rate of a given carrier  
162 gas is defined for a given analysis, it does not change with column length and is proportional to the  
163 column inner diameters for all translations [8].

163

164

165

#### 164 *3.1.1 – Analysis at different initial flow rates with conventional column at constant temperature rate*

166

167 The lavender e.o. was first analysed with the conventional  $d_c$  column under the temperature  
168 and flow conditions applied in routine analysis, i.e. helium flow rate 1 mL/min and 2°C/min heating  
169 rate. Under these conditions the chiral markers were well separated with an analysis time of 35.2  
170 minutes. Table 1 reports order of elution, retention times ( $t_R$ ) and resolutions ( $R_S$ ) of the  
171 enantiomers of the chiral markers investigated. Figure 1a reports the Es-GC pattern of the lavender

171 essential oil investigated, analysed under routine analysis conditions. The applied CD derivative  
172 afforded baseline separation of all chiral compounds, with the exception of  $\alpha$ -pinene (**1**)  
173 enantiomers, which were only partially resolved ( $R_s$  around 1 under all conditions applied), and of  
174 1-octen-3-ol (**6**) enantiomers, that were not separated at all, while the (*S*)-enantiomers of camphor  
175 (**7**), lavandulol (**13**) and lavandulyl acetate (**12**) were not detectable. Moreover, in this analysis (*R*-  
176 lavandulol (**13b**) and (*R*)-lavandulyl acetate (**12b**) coeluted.

177 As the starting point for method optimisation, the usual routine analysis conditions adopted  
178 in the authors' laboratory were applied except for the initial flow rate, which was doubled to 2  
179 mL/min (Table 1) to reduce the time needed for method development. However, this choice did not  
180 affect the final optimal conditions.

181

### 182 3.1.2 - Determination of the optimal multi-rate temperature program at a fixed initial flow

183 The investigated e.o. was then analysed by applying a set of different single-ramp heating  
184 rates, namely 2.6, 3.3, 5.0, 7.5, 10, and 15 °C/min (°C/ $t_M$ ). The results for 2.6, 3.3 and 5.0°C/min  
185 are reported in Table 1; those for 7.5, 10, and 15 °C/min are not reported, because with these rates  
186 an increasing number of chiral e.o. components, e.g.  $\alpha$ -pinene (**1**), borneol (**9**), linalyl acetate (**10**),  
187 terpinen-4-ol (**11**), were not separated, and in addition, some enantiomers of different markers  
188 and/or components coeluted.

189 The rate 2.6°C/min gave the most satisfactory separation, with an analysis time of about  
190 25.5 min and at the same time a good separation of all compounds, including the (*R*)-limonene  
191 (**5b**)/ocimene, 1-octen-3-ol (**6**)/ $\gamma$ -terpinene and (*S*)-linalool (**8a**)/(*S*)-borneol (**9a**) pairs that were not  
192 separated at 2°C/min. The analysis at 3.3°C/min took about 21 min, but resulted in a poorer  
193 resolution of  $\alpha$ -pinene (**1**) enantiomers and in the co-elution of (*R*)-lavandulol (**13b**) and (*R*-  
194 lavandulyl acetate (**12b**). Lastly, at 5°C/min the analysis time was about 16 min, (*R*)-lavandulol  
195 (**13b**) and (*R*)-lavandulyl acetate (**12b**) were very well separated, but linalyl acetate enantiomers  
196 were not discriminated,  $\alpha$ -pinene (**1**) enantiomers were separated only very slightly, and (*S*-  
197 camphene (**2a**) and (*R*)-  $\alpha$ -pinene (**1b**) co-eluted.

198 These experiments showed that, besides separation of the chiral-marker enantiomers,  
199 lavender e.o. contains three critical pairs of components:  $\alpha$ -pinene (**1**)/camphene (**2**), 1-octen-3-ol  
200 (**6**)/ $\gamma$ -terpinene, and (*R*)-lavandulol (**13b**)/(*R*)-lavandulyl acetate (**12b**), whose separations take place  
201 at different heating rates (2.6, 3.3 and 2.6°C/min, respectively). To obtain the best resolution of  
202 critical pairs in the shortest time, the following temperature program were planned to be explored.  
203 First ramp: 2.6°C/min from 50°C to 74°C (shortly after elution of  $\alpha$ -pinene-camphene group) to  
204 obtain the best resolution of 2a-1b-1a analytes; second ramp: 3.3°C/min from 74°C till elution of 1-  
205 octen-3-ol (**6**)/ $\gamma$ -terpinene-pair; third ramp: 2.6°C/min after elution of 1-octen-3-ol (**6**)/ $\gamma$ -terpinene-  
206 pair till elution of (*R*)-lavandulol (**13b**)/(*R*)-lavandulyl acetate (**12b**)-pair. To find elution  
207 temperature of 1-octen-3-ol (**6**)/ $\gamma$ -terpinene-pair, the second ramp was run till the end of the  
208 analysis. This dual-ramp program not only provided an acceptable resolution of the 1-octen-3-ol  
209 (**6**)/  $\gamma$ -terpinene-pair, but also resulted in a good resolution of the(*R*)-lavandulol (**13b**)/(*R*-  
210 lavandulyl acetate (**12b**)-pair. The final temperature program consisted of the following ramps from  
211 50 to 74°C (elution temperature of (*R*)- $\alpha$ -pinene (**1b**), retention time 8.62 min) at 2.6°C/min, then  
212 to 115°C (elution temperature of (*R*)-lavandulol (**13b**), retention time 21.79) at 3.3°C/min, then to  
213 220°C at 15°C/min to clear the column. Figure 1b reports the Es-GC pattern of the lavender e.o.  
214 analysed under the optimized multi-rate temperature program.

215

### 216 3.1.3 Determination of EOF and SOF for multi-rate heating

217 The next step was to optimize the flow rate by determining the initial EOF (initial flow that  
218 maximizes column efficiency and peak resolution) and calculating the initial SOF (initial flow  
219 which minimizes analysis time at fixed efficiency) [7]. In any analysis (isothermal or temperature-  
220 programmed), optimal flow rate (EOF or SOF) is different for different solutes. As a result, it is  
221 impossible to maintain a flow that is optimal for all solutes. A reasonable compromise would be to

222 apply the flow that is optimal for the most critical pair (or the most important pair). Further  
 223 considerations have to be done in a temperature-programmed analysis. Optimal flow rate for a  
 224 given solute pair is not a fixed quantity, but declines with the temperature although not as fast as the  
 225 actual flow declines in a constant pressure mode [16]. As a result there is a mismatch between  
 226 actual and optimal flow in both constant flow and constant pressure modes. On the other hand,  
 227 column efficiency is a weak function of flow rate as long as it is reasonably close to optimal. For  
 228 this reason, it is not worth to use sophisticated flow and temperature programs enabling to achieve  
 229 perfectly optimal conditions for a given solute pair at every temperature during the analysis.  
 230 Constant pressure and constant flow modes are both sufficiently effective. In a constant pressure  
 231 mode, one can therefore speak of optimal *initial* flow rate. This can be EOF that causes the highest  
 232 column efficiency for a given solute pair and, as a result, its highest resolution (see below). This can  
 233 also be initial SOF that causes the shortest analysis time for a given resolution of the critical pair. It  
 234 is important to emphasize that so selected EOF is not necessarily optimal at the conditions at the  
 235 beginning of the run, rather, it is the value that simply leads to the highest resolution of critical pair.  
 236 The same considerations can be done for SOF.

237 Ten different pressures were applied to the column, resulting in different initial flow rates.

238 The GC method translator was used to translate the temperature program for each pressure,  
 239 in order to maintain the same normalized temperature program in all cases. Table 2 reports the  
 240 initial flow rates, the corresponding translated temperature programs, and the resulting analysis  
 241 times. These results were used to determine initial EOF (initial flow rate that maximizes column  
 242 efficiency by minimizing its plate height [8, 10]).

243 In temperature-programmed analysis,  $H$  for a peak having standard deviation ( $\sigma$ ) and elution  
 244 retention factor  $k$  can be found from the Habgood-Harris formula [17, 18],

$$245 \quad N = \left( \frac{(1+k)t_M}{\sigma} \right)^2 \quad (1)$$

246 where  $N$  is the plate number corresponding to the peak and  $t_M$  is the hold-up time measured at a  
 247 fixed temperature equal to the peak elution temperature. When  $N$  is known,  $H$  can be found as

$$248 \quad H = \frac{L}{N} \quad (2)$$

249 where  $L$  is the column length. Although Eqs. (1) and (2) offer a manageable approach to measuring  
 250  $N$ ,  $H$  and initial EOF in a temperature programmed analysis, a shortcut is possible when the only  
 251 goal is to find EOF.

252 Resolution,  $R_s$ , of two peaks, 1 and 2, can be expressed as

$$253 \quad R_s = \frac{t_{R2} - t_{R1}}{2(\sigma_1 + \sigma_2)} = \frac{(1+k_{app2})t_{M2} - (1+k_{app1})t_{M1}}{2(\sigma_1 + \sigma_2)} \quad (3)$$

254 where

$$255 \quad k_{app} = \frac{t_R}{t_M} - 1 \quad (4)$$

256 is the apparent retention factor of a peak (which can be substantially different from the elution  
 257 retention factor,  $k$ , in Eq. (1)). Closely eluting peaks have nearly equal elution temperatures and  
 258 widths. As a result,  $t_{M2} \approx t_{M1}$ ,  $\sigma_2 \approx \sigma_1$ . This allows Eq. (3) to be simplified as

$$259 \quad R_s = \frac{\Delta k_{app} t_M}{4\sigma}, \quad \Delta k_{app} = k_{app2} - k_{app1} \quad (5)$$

260 which together with Eq. (1) yields<sup>1</sup>:

---

<sup>1</sup> In isothermal analysis where  $k_{app} = k$ , this formula converges to a familiar expression [19]  $R_s = \frac{\Delta k}{1+k} \cdot \frac{\sqrt{N}}{4}$ .



261 
$$R_s = \frac{\Delta k_{\text{app}}}{1+k} \cdot \frac{\sqrt{N}}{4} \quad (6)$$

262 Solving this equation and Eq. (2) for  $H$ , one has:

263 
$$H = \frac{\Delta k_{\text{app}}^2 L}{16(1+k)^2 R_s^2} \quad (7)$$

264 Parameters  $L$ ,  $k$  and  $\Delta k_{\text{app}}$  are fixed quantities for all analyses that utilize the same column  
 265 and that are translations of one another [11]. In view of that, the last formula implies that  $H$  is  
 266 inversely proportional to the square of  $R_s$ , i.e.

267 
$$H \propto \frac{1}{R_s^2} \quad (8)$$

268 This can also be expressed as

269 
$$h = \frac{1}{R_s^2} \quad (9)$$

270 where

271 
$$h = \frac{16(1+k)^2}{\Delta k_{\text{app}}^2 L} \cdot H \quad (10)$$

272 can be viewed as relative plate height. Eqs. (8) and (9) show that, as for the flow corresponding to  
 273 the minimum plate height ( $H$ ), EOF also corresponds to the minimum relative plate height ( $h$ ) and  
 274 to the maximum resolution ( $R_s$ ) of the target peak pair.

275 The goal of optimising the initial flow in this analysis was to obtain the best separation-time  
 276 trade-off for  $\alpha$ -pinene enantiomers, i.e. the most critical pair in the analysis.

277 Resolutions of  $\alpha$ -pinene enantiomers for all initial flow rates tested,  $F_{\text{init}}$ , are listed in Table  
 278 2. Plots of functions  $R_s(F_{\text{init}})$  and  $h(F_{\text{init}})$  are shown in Figure 2. The plots show that the EOF is close  
 279 to 1 mL/min in combination with 1.57 and 2°C/min as the first two heating rates (Table 2).

280 As has been reported [8], SOF can be calculated from EOF as  $\text{SOF} = \sqrt{2} \text{ EOF}$ . In our study,  
 281 in which the initial EOF was 1 mL/min, the initial SOF is therefore 1.4 mL/min, and the  
 282 corresponding first two heating rates are 2.02 and 2.57°C/min (Table 2). Under these conditions,  
 283 analysis time was 29.3 min. Table 3 reports retention times, enantiomer resolution and  $\sigma$  values of  
 284 chiral markers of the lavender e.o. analysed under the optimal conditions determined. The lavender  
 285 e.o. profiles at EOF and SOF are shown in Figure 1c and 1d, respectively.

### 286 3.2 Translation of the method to a narrow bore column with FID.

287 The optimised SOF method with conventional (25 m × 0.25 mm × 0.25 μm) column was  
 288 then translated to a narrow-bore (NB) column (11.13 m × 0.1 mm × 0.1 μm) coated with the same  
 289 stationary phase. Parameters of the translated method are shown in Table 2 and the e.o. profile in  
 290 Figure 3a. As part of the method translation, flow rate was reduced in proportion with the column  
 291  $d_c$ , i.e. from 1.4 mL/min to 0.56 mL/min, thus assuring SOF operation of the NB column. Under  
 292 these conditions, and because both columns had very similar length/  $d_c$  ratio, translation did not  
 293 affect resolution of any peak pair. Table 3 confirms this expectation (taking 10-20 % inaccuracy in  
 294 measurement of peak resolution into account). Table 3 also reports peak retention time and  $\sigma$  values  
 295 in the translated method. Retention data in Table 3 show that translation reduced analysis time by  
 296 2.7 times, without loss in peak resolution.

### 297 3.3. Evaluation of MS as detector

298 The SOF analysis conditions with FID were then translated to the Es-GC-MS of the same  
 299 lavender e.o.. Table 3 reports parameters  $t_R$ ,  $\sigma$  and  $R_s$  of the marker components of the e.o.  
 300 investigated with the NB column using both FID and MS as detectors. The translation further  
 301 reduced analysis time without losing peak resolution. As is shown in Table 3, translation from the  
 302 FID method with conventional column at SOF to the MS method with NB column at SOF overall  
 303  
 304

305 reduced analysis time by 3 times (retention time of the last peak was reduced from 29.28 min to  
306 10.09 min).

307

#### 308 **4. Conclusions**

309 The results show how effective optimization of a Es-GC method and the translation  
310 approach can be in reducing analysis time. The use of the optimised Es-GC conditions enabled  
311 separation and efficiency to be kept constant in the analysis of a lavender e.o., taken as model,  
312 while drastically reducing analysis time from about 37.6 minutes for the routine method to 29.3  
313 minutes for the optimized method with conventional  $d_c$  column, and to 10.8 (FID) and 10.1 (MS)  
314 minutes with the corresponding NB column; the time required for the whole chromatographic run  
315 was reduced from about 87 minutes for the routine method to 40 minutes for the optimized method  
316 and 15 (FID) or 13.5 (MS) minutes respectively for the NB columns.

317 These results also show that Es-GC analysis with CD as chiral selectors can be speeded up,  
318 not only by using MS as a further dimension for chiral discrimination [5] but also by effectively  
319 tuning the chromatographic conditions with conventional column, and transferring the method to  
320 short narrow-bore columns, keeping separation unvaried.

321

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- 349

350 Captions to figures

351

352 Figure 1: Es-GC profile of the lavender e.o. analysed under different conditions with the  
353 conventional  $d_c$  column. For analysis conditions see text and table 2. Peak identification: 1:  $\alpha$ -  
354 pinene, 2: camphene, 3:  $\beta$ -pinene, 4:  $\alpha$ -phellandrene, 5: limonene, 6: 1-octen-3-ol, 7: camphor, 8:  
355 linalool, 9: borneol, 10: linalyl acetate, 11: terpinen-4-ol, 12: lavandulol, 13:  $\square$ -terpineol, 14:  
356 lavandulyl acetate; a: (*S*)-enantiomer, b: (*R*)-enantiomer

357

358 Figure 2: Diagrams of the variation in resolution ( $R_s$ ) of  $\alpha$ -pinene enantiomers and relative  
359 plate heights ( $h$ ) for  $\alpha$ -pinene under different initial flow rates and corresponding multi-rate  
360 temperature programs (see table 2).

361

362 Figure 3: FID and MS Es-GC profiles of the lavender e.o. analysed under SOF conditions  
363 with the narrow-bore column. For analysis conditions see text and table 2. For peak identification  
364 see caption of Figure 1.

Table 1: Retention time ( $t_R$ ) and resolution ( $R_s$ ) of the enantiomers of the lavender e.o. chiral markers under two initial flow rate and a several single-ramp heating rates. Conditions: conventional column, FID. Legend: <sup>1,2,3</sup> = coeluting peaks; 1E = only one enantiomer detected; NR = not resolved.

Initial flow rate (mL/min)		1		2		2		2		2	
Heating rate (°C/min)		2.0		2.0		2.6		3.3		5.0	
Compound		$t_R$ (min)	$R_s$	$t_R$ (min)	$R_s$	$t_R$ (min)	$R_s$	$t_R$ (min)	$R_s$	$t_R$ (min)	$R_s$
1a	(S)- $\alpha$ -Pinene	13.14	1.0	9.84	1.0	8.70	1.0	7.85	0.9	6.45	0.6
1b	(R)- $\alpha$ -Pinene	13.02		9.74		8.62		7.78		6.41 <sup>1</sup>	
2a	(S)-Camphene	12.90	7.5	9.52	6.9	8.49	6.2	7.70	6.8	6.41 <sup>1</sup>	5.0
2b	(R)-Camphene	13.65		10.21		9.04		8.15		6.71	
3a	(S)- $\beta$ -Pinene	14.93	4.5	11.33	4.9	9.96	4.4	8.92	4.0	7.27	3.3
3b	(R)- $\beta$ -Pinene	14.40		10.82		9.56		8.60		7.06	
4a	(S)- $\beta$ -Phellandrene	20.85	5.1	16.88	5.9	14.38	5.5	12.49	4.9	9.64 <sup>2</sup>	2.7
4b	(R)- $\beta$ -Phellandrene	20.22		16.20		13.87		12.11		9.48	
5a	(S)-Limonene	20.65	6.5	16.65	6.8	14.21	6.7	12.37	6.5	9.64 <sup>2</sup>	6.0
5b	(R)-Limonene	21.55		17.48		14.91		12.92		9.98	
6	1-Octen-3-ol	24.43	NR	20.67	NR	17.22	NR	14.67	NR	11.04	NR
7b	(R)-Camphor	25.86	1E	21.40	1E	18.02	1E	15.51	1E	11.88	1E
8b	(S)-Linalool	28.23	6.3	24.26 <sup>1</sup>	7.0	20.05	6.1	16.96	5.5	12.63	4.1
8b	(R)-Linalool	27.28		23.27		19.28		16.37		12.23	
9a	(S)-Borneol	28.86	2.9	24.26 <sup>1</sup>	4.5	20.30	3.5	17.33	2.7	13.10	2.3
9b	(R)-Borneol	29.30		24.80		20.66		17.59		13.25	
10a	(S)-Linalyl acetate	31.54	2.0	26.92	3.0	22.34	2.6	18.99	2.0	14.07 <sup>3</sup>	NR
10b	(R)-Linalyl acetate	31.20		26.53		22.06		18.74		14.07 <sup>3</sup>	
11a	(S)-Terpinen-4-ol	31.84	2.0	27.50	2.2	22.68	2.2	19.14	1.9	14.21	1.6
11b	(R)-Terpinen-4-ol	32.12		27.80		22.91		19.31		14.32	
12b	(R)-Lavandulyl acetate	33.02 <sup>1</sup>	1E	28.17	1E	23.37	1E	19.83	1E	14.84	1E
13b	(R)-Lavandulol	33.02 <sup>1</sup>	1E	29.02	1E	23.74	1E	19.87	1E	14.59	1E
14a	(S)- $\alpha$ -Terpineol	34.53	5.0	30.24	6.1	24.78	5.5	20.77	4.9	15.27	3.9
14b	(R)- $\alpha$ -Terpineol	35.19		30.96		25.30		21.17		15.52	

Table 2: Method parameters (initial flow rates and translated heating rates) and measured parameters (analysis times and resolutions of  $\alpha$ -pinene enantiomers).

Column dimensions (detector)	25 m $\times$ 0.25 mm (FID)											10 m $\times$ 0.1 mm	
													(FID)
Initial flow rate (mL/min)	2.0	0.3	0.5	0.7	1.0 (EOF)	1.4 (SOF)	1.7	2.3	2.5	2.8	4.0	0.56 (SOF)	0.56 (SOF)
Temperature program													
Initial Temperature ( $^{\circ}$ C)	50	50	50	50	50	50	50	50	50	50	50	50	50
Heating Rate1 ( $^{\circ}$ C/min)	2.60	0.58	0.90	1.19	1.57	2.02	2.32	2.86	3.02	3.25	4.08	5.53	5.90
Intermediate temperature1 ( $^{\circ}$ C)	74	74	74	74	74	74	74	74	74	74	74	74	74
Heating Rate2 ( $^{\circ}$ C/min)	3.30	0.74	1.14	1.51	2.00	2.57	2.95	3.63	3.83	4.13	5.17	7.04	7.50
Intermediate temperature2 ( $^{\circ}$ C)	115	115	115	115	115	115	115	115	115	115	115	115	115
Heating Rate3 ( $^{\circ}$ C/min)	15.00	3.34	5.20	6.87	9.08	11.67	13.40	16.49	17.43	18.77	23.52	31.96	34.10
Final Temperature ( $^{\circ}$ C)	220	220	220	220	220	220	220	220	220	220	220	220	220
Final Time (min)	2.00	8.98	5.77	4.37	3.30	2.57	2.24	1.82	1.72	1.60	1.27	0.94	0.90
Analysis time (min)	22.76	102.13	65.93	49.82	37.65	29.28	25.49	20.69	19.59	18.19	14.50	10.78	10.09
Resolution of $\alpha$ -pinene	0.96	1.01	1.04	1.06	1.07	1.04	1.00	0.93	0.90	0.88	0.72	1.09	1.10

Table 3: retention time ( $t_R$ ), resolution ( $R_s$ ) and  $\sigma$  values of the enantiomers of the lavender e.o. chiral markers analysed under different conditions. Legends: 1E = only one enantiomer detected; NR = not resolved

Column, initial flow rate		25m, 1 mL/min (EOF)			25m, 1.4 mL/min (SOF)			10m, 0.56 mL/min (SOF)					
		$t_R$	$\sigma$	$R_s$	$t_R$	$\sigma$	$R_s$	FID			MS		
Compound		$t_R$	$\sigma$	$R_s$	$t_R$	$\sigma$	$R_s$	$t_R$	$\sigma$	$R_s$	$t_R$	$\sigma$	$R_s$
1a	(S)- $\alpha$ -Pinene	14.43	1.91	1.1	11.21	1.54	1.0	4.07	0.48	1.1	3.81	0.47	1.0
1b	(R)- $\alpha$ -Pinene	14.29	1.95		11.10	1.56		4.04	0.51		3.78	0.47	
2a	(S)-Camphene	14.07	1.96	6.8	10.93	1.64	6.5	3.97	0.53	7.5	3.71	0.55	6.9
2b	(R)-Camphene	14.98	2.04		11.64	1.63		4.23	0.53		3.97	0.55	
3a	(S)- $\beta$ -Pinene	16.50	2.11	4.7	12.82	1.67	4.7	4.66	0.54	5.4	4.37	0.48	5.4
3b	(R)- $\beta$ -Pinene	15.84	2.05		12.31	1.60		4.47	0.51		4.19	0.50	
4a	(S)- $\beta$ -Phellandrene	23.24	2.00	5.5	18.06	1.55	5.5	6.61	0.53	6.0	6.19	0.41	6.2
4b	(R)- $\beta$ -Phellandrene	22.51	2.00		17.49	1.55		6.40	0.55		6.00	0.52	
5a	(S)-Limonene	23.01	1.99	7.3	17.88	1.55	7.3	6.54	0.52	7.8	6.13	0.47	8.4
5b	(R)-Limonene	24.01	2.17		18.66	1.63		6.84	0.60		6.40	0.51	
6	1-Octen-3-ol	27.21	1.87	NR	21.16	1.47	NR	7.79	0.53	NR	7.29	0.46	NR
7b	(R)-Camphor	28.53	2.26	1E	22.18	1.71	1E	8.13	0.67	1E	7.62	0.59	1E
8b	(S)-Linalool	31.14	1.90	6.0	24.21	1.49	6.0	8.93	0.51	5.6	8.36	0.47	5.8
8b	(R)-Linalool	30.09	3.32		23.41	2.45		8.61	1.17		8.07	1.00	
9a	(S)-Borneol	31.67	2.47	3.1	24.62	1.82	3.1	9.05	0.73	3.1	8.48	0.82	2.6
9b	(R)-Borneol	32.14	2.26		24.99	1.75		9.19	0.64		8.60	0.60	
10a	(S)-Linalyl acetate	34.46	1.64	3.0	26.79	1.30	3.0	9.84	0.48	3.1	9.20	0.51	2.7
10b	(R)-Linalyl acetate	34.09	2.17		26.50	1.65		9.73	0.61		9.11	0.55	
11a	(S)-Terpinen-4-ol	34.80	2.12	2.1	27.06	1.65	2.0	9.98	0.60	2.0	9.34	0.49	2.2
11b	(R)-Terpinen-4-ol	35.09	2.03		27.28	1.72		10.06	0.56		9.41	0.52	
12b	(R)-Lavandulyl acetate	35.90	2.06	1E	27.91	1.65	1E	10.25	0.53	1E	9.59	0.56	1E
13b	(R)-Lavandulol	36.06	1.82	1E	28.04	1.47	1E	10.33	0.44	1E	9.67	0.43	1E
14a	(S)- $\alpha$ -Terpineol	37.22	1.45	4.6	28.94	1.15	4.5	10.66	0.36	5.6	9.97	0.35	5.3
14b	(R)- $\alpha$ -Terpineol	37.65	1.36		29.28	1.08		10.78	0.32		10.09	0.31	

Notes: All  $t_R$  values are in minutes, all  $\sigma$  values are in seconds.

Figure 1

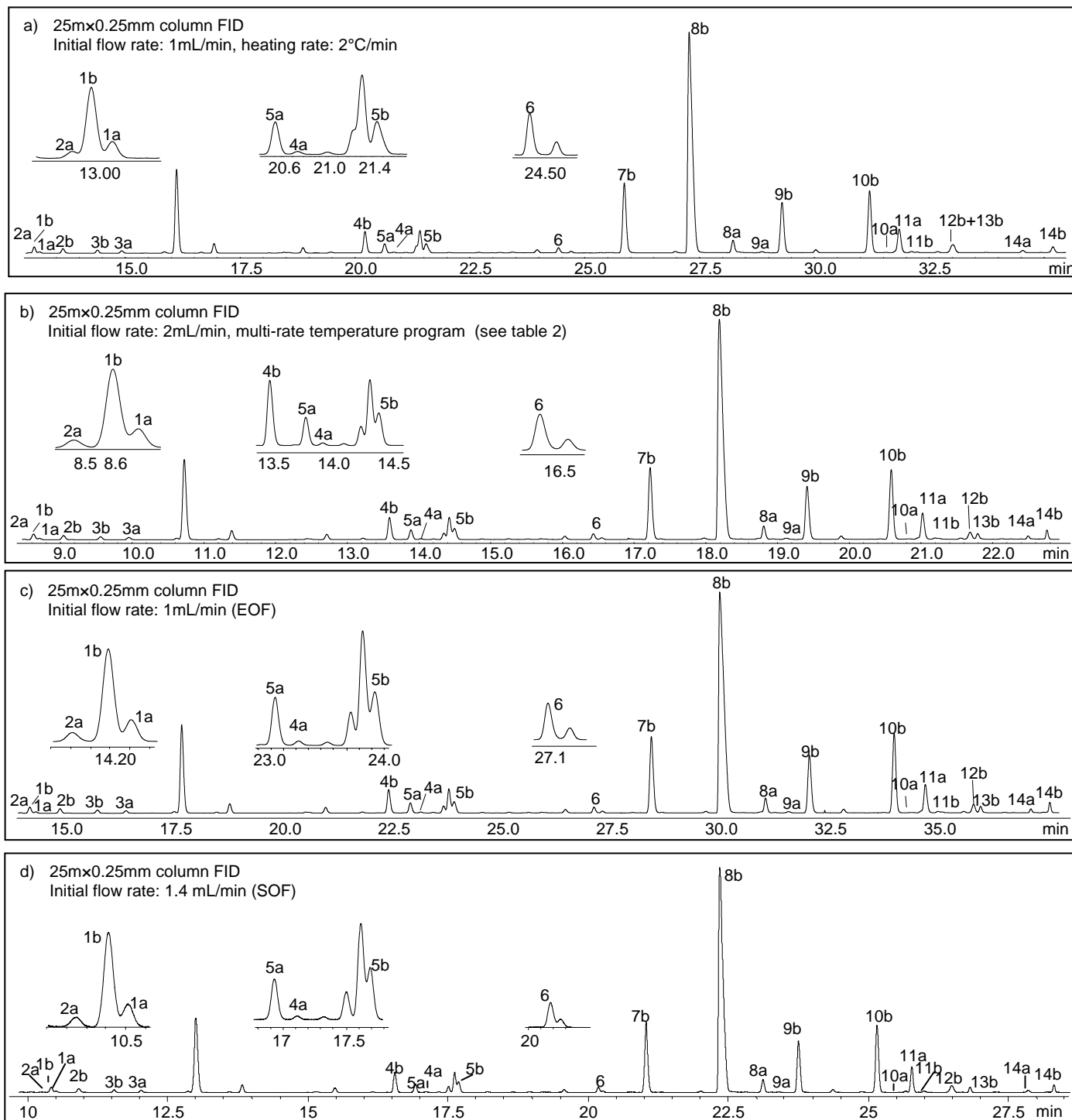




Figure 2

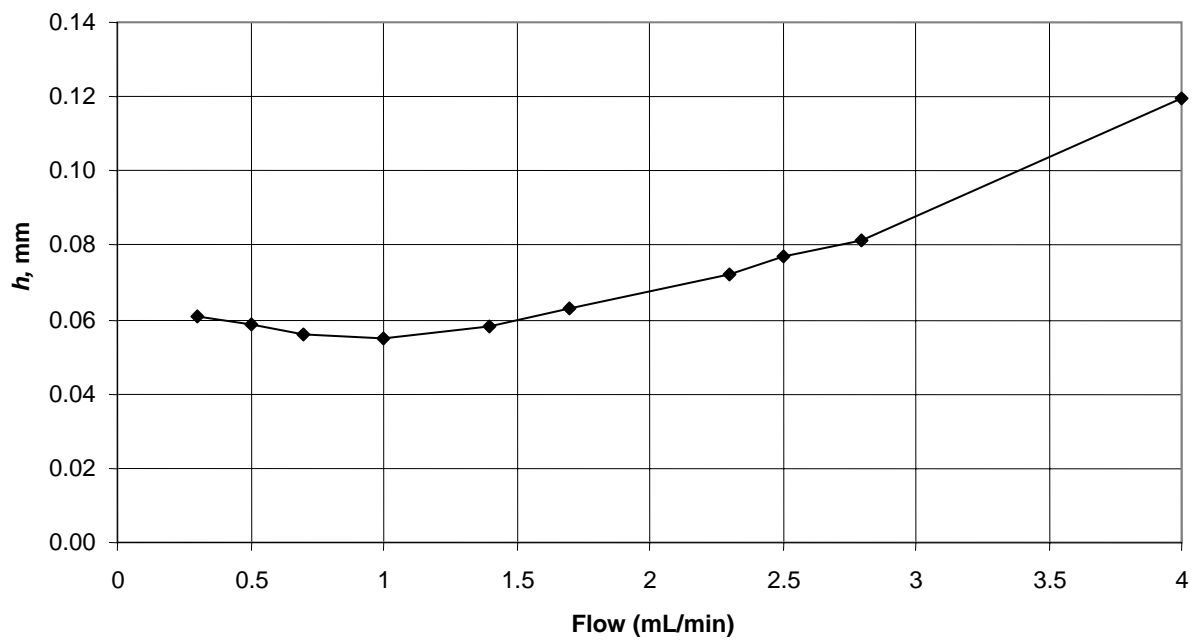
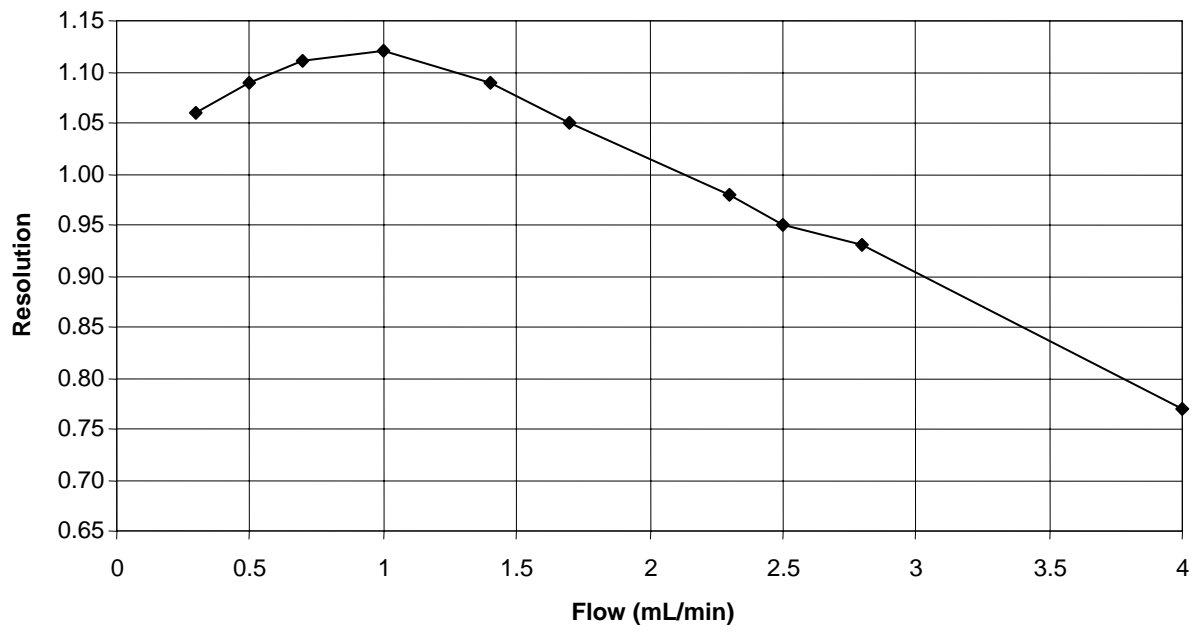


Figure 3

