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# Analysis of isomeric tropane alkaloids from *Schizanthus grahamii* by very fast gas chromatography

This study presents a very fast GC analysis applied for the baseline separation of isomeric tropane alkaloids extracted from the stem-bark of *Schizanthus grahamii* (Solanaceae). The work provided a challenging application where isothermal analysis in conjunction with very short narrow bore columns (3 m × 100 μm ID and 1.5 m × 50 μm ID) was particularly suited for the speeding up. Experimental parameters were used in the optimisation steps, including selection of stationary phase, temperature, internal column diameter and optimal practicable gas velocity. Some considerations about sample injection in fast isothermal analysis are also briefly presented. Finally, the investigated approach allowed a very fast baseline separation of four positional and configurational isomers in less than 9 s.

**Keywords:** *Schizanthus grahamii* / Speed optimisation / Tropane isomers / Very fast gas chromatography

## 1 Introduction

The last few years have seen an increasing interest in the development of strategies leading to fast gas chromatographic methods [1–5]. New terms such as very fast and ultrafast GC have been introduced but no definitely established terminology has been retained by the scientific community so far [6–8]. Several options for speeding up GC analysis have been reported. These approaches include short columns [9, 10], small internal diameters [11], columns with reduced film thickness [12, 13], high flow rate above optimal carrier gas velocity [14], vacuum outlet operation [15, 9], fast temperature programming rate (resistive heating) [11, 16], turbulent flow [17, 18] and helically coiled columns [19]. In all cases, whenever possible, hydrogen should be used as carrier gas together with selective stationary phases.

The most straightforward approach towards fast GC is certainly to use short columns with reduced internal diameter and thin films. Recently, their potential and effectiveness have been demonstrated in routine analyses [20, 21]. Nevertheless, there are two major problems with narrow bore columns. On the one hand, sample capacity is notably decreased which also affects the column dynamic range, and on the other hand, it involves a large pressure drop. Furthermore, introducing minute amounts of sample in very narrow bands onto such columns still remains a demanding task.

The present report focuses on a strategy adopted for the very fast separation of four positional and configurational tropane isomers (Fig. 1) extracted from the stem-bark of *Schizanthus grahamii*, an endemic solanaceous plant from Chile. The aim was to attain the fastest separation maintaining a given resolution ($R_S \geq 1.5$) between the critical peak pair. For this purpose, a suitable stationary phase, the choice of temperature and the use of optimal practicable gas velocities were successively selected for isothermal GC.

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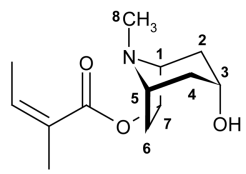
**Abbreviation: HETP**, height equivalent to one theoretical plate

## 2 Experimental

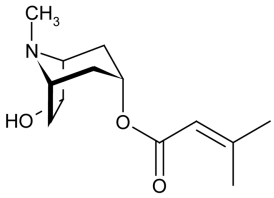
### 2.1 Plant material

*S. grahamii* Gill. was collected in Rengo (Central Chile) in January 2000. The stem-bark (2.6 kg) was extracted with EtOH at room temperature. After filtration, the alcoholic solution was evaporated to dryness. The residue was taken up in 0.1 M HCl and washed with $CH_2Cl_2$. The aqueous solution was then basified to pH 12 with $NH_4OH$ and extracted with $CH_2Cl_2$, yielding a gummy alkaline residue (6.6 g). Further purification on an aluminium oxide column was performed according to Muñoz *et al.* [22]



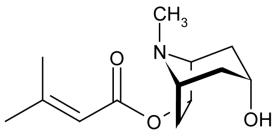
**1**

3α-hydroxy-7β-angeloyloxytropane



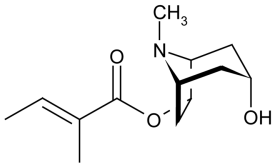
**2**

3α-senecioyloxy-7β-hydroxytropane



**3**

3α-hydroxy-7β-senecioyloxytropane



**4**

3α-hydroxy-7β-tigloyloxytropane

**Figure 1.** Structures of the four isomeric hydroxytropane esters from *S. grahamii* Gill.

leading to a purified fraction containing the four isomeric alkaloids **1**–**4** (Fig. 1).

### 2.2 GC-MS analyses

GC-MS analyses were carried out on a Hewlett-Packard 5890 series II chromatograph coupled to an HP 5972 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) which operated in the electron impact (EI) ionisation mode at 70 eV. Spectra were recorded in the mass range *m/z* 30–400 Th at 1.3 scan/s and the MS transfer line was set at 250°C. The instrument was equipped with a split/splitless injector. Injections were carried out with an HP 6890 series fast automatic liquid sampler (Agilent Technologies). The carrier gas was delivered in the constant flow mode (He, 1 mL/min).

Two capillary columns were used, namely HP5-MS (Agilent Technologies) and DB-1701 (J&W Scientific, Folsom, CA, USA) with the same geometry: 30 m × 0.25 mm ID × 0.25 μm film thickness.

Split injections with a splitting ratio of 30:1 were performed at an injector temperature of 250°C with a straight 4 mm ID liner packed with a 1 cm deactivated glass wool plug placed 10 mm above the column entrance. One microlitre injections (10 mg extract dissolved in 10 mL MeOH) were performed with a 10 μL syringe equipped with a 42 mm × 0.63 mm OD needle with cone tip (Agilent Technologies). Isothermal analyses were performed between 140 and 250°C.

### 2.3 Fast GC-FID

A model 6850 gas chromatograph (Agilent Technologies) was used for fast analyses. The instrument was equipped with a split/splitless injection port. The FID was set at 280°C and air, hydrogen and make up ($N_2$) flows were adjusted to 450, 40 and 40 mL/min, respectively. Split injections with an injector temperature of 300°C were performed with high split ratios ranging from 500:1 up to 2000:1. 0.1 μL injections (5–30 mg extract dissolved in 10 mL $CHCl_3$) were performed *via* a 6850 auto-sampling unit (band formation) with a 5 μL syringe (42 mm × 0.63 mm OD with an HP point style). A 1 mm ID open liner packed with a deactivated glass wool plug was used and placed 1 cm above the column entrance. Two DB-5 columns, 6 m (column a) and 3 m (column b) × 0.1 mm ID × 0.1 μm df (Agilent Technologies) were employed together with a 1.5 m × 0.05 mm ID × 0.05 μm df BGB-1701 column (BGB Analytik, Böckten, Switzerland). Hydrogen from a Whatman 75–34 generator (Agilent Technologies) was used as carrier gas with a maximal deliverable head pressure of 4.5 bar. Isothermal analyses were carried out between 140 and 250°C.

## 3 Results and discussion

In preliminary experiments (data not shown) several capillary columns (30 m × 0.25 mm ID × 0.25 μm df) coated with different stationary phases were evaluated and resolutions between the isomers were measured by conventional GC-MS. These results showed that the best selectivities were obtained with the apolar 5%-phenyl–95%-methylpolysiloxane and the midpolar 14%-cyanopropylphenyl–86%-dimethylpolysiloxane columns. The latter were selected to determine operating conditions for the speed-up process in isothermal analysis employing short narrow bore columns.

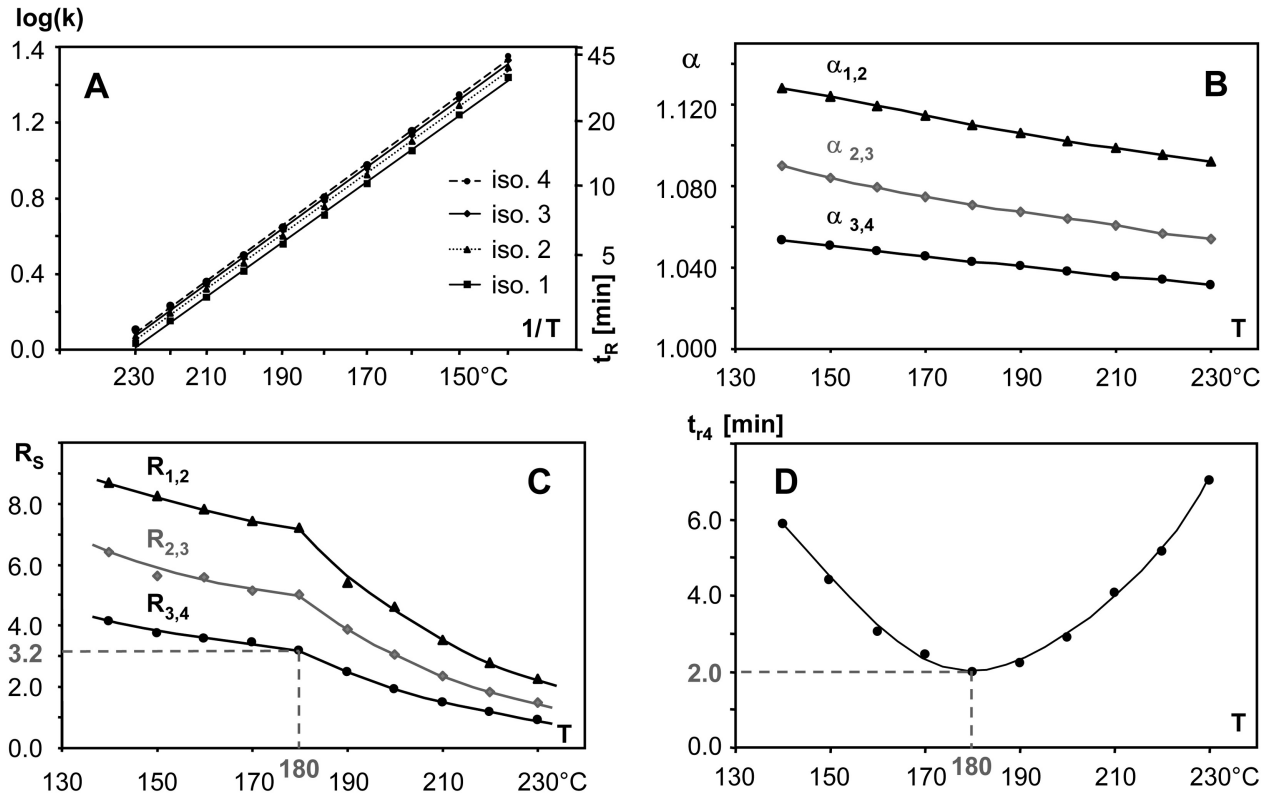



**Figure 2.** (A) Plot of logarithm of the retention factor *versus* the reciprocal column temperature (the abscissa is labelled in °C); (B) plot of selectivity *versus* temperature; (C) plot of resolution *versus* temperature; (D) curve of the calculated retention time of the last eluting isomer allowing a baseline separation in function of temperature; measures carried out by GC-MS on a 30 m × 0.25 mm ID × 0.25 μm df HP5-MS stationary phase.

### 3.1 Separation on 5%-phenyl–95%-methylpolysiloxane columns

As shown in Fig. 2A, the plot of the logarithm of *k versus* the reciprocal $T_c$ (in K) gave, in a good approximation, parallel straight lines ($R^2$ between 0.9992 and 0.9993) and confirmed that no inversion in elution order took place within the selected temperature range. The most favourable elution temperature was chosen by measuring selectivity (α) at various temperatures. Figure 2B demonstrated that α decreases with the increase of temperature and that isomers **3** and **4** are the critical pair to separate. As selectivity does not take into account the quality of the separation, the evolution of resolution *versus* temperature was plotted (Fig. 2C). It showed that $R_s$ slightly decreased until 180°C and considerably until 230°C. In order to find out the optimal temperature for the fastest baseline separation, the calculated retention time of the last isomer which would assure a 1.5 resolution between alkaloids **3** and **4** was reported in function of column temperature (Fig. 2D). For this purpose, retention times of **4** measured in Fig. 2A were divided by the quadratic ratio of the resolution value over 1.5. Indeed, for a resolution of 3.2 ($R_{3,4}$ in Fig. 2C) at 180°C, the separation is overresolved by a factor of 2.1 and thus efficiency and analysis time can be reduced by a factor of 4.5. The curve of this calculated retention time *versus* $T_C$ passed through a minimum near 180°C, at which a baseline separation between analytes **3** and **4** should be achieved in roughly 2 min. Consequently, this temperature was considered for further experiments.

The most straightforward way to reduce the analysis time is to use short columns with small internal diameters. Indeed it is known that with thin film open tubular columns and well-retained compounds, the height equivalent to one theoretical plate (HETP) is roughly equal to its internal diameter $d_C$ [23]. Consequently, to speed up the separation, two DB-5 100 μm ID columns (column a: 6 m and column b: 3 m) were used. At a given temperature, if the phase ratio is maintained constant, *k* is also constant even for different column geometries. In that case, efficiencies between two columns of same length can be measured through their internal diameter

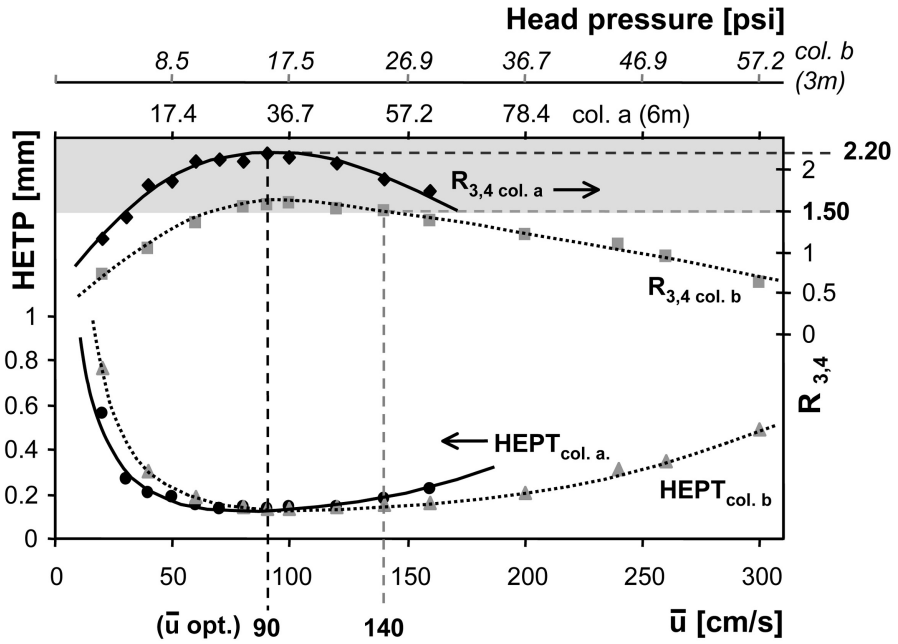



**Figure 3.** Golay plot (HETP *vs.* $\bar{u}$) of isomer **4** with the corresponding resolution between isomer **3** and **4** ($R_{3,4}$). Column a: DB-5 6 m × 0.1 mm ID × 0.1 μm df; Column b: DB-5 3 m × 0.1 mm ID × 0.1 μm df. Corresponding head pressures are also labelled.

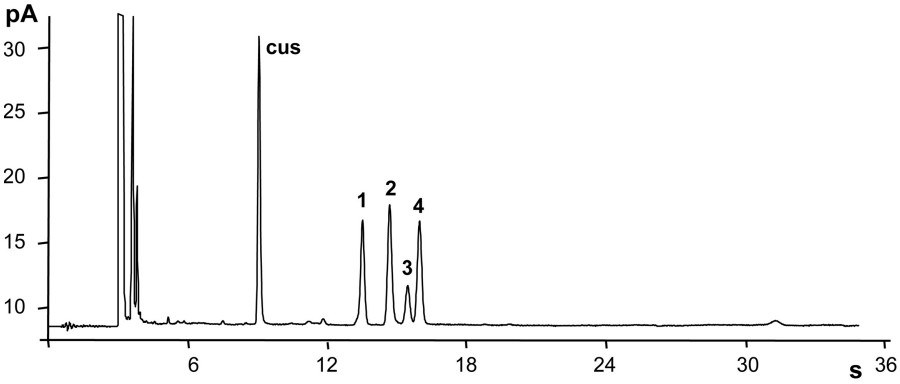



**Figure 4.** GC-FID separation obtained with the 3-m-long narrow bore column (column b) at a linear gas velocity ($H_2$) of 140 cm/s.

ratio. Thus, compared to the conventional 0.25 mm ID capillary column, a 2.5-fold speed gain may be expected. Owing to its low viscosity and large diffusion coefficient, hydrogen instead of helium was used as carrier gas and, therefore, larger optimum linear velocity can be applied. Considering both advantages, a narrow bore column and $H_2$ as carrier gas, a speed gain by a factor of 5 was expected. Starting from the calculated retention time of the last eluting alkaloid (**4**) (2 min in Fig. 2D) under conventional GC-MS, an analysis time beneath 24 s was anticipated.

$H_{min}$ and resolution of the critical pair of alkaloids were measured at the previously selected temperature (180°C) on both microbore columns. Plots of plate height together with the corresponding $R_{3,4}$ value *versus* average linear velocity $\bar{u}$ (Golay plots) were drawn for isomer **4** in Fig. 3. It showed that on a 6-m-long narrow bore column (column a) the separation was still over-resolved at the optimal linear velocity ($\bar{u}_{opt}$ = 90 cm/s). As a 2.2 resolution between **3** and **4** was measured on this column, it was cut in half, which should still assure a baseline separation. With this reduced length, the optimal linear velocity was slightly shifted upwards to 100 cm/s. As there was still some excessive resolution, it was possible to increase the linear gas velocity up to 140 cm/s enabling to baseline separate the critical peak pair in less than 18 s (Fig. 4).

### 3.2 Separation on 14%-cyanopropylphenyl–86%-dimethylpolysiloxane columns

As above, plots of log(*k*) *versus* reciprocal column temperature (Fig. 5A) demonstrated that no elution inversion occurred within the investigated temperature range. Plots of selectivity in function of temperature (Fig. 5B) showed that the critical peak pair was not the same throughout the examined temperature range. Indeed, above 185°C the lowest selectivity was between **3** and **4**,

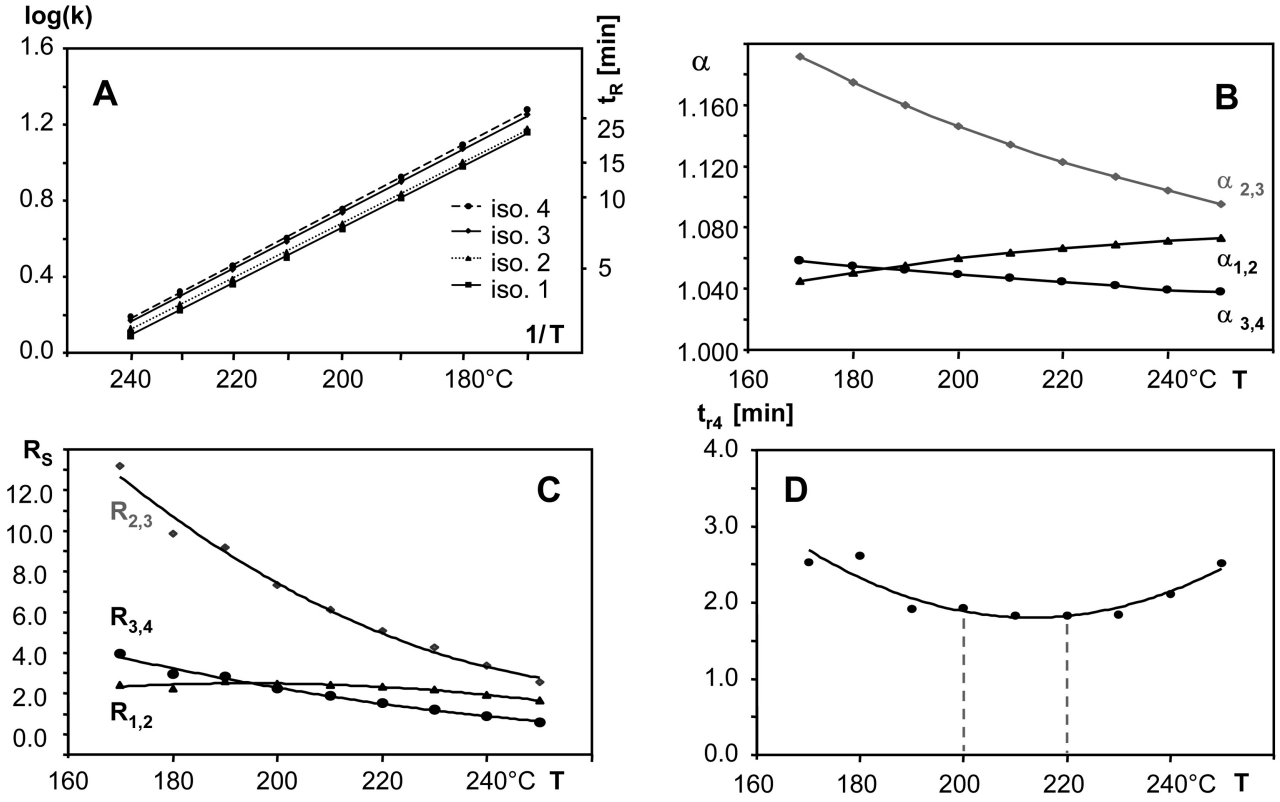



**Figure 5.** (A) Plot of logarithm of the retention factor *versus* the reciprocal temperature (the abscissa is labelled in °C); (B) plot of separation factor *versus* temperature; (C) plot of resolution *versus* temperature; (D) curve of the calculated retention time of the last eluting isomer allowing a baseline separation in function of temperature; measures effected by GC-MS on a 30 m × 0.25 mm ID × 0.25 μm df DB-1701 stationary phase.

while below this temperature it was between **1** and **2**. The evolution of resolution with temperature (Fig. 5C) revealed that the best compromise was nearby 200°C. To select the fastest conditions for a baseline separation, the calculated retention time ($t_{R4}$) *versus* column temperature was plotted (Fig. 5D). It appeared that the analysis time can slightly be decreased ($t_{R4}$ < 2.0 min) compared to the HP5-MS column if a baseline separation is also maintained for the first eluting isomeric peak pair. To ensure a significant speed gain, a 50 μm ID column (BGB-1701, 1.5 m × 50 μm ID) was used. Optimal flow conditions were determined by plotting Golay curves for compounds **2** and **4** at 210°C (Fig. 6A) and 200°C (Fig. 6B). $R_{1,2}$ and $R_{3,4}$ are presented on the same graphs. They confirmed the results obtained under conventional GC-MS showing that resolution between **3** and **4** was more critical than between **1** and **2** above 200°C and that this difference is more pronounced at 210°C while the overall resolution is higher at 200°C. $H_{min}$ measured (0.066 mm at both temperatures, for isomer **4**) was close to the theoretical value and was reached at average linear velocities between 100 and 120 cm/s. Finally, the fastest conditions were obtained at 200°C with a baseline separation of both critical pairs **1**,**2** and **3**,**4** at 150 cm/s. The chromatogram obtained under these specific conditions is shown in Fig. 7. The separation was performed in less than 9 s.

The number of effective plates determined on the last eluting isomer was raised up to 21 800 plates. Peak width at half height of the four alkaloids ranged between 85 and 105 ms, which lies in the very fast GC domain [6]. Compared to optimal gas velocity conditions, an efficiency loss of roughly 4% only was noticed, while the speed of analysis could be increased by about 30%. This is due to the shallower Golay type curve beyond $\overline{u}_{opt}$ of such a narrow bore column.

To maintain such performances, small amounts of sample must be injected rapidly in narrow bands. Therefore, the following injection conditions were used: a midpolar solvent generating low vapour volume ($CHCl_3$), a small injected solvent volume (0.1 μL generating approximately 12 μL solvent vapour only), a high split ratio leading to high split flows (150–380 mL/min), a liner with reduced inner diameter (1 mm ID corresponding to a volume of 65 μL) and a fast autosampler for a fast needle dwell time (100 ms). Consequently, it was estimated that the whole liner volume was effectively and rapidly purged so that the contribution of injection to band

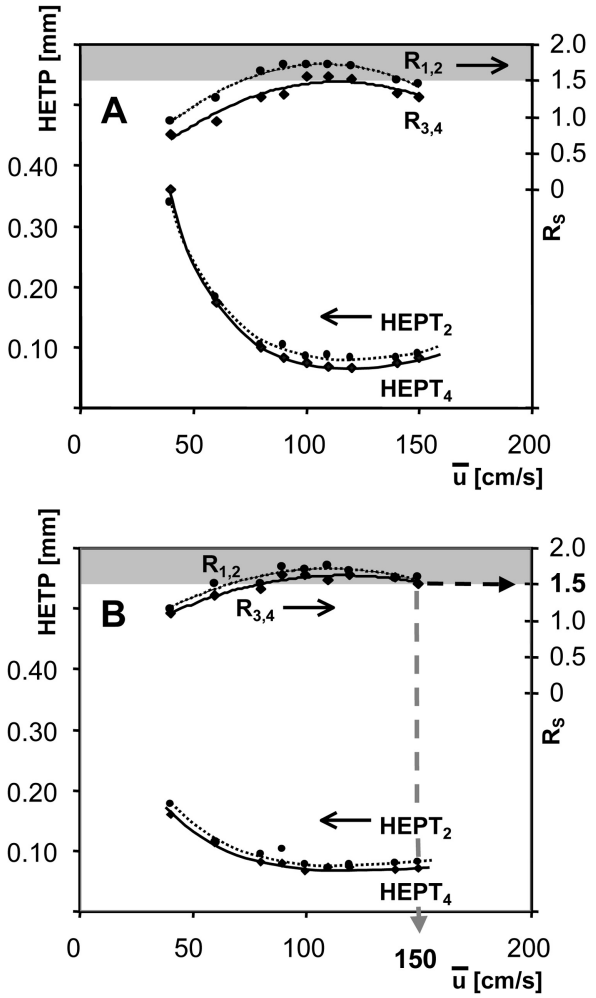



**Figure 6.** Plot of HETP *versus* average linear velocity measured for isomers **2** (dashed line) and **4** together with the corresponding resolution $R_{1,2}$ and $R_{3,4}$; column: 1.5 m × 0.05 mm ID × 0.05 μm df coated with BGB-1701; (A) measurements at 210°C; (B) measurements at 200°C where the required baseline separation was obtained at 150 cm/s ($H_2$).

broadening was small. Finally, with such high split ratio (1000:1 in case of Fig. 7) the RSD% of absolute peak areas of the four isomers and cuscohygrine (cus) was inferior to 5% ($n$ = 5).

## 4 Concluding remarks

A systematic investigation of isothermal fast GC operating conditions was described to drastically reduce analysis time of a purified plant extract containing isomeric tropane alkaloids. For this purpose, appropriate column selectivity, column temperature, optimal practicable linear gas velocity and column lengths were selected to maintain a baseline separation between all isomers. Despite the adopted strategy being restricted to simple mixtures, it is permitted to shorten the analysis time of four positional and configurational isomers to less than 9 s. It implied that the band broadening induced by the sample introduction was minimised. This was achieved by choosing narrow liners, high split flows and a fast syringe handling. Finally, even if sample preparation is usually the most time consuming step in natural product research, results presented herein demonstrated that phytochemical investigations can positively benefit from fast GC to reduce the overall analysis time.

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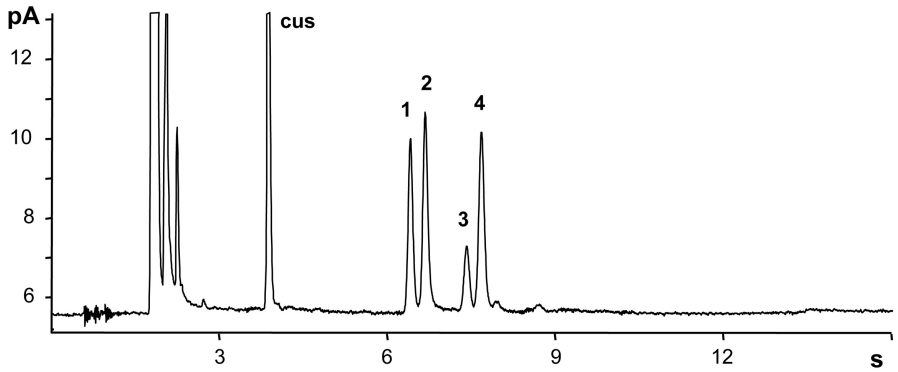


**Figure 7.** Very fast GC-FID separation at 200°C with the BGB-1701 1.5 m × 0.05 mm ID × 0.05 μm df column operating at an average linear gas velocity ($H_2$) of 150 cm/s.

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