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Dynamic interconversion of chiral oxime compounds in gas chromatography

Chewe Chifuntwe[a], Feng Zhu[b], Helmut Huegel[a], Philip J. Marriott[c,*]

[a] School of Applied Sciences, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia
[b] Department of Chemistry, Foshan University, Foshan 528000, China
[c] Australian Centre for Research on Separation Science, School of Applied Sciences, RMIT University, City Campus, GPO Box 2476V, Melbourne, Victoria 3001, Australia

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ABSTRACT

A number of chiral oxime compounds have been synthesised and their gas chromatographic analysis on both a polyethelene glycol phase column and two chiral column phases was investigated. Of particular interest to this work is the observation of dynamic interconversion behaviour, both in a single dimensional analysis, and by using comprehensive two-dimensional gas chromatography (GC × GC). A number of non-chiral compounds were studied as a means to understand the nature of the behaviour observed. As expected, the achiral compound on both the wax column and the chiral column generated two isomeric compounds—the E and Z isomers. On the wax column, a characteristic interconversion zone representing the dynamic process was observed, with extent of interconversion dependent on the conditions used. For the chiral compounds, two isomers and the interconversion zone were exhibited on the wax column, however on the chiral column 4 isomeric peaks were found—the (R) and (S) enantiomers of each of the E and Z isomers. In the case of the chiral column, the extent of interconversion was negligible, and this appears to correlate with the use of low polarity columns. In order to encourage dynamic interconversion, a polyethylene glycol column was coupled to the chiral column, by placing it either before or after the chiral column. In this case a monitor detector was employed between the two columns in order to isolate the effects of the first column from the behaviour on the second. In a further study, the most appropriate column arrangement from the earlier study was placed into a comprehensive two-dimensional gas chromatography instrument, with a wax-phase column in the second dimension. The unique location of peaks for each of the molecules in 2D space and patterns for the interconversion processes is interpreted phenomenologically.

1. Introduction

The study of species that undergo interconversion or some other type of structural change during chromatographic elution is a small but interesting topic area. Perhaps two different processes can be identified. One is a structural change that is irreversible, such as in decomposition processes. This is exemplified by dicyclopentadiene decomposition to cyclopentadiene [1]. This general process will not be further discussed here. The other process is that of dynamic molecular interconversion, where each molecule undergoes a mechanism of structural change into their counterpart, with the most important features being that (i) the two molecules should be resolvable on the phase and under the conditions used, and (ii) the physical conditions allow the observation of the interconversion process (i.e. the temperature and/or time of analysis is adequate to permit interconversion). The general scheme of this process as it is revealed in the chromatography experiment is shown in Fig. 1. This is an actual chromatogram of an interconverting system A ⇆ B, with sketched hypothetical distributions of each of the isomers underlying the overall response. The interconversion region corresponds to compounds that undergo an isomerisation step, and therefore are largely resolved from each of the original antipodes A and B. Thus the total band of the compound comprises the original narrow peaks of A and B, and the broad zone of interconversion.

A number of researchers have been predominant in this area, with the Schurig group in particular investigating a wide range of molecular processes, including chiral molecules in enantioselective separation methods, and under a variety of separation techniques including HPLC and GC [2–8]. Schurig has reviewed this general area [9–11]. Hochmuth and König [12] reported rotational energy barriers for chiral cyclophanes by using GC. The dynamic effect of secondary equilibria in reversed-phase HPLC of prolines was discussed by Melander et al. [13]. Trapp developed the theoretical interpretation of the interconversion process and a computer program to derive physical constants [14–16], whilst Krupcik compared experimental data and simulated results for various interconversion examples [17].

* Corresponding author. Tel.: +61 3 99252632; fax: +61 3 99253747.
E-mail address: philip.marriott@rmit.edu.au (P.J. Marriott).

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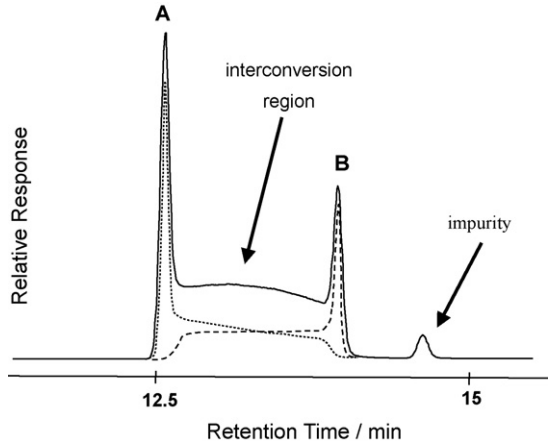

Fig. 1. Chromatogram of interconversion process between two isomers A and B, with the net effect shown in bold, and sketched outlines showing the A isomer distribution dotted, and B isomer dashed.

Marriott et al. reported the observation of sterically hindered rotation of phenantherene and anthracene molecules, organometallics and inorganic complexes in a series of papers, studying the oxime system by using one-dimensional gas chromatography systematically [18–20].

Enantioselective stopped-flow multidimensional gas chromatography (MDGC) was used to determine enantiomerization (inversion) barriers for 1-chloro-2,2-dimethylaziridine in the gas phase, with a range of $\Delta G^{\#}$ values from 70 kJ mol^{-1} to 200 kJ mol^{-1}. After GC separation of the enantiomers in the first column, gas phase enantiomerization of the heart-cut fraction of one single enantiomer was performed in the second (reactor) column at increased temperature. Subsequently this fraction was separated into the enantiomers in the third column [21]. This process was recently reviewed [22].

More recently, Marriott et al. [23,24] applied the multidimensional GC method of comprehensive two-dimensional gas chromatography (GC × GC) for the study of interconversion processes of oximes. In the GC × GC experiment a modulator periodically (e.g. every 2–8 s) collects effluent from the first dimension (^{1}D) column and pulses it rapidly to a ^{2}D short, fast elution column. High frequency detector transduction allows these peaks to be accurately recorded. For the interconversion process, by proper choice of the ^{2}D column phase, it is possible to resolve the different isomer forms of the compound, to provide the instantaneous distribution of species at any given point over the chromatographic profile [23,24]. Trapp et al. interpreted the GC × GC experiment based on theoretical treatment and derived kinetic data for the isomerisation process [25]. A review of molecular structure–retention relationships in GC × GC [26] proposed the separation of the two pairs of diastereomers for chalcogran under conditions of dynamic interconversion, with the assumption of adequate resolution of all species on the two columns, however this system has not been experimentally verified.

Based on this background, the present study extended the oxime study to chiral molecular species, and incorporated enantioselective GC column phases as the separation medium.

2. Experimental

2.1. Reagents and chemicals

The aldehydes phenylacetaldehyde, 2-methylbutyraldehyde and 2-methylpenatanaldehyde used to synthesise the oximes were purchased from Aldrich whilst heptanaldehyde was purchased from Merck, and were used without further distillation. Absolute ethanol (Ajax), hexane (Ajax), hydroxylammonium chloride (BDH) and sodium hydroxide (BDH), all of analytical reagent grade, were also used in the synthesis of oximes.

2.2. Synthesis

Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime, and phenylacetaldoxime were synthesised from their respective aldehydes, following a modified procedure from Boucher et al. [27] in which an 80 mmol solution of hydroxylammonium chloride in 20 mL of ethanol was stirred at room temperature. To this solution was added 20 mL of an 80 mmol aqueous solution in sodium hydroxide, followed by a 40 mmol solution of aldehyde dissolved in ethanol. The reaction mixture was stirred at room temperature for 4 h. After 4 h the solution was filtered to remove the sodium chloride precipitate, and ethanol was removed by rotary evaporation at 50 °C under reduced pressure. The oxime was extracted with either hexane or petroleum ether, dried with anhydrous $MgSO_4$ and the solution filtered. Solvent was removed by rotary evaporation under reduced pressure at 50 °C to afford the crude product. The crude product was purified by bulb-to-bulb distillation in a kugelrohr and characterised by NMR and GC–MS.

2.3. Sample preparation

Following synthesis samples of 2-methylbutyraldehyde oxime and 2-methylpentanaldehyde oxime were prepared for GC analysis in dichloromethane (B&J ACS; HPLC grade) with *N*,*N*-dimethylformamide (Ajax; AR grade) used as an internal standard, heptanaldehyde oxime was prepared in absolute ethanol (Scharlau; HPLC grade) with 1-hexanol (Ajax; LR grade) and 1-octanol (Ajax; LR grade) as internal standards, whilst phenylacetaldoxime was prepared in 1-pentanol with 2-phenylethanol as an internal standard.

2.4. Instrumentation

2.4.1. NMR

All oximes were prepared in $CDCl_3$-*d* solvent (Merck) and characterised by NMR on either a Brucker Avance 300 or Bruker 500 instrument. 2-Methylbutyraldehyde oxime and 2-methylpentanaldoxime were characterised by using the Brucker Avance 300 NMR whilst heptanaldehyde oxime and phenylacetaldoxime were characterised by using the Bruker 500 NMR. Spectra agreed with literature data, and will not be separately discussed here.

2.4.2. GC–MS

A quadrupole mass spectrometry system (GC–qMS) was used for characterisation of the synthesised oximes. The system consisted of an Agilent model 6890 GC fitted with an Agilent model 7683 autoinjector, coupled to a model 5973 MS detector with fast electronics upgrade (Agilent Technologies, Burwood, Australia). A BPX5 column (SGE International, Ringwood Australia) of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness (d_f) was used. Data acquisition and processing was afforded by Agilent ChemStation software.

2.4.3. GC-FID

Once the oximes were characterised, preliminary single dimension GC experiments were conducted on an Agilent model 5890 GC system, Subsequently, a Shimadzu model GC-17A with autosampler model AOC-17 (Shimadzu, Rydalmere, Australia) was used for

single dimension GC experiments. Both systems were fitted with a flame ionisation detector (FID).

Single dimension GC experiments were conducted to find the most suitable wax and chiral columns to employ for our experiments. The column found to be the most suitable for chiral analysis was a MEGA (diethyl-*t*-butyl-β-cyclodextrin phase) capillary column, with the tested column having dimensions 23 m × 0.25 mm i.d. × 0.25 μm d_f.

For interconversion experiments, a polyethylene glycol (wax) phase was found to be most suitable to promote the interconversion process, and so a SolGel wax-phase (SGE International; 30 m × 0.25 mm i.d. × 0.25 d_f) column was used. In order to combine both chiral separation and molecular interconversion properties of the respective columns, the two columns were coupled together to form one long column (dual column).

For coupled column analysis (dual ^{1}D columns), the above MEGA and SolGel wax columns were joined in either chiral–wax or wax–chiral order. To investigate the contribution of each column to the overall chromatogram a FID was positioned at the interface of the coupled columns. Whilst other chiral columns were tested (e.g. Rt-BDex cst from Restek Corp, Bellefonte, PA) they were not successful in resolving both of the enantiomers of the E and Z isomers, of the chiral compounds of interest.

A range of GC conditions were employed, according to the needs of the analysis, to either limit or promote interconversion. This was accomplished by a combination of temperature and pressure (flow rate) settings. Generally isothermal conditions were used, but on occasions temperature programmed analyses were conducted. Hydrogen carrier gas was used throughout. The injector and detector temperatures were both 230 °C. Shimadzu Class GC-10 software was used for data acquisition.

2.4.4. GC × GC-FID

The gas chromatograph, equipped with a flame ionisation detector (GC × GC-FID), used in the study was an Agilent 6890 system with a Longitudinal Modulation Cryogenic System (LMCS, Chromatographic Concepts Pty Ltd., Doncaster, Australia). The dual ^{1}D (wax–chiral) column arrangement was used for GC × GC studies. Since the ^{2}D column should be of wax type to ensure adequate ^{2}D separation, either a Stabilwax (1.1 m × 0.1 mm i.d. × 0.1 μm d_f; Restek) or a BP20 (3.1 m × 0.1 mm i.d. × 0.1 μm d_f; SGE) column was used, the latter providing better resolution.

The injector and detector temperatures were 230 °C and 250 °C, respectively. A sampling frequency of 100 Hz was used for GC × GC analysis. A high sampling frequency is required to monitor very fast GC peaks at the end of the ^{2}D column. Hydrogen was used as a carrier gas at various flow rates as indicated in the figures, and the temperature was usually isothermal, at various temperatures as indicated in figure captions. A range of modulation periods (P_M) between 2 s and 5 s were used, according to the retention time on the ^{2}D column and the duration of the overall peak distribution on the ^{1}D column. The temperature of the modulator system (T_M) was held at 0 °C, with CO_2 used as a coolant in the LMCS and nitrogen as a flush gas at a pressure of 15 psi. The modulator in GC × GC operation was commenced at a time usually 2 min prior to the elution of the first peak of interest. Agilent ChemStation software was used for modulation control, data acquisition and processing.

2.5. Description of instrument arrangements and conditions

For analyses employing dual column analysis, a FID was positioned at the interface and/or at the end of the dual column arrangement. Fig. 2 is a schematic diagram describing the different geometries of the column arrangements developed in this study. Collectively, the system can be operated as:

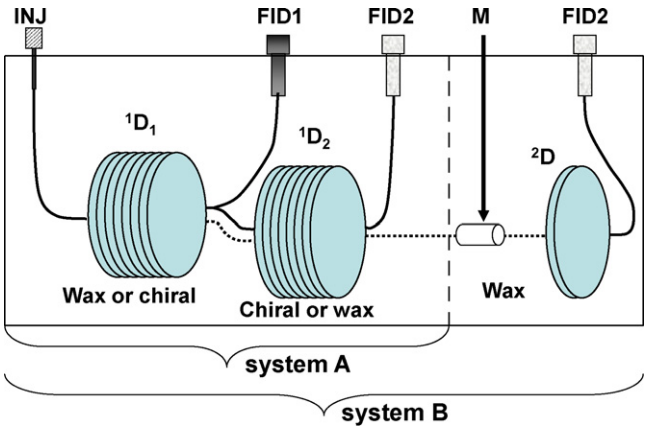

Fig. 2. Dual column arrangement. (System A) Single dimension coupled column with an FID detector (FID1) between column $^1D_1$ and $^1D_2$, and FID detection (FID2) after $^1D_2$. The two columns can be either a wax–chiral set, or a chiral–wax set. The single column arrangement is trivial. (System B) Two-dimensional coupled column with a single FID detector (FID2) after the ^{2}D column, as shown by the dotted line. Only a wax–chiral column arrangement for $^1D_1 + {}^1D_2$ was employed in this case, with a wax ^{2}D column. A modulator M is located between the ^{1}D columns and the ^{2}D column.

(i) a single GC column ($^1D_1$), with a single FID (FID1);
(ii) a dual GC column ($^1D_1 + {}^1D_2$), with a single FID (FID2);
(iii) a dual GC column ($^1D_1 + {}^1D_2$), with a dual FID (FID1 + FID2);
(iv) a dual GC column as first dimension ($^1D_1 + {}^1D_2$), with a single column second dimension (^{2}D) and a single FID (FID2), for GC × GC operation.

Fig. 2 (System A) describes a one-dimensional system even when comprising two columns, since the columns are essentially directly connected and there is no heart-cutting or other process at their interface. Fig. 2 (System B) describes a comprehensive 2D system, since the modulator performs the classical function of subsampling the first dimension effluent into the ^{2}D column for rapid separation.

For 1D analysis, a variety of single chiral columns, and two different wax-type columns were used. For dual column operation, the wax and chiral columns were swapped around as required with either wax–chiral or chiral–wax arrangement. For GC × GC operation, it was decided to choose the wax–chiral column arrangement for ($^1D_1 + {}^1D_2$), with both short (1.1 m) Stabilwax and longer (3.1 m) SolGel wax columns as ^{2}D, each of 0.1 mm i.d. and 0.1 μm d_f. The longer column provided improved separation in the second dimension. Table 1 presents an overview of different experiments that were conducted for the different columns and system arrangements. The most important variables are temperature setting and retention times, and these can generally be found in the figure legends and from the chromatographic traces. Thus either pressure or linear carrier flow rates are only of value as a means to change overall retention.

2.6. Data conversion

Data were converted to .csv format, and exported in ascii format for processing using Origin (Microcal Software, Northampton, MA, USA). Contour plots were displayed using Transform software (Fortner Research, VA, USA).

Table 1
Details of experimental arrangements investigated, and conditions employed.

Experiment	Compounds
1D GC; 5890 GC; BP5 and wax columns	Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime, and phenylacetaldoxime
1D GC; GC-17 GC; single column; chiral columns and wax columns	Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime, and phenylacetaldoxime
1D GC; GC-17 GC; dual columns; wax–chiral & chiral–wax columns	Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime
1D GC; dual FID GC-17 GC; dual columns; wax–chiral & chiral–wax columns	Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime
GC × GC; 6890 GC; dual ^{1}D columns (wax–chiral) and ^{2}D wax column;	Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime

Conditions: Generally conditions were varied over a range of isothermal temperatures from 70 °C to 150 °C, and pressure settings were generally varied over the range from 10 psi to 50 psi. Resulting flow rates depend upon pressure, column lengths and i.d., and the temperature. In general, neither the linear carrier flow rate nor pressure setting are critical in determining the extent of reaction, but rather the temperature setting and the time on the column (as given by the retention time of the species) are the critical parameters. Hence for ease of presentation, the pressure settings will generally be provided.

3. Results and discussion

3.1. Batch heat-treatment of heptanaldoxime and phenylacetaldoxime

Samples of heptanaldoxime and phenylacetaldoxiome were heated in ethanol or 1-pentanol solvent at 90 °C or 100 °C, respectively for a range of times (0–4 h, then fully equilibrated, or 0–7 h, respectively) in order to promote the thermodynamic equilibration in the mixture. If the isomers are at equilibrium at this temperature chosen, then no change in isomer ratio will be expected. In case of heptanaldoxime which was not bulb-to-bulb distilled, heating produced a marked alteration in the ratio observed, and eventually the isomer ratio was ca. 1.4:1.0 compared with an initial ratio of 0.06:1.0 which is that produced by the synthesis reaction of the compound. Bulb-to-bulb distillation essentially produced a product that was equilibrated and so was found to be unchanged by heating in ethanol for an extended period. A similar observation was noted for phenylacetaldoxime. Further work will not be reported for phenylacetaldoxime, due to the relatively high temperature of elution of the compound, that leads to substantial interconversion.

3.2. Relative elution and interconversion on single column systems

First, the chiral column was found to not generate the characteristic isomerism profile, and rather only separates the compounds. The chiral column will resolve both E and Z isomers, and may do this more effectively than found for the SolGel wax or the BP20 wax phases. For chiral molecules, it is generally found that the E isomers are resolved into their (R) and (S) forms better than the Z isomers are. For instance, for 2-methylbutraldehyde oxime on the chiral phase at 90 °C and 20 kPa H_2 pressure, the R_s of E isomers was found to be about 8.5, whereas for the Z isomers R_s was only approximately 1.2–1.5. The Z isomer normally elutes earlier than the earliest eluting E enantiomer. In contrast, for 2-methylpentanaldehyde oxime the Z isomers often elute after the first E enantiomer. Indeed, the Z isomers appears to exhibit a much greater change in retention factor as temperature is varied, such that it can elute earlier than the first E enantiomer, and then coelute with this isomer, and eventually can coelute with the later of the E enantiomers as the temperature is increased (see Section 3.4.2). This is more pronounced for the 2-methylpentanaldehyde oxime compound than for the 2-methylbutraldehyde oxime.

An additional observation is that low polarity phases appear to produce very small extents of isomerisation. Thus the BPX5 phase is able to resolve the E and Z isomers but little interconversion is seen. The wax-type (polyethylene glycol) phases, SolGel wax and BP20, lead to much greater interconversion. The chiral phases tested likewise tend to lead to negligible isomerisation, presumably because the chiral selector is dissolved in a low polarity polymer phase. But only the Mega phase was able to give effective resolution of the enantiomers of the E and Z isomers and so was chosen for further study. Unfortunately, there does not seem to be a phase that simultaneously is able to resolve the geometrical isomers and enantiomers, with also a strong tendency to generate isomerisation. This would be expected for a phase that had the cyclodextrin embedded in a polar wax-type phase, and columns with this characteristic do not seem to be widely available. This is perhaps surprising, given that wax-type phases are compatible with essential oil components, and these compounds often exhibit chirality, with a need to estimate chiral composition of the compounds. Thus the approach reported here is to join a chiral to a wax-phase column, so that both chiral resolution and interconversion can be observed, though this is a sequential process rather than simultaneous.

On an achiral column, the chiral molecules lead to only two peaks, E and Z, with almost negligible interconversion barrier under conditions which do not promote this process. For 2-methylbutyraldehyde oxime the Z isomer elutes rather later than the E isomer, so the relative retentions of Z and E are reversed on these chiral and achiral columns.

The effect of increased oven temperature (T) on extent of interconversion, for the chiral 2-methylbutyraldoxime compound on the wax column, can be seen in Fig. 3. Even at 80 °C (Fig. 3(A)) there is evident interconversion, and at 150 °C (Fig. 3(D)), the distribution resembles a smooth but broad band, with no evidence of the E and Z antipodes. This corresponds to rapid interconversion. However interconversion is not so rapid that the band then becomes a very narrow peak of similar magnitude to a non-interchanging internal standard which would imply $t_{RE} \sim t_{RZ}$.

3.3. The dual column system

There are three approaches which can be taken to interpretation of the results of the dual column system.

(i) decide what happens to the Z isomer;
(ii) decide what happens to the E isomer;
(iii) decide how to treat the interconversion region on the first column, as it enters then is eluted from the second column.

In this case it is informative to study the effect of each column separately. The various studies below report such observations.

The two columns perform different functions; the chiral column gives enantiomer separation and E/Z separation, but little isomerisation; the wax phase is chosen because it apparently produces a much larger extent of isomerisation, but also gives E/Z separation.

The assumption here is that E/Z isomerisation occurs only within the particular enantiomer (R) or (S), i.e. (S)E ⇆ (S)Z or (R)E ⇆ (R)Z, and E/Z isomerisation is not accompanied by enantiomerisation.

The dual column arrangement used here therefore will comprise a chiral column and a wax column, coupled in either order. The dual column arrangement was first used to analyse achiral oximes in order to facilitate understanding of the processes that occur with-

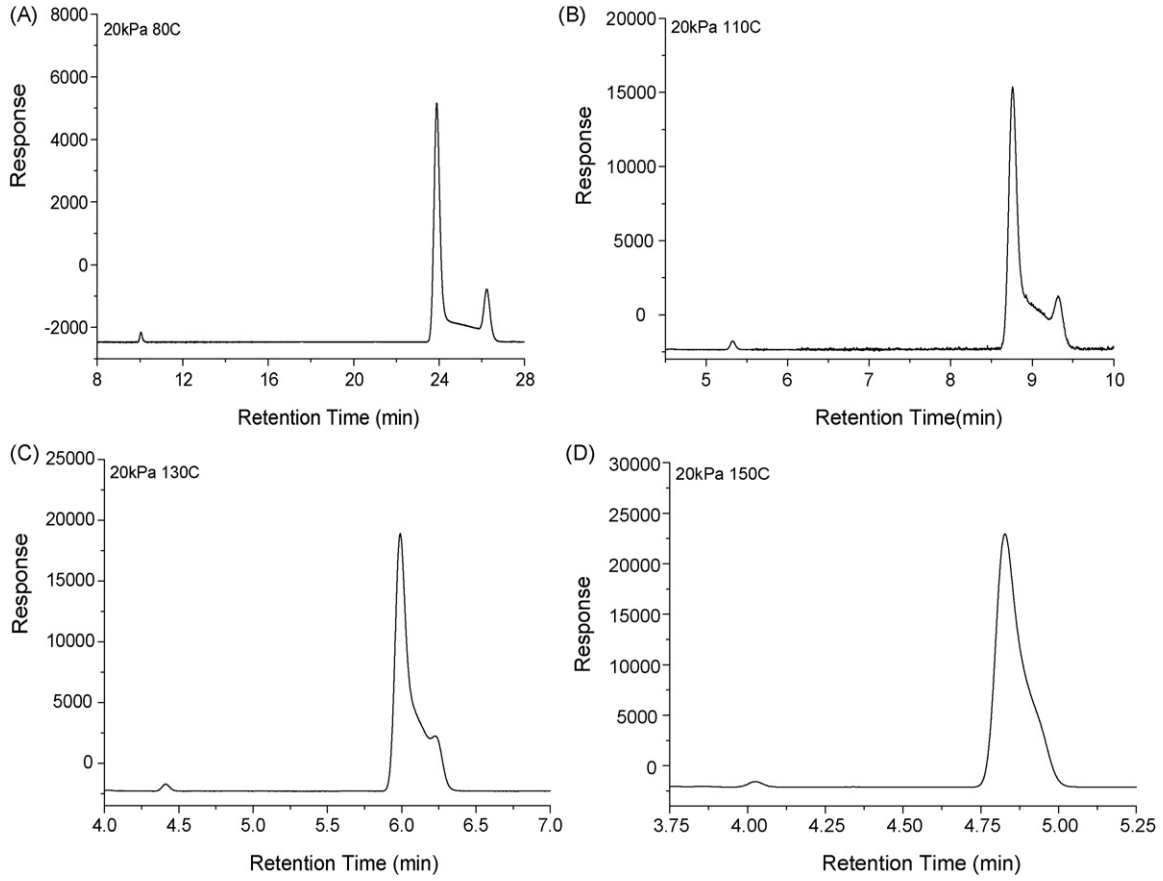

Fig. 3. Isobaric (20 kPa) 2-methylbutyraldehyde oxime analysis on a wax column. Isothermal oven temperatures are (A) 80 °C; (B) 110 °C; (C) 130 °C; (D) 150 °C.

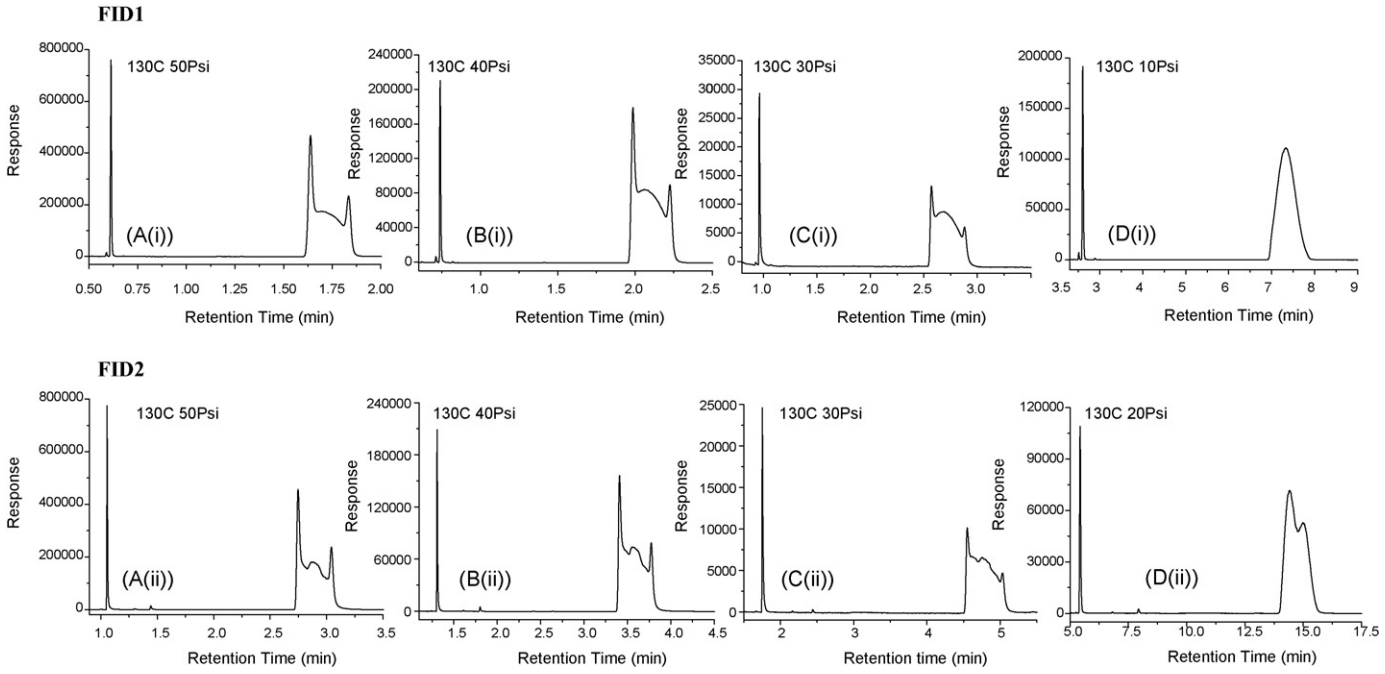

Fig. 4. Isothermal (130 °C) heptanaldoxime analysis. Dual detector wax–chiral column system, respectively. (i) FID1 detector (DET-1); (ii) FID2 detector (DET-2), carrier gas pressures are (A) 50 psi; (B) 40 psi; (C) 30 psi; (D) 10 psi. As an indication of the linear carrier velocities in each case, these were estimated to be 191, 97, 74 and 25 cm/s, respectively.

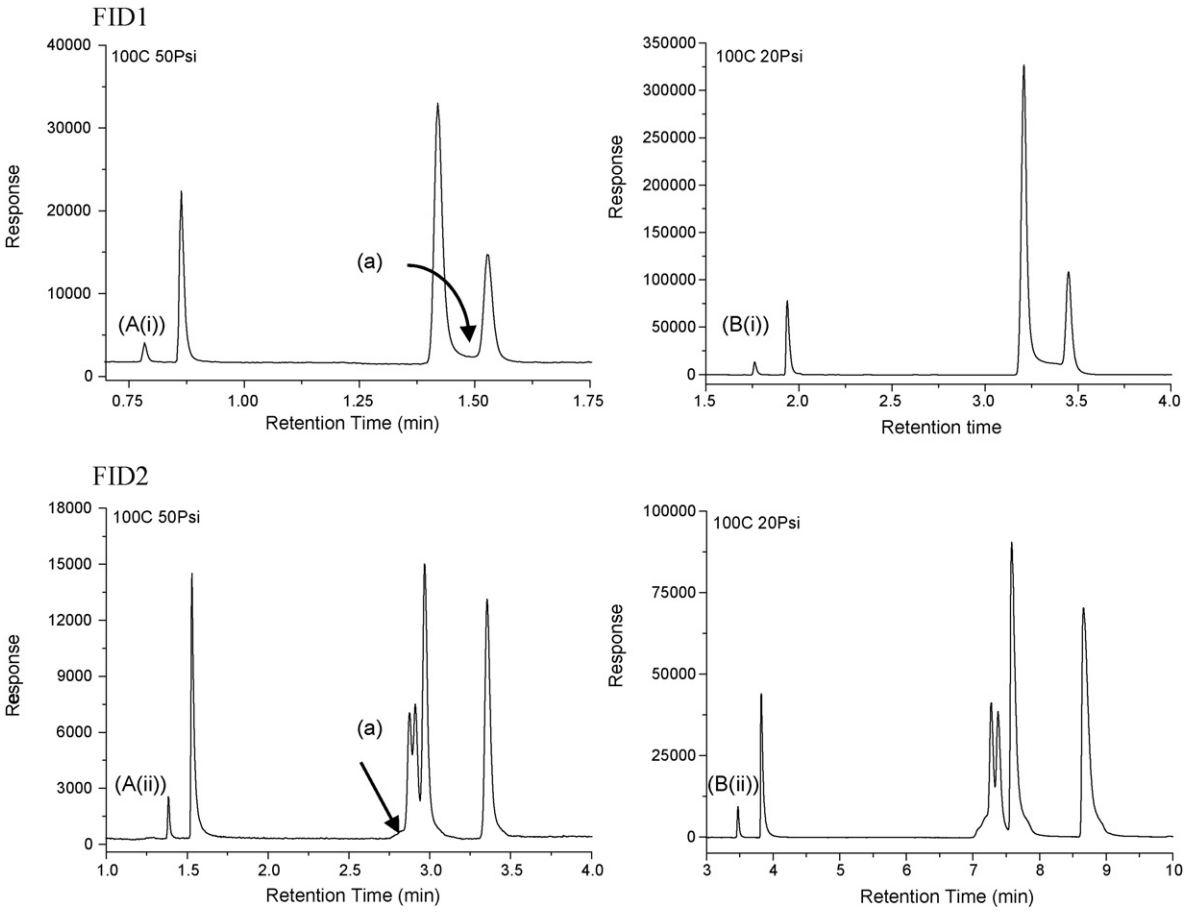

Fig. 5. Isothermal (100 °C) 2-methylbutyraldehyde oxime analysis. Dual detector wax–chiral column system. (i) FID1 detector; (ii) FID2 detector. Carrier gas pressures are (A) 50 psi and (B) 20 psi. (a) Indicates the interconversion region which for data in (ii) shows this region moves earlier than the Z isomer peaks.

out the complexity that comes with chiral separation, then chiral oxime compounds were studied. The need for both wax–chiral and chiral–wax columns to be tested is due to the different types of processes that can be observed on each column. A detector is provided between the columns in some experiments, and the dual FID system was developed when it was decided that a separate evaluation of the effect of each column on the separation was required. For the GC × GC experiment (see Section 3.4) the most appropriate or effective column order could then be selected.

3.3.1. Achiral compounds

3.3.1.1. Wax–chiral arrangement. The wax column will separate the E/Z isomers, and depending on conditions of temperature (T) and time (t) spent in the column, promote interconversion to varying extents.

The chiral column will then

(i) separate the individual E and Z enantiomers according to the retention factor of each; and
(ii) cause the interconversion region to move apart as specific bands. Here it cannot be stated that the chiral column will 'resolve' the interconversion region, because this is a broad overlapping band, and it is possible that the broad E and Z bands that comprise the interconversion zone will not be fully resolved.

Fig. 4 is an example of the achiral compound heptanaldoxime that progressively undergoes an increasing extent of isomerisation as the carrier flow (as indicated by the pressure setting) is decreased from 50 psi to 10 psi). The first column (wax) reveals a smooth distribution comprising the interconversion zone, which reflects the type of 'plateau' conventionally found for this system and as seen in Fig. 1. The trace at the end of the second column (chiral) is now no longer a smooth plateau shape, but is distorted by the separation that the chiral column generates for the interconversion zone (see Fig. 4(C)(ii)). The distortion will result from the specific retentions of the E and Z isomers comprising the interconversion zone, on the chiral column. Note that even though there is considerable interconversion on the wax column, there is little additional interconversion on the chiral column, as indicated by comparison of Fig. 4(C)(i) and (C)(ii); the E and Z antipodes change very little in respect of their areas, compared to the total area of the band. The relative retentions of the E and Z isomers do not appear to have varied greatly on the chiral phase, but Z elutes slightly closer to E. For the wax column, $k_E \sim 1.65$, and $k_Z \sim 1.98$; for the chiral column, $k_E \sim 1.6$, and $k_Z \sim 1.7$. Fig. 4(D)(i) generates a single peak, although its width (~1 min) implies that there is still considerable influence of the E and Z isomers in defining the total width. Fig. 4(D)(ii) shows the E and Z isomers delivered to the chiral column, but now some separation occurs (without additional interconversion). The width of the band is now about 2.5 min.

3.3.1.2. Chiral–wax arrangement. The chiral column will cause E/Z separation, but little interconversion. Then on the wax column the individual E and Z isomers will undergo an isomerisation process, but essentially now on physically resolved isomers from the first (chiral) column. Thus an observation somewhat like a decomposition process [1] might be expected due to the prior resolution

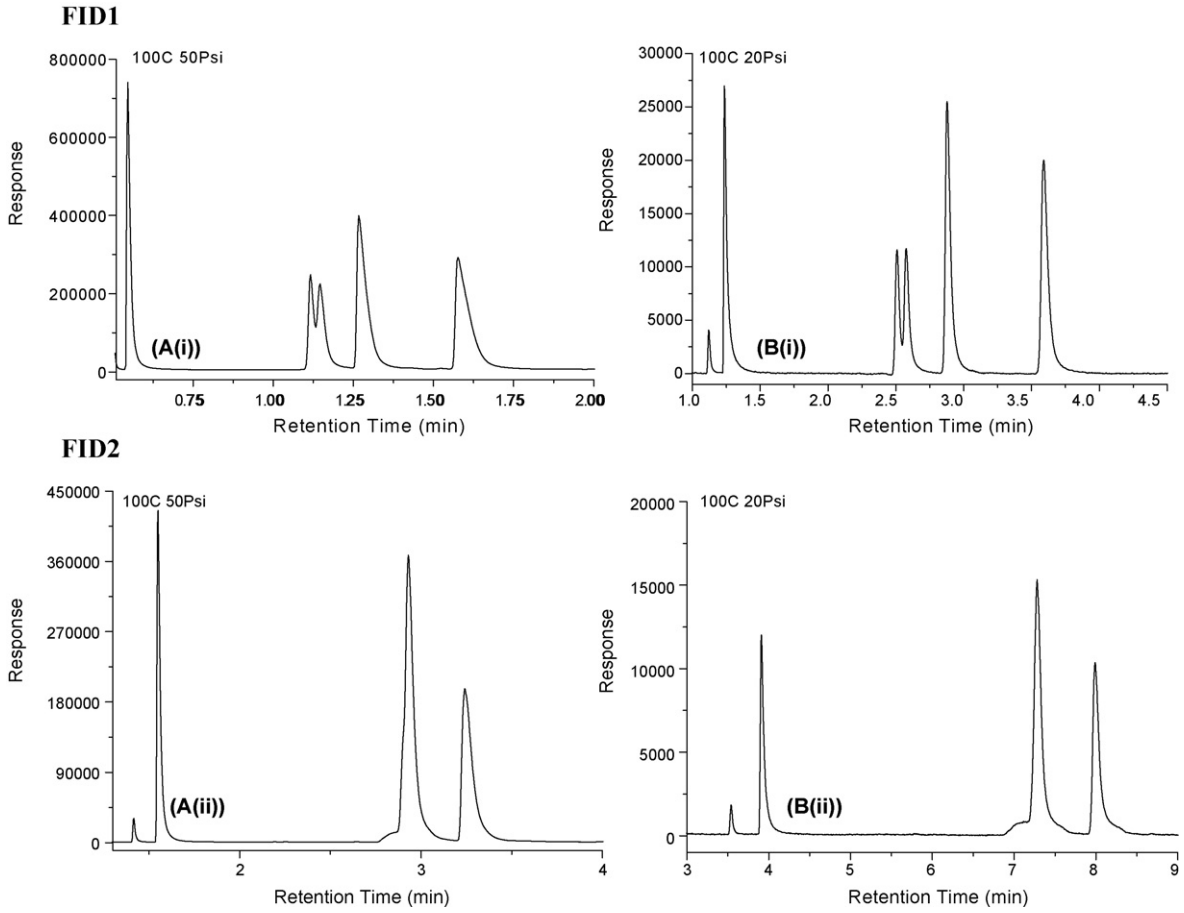

Fig. 6. Isothermal (100 °C) 2-methylbutyraldehyde oxime analysis. Dual detector chiral–wax column system. (i) FID1 detector; (ii) FID2 detector. Carrier gas pressure at (A) 50 psi and (B) 20 psi.

on the wax column, rather than the more familiar reversible interconversion process of the oximes. The extent of resolution of the E and Z isomers on the wax column will most likely be different to that of the input distribution from the chiral column. This will be determined by the individual retention factors on each column.

This means it should be possible to consider this to be a sum of the effect of the wax column on the E isomer, and the effect of the wax column on the Z isomer. The actual distribution will therefore not be the same as that for the analysis of E and Z on a single wax column but will more likely be a complex linear combination of the two effects.

3.3.2. Chiral compounds

3.3.2.1. Wax–chiral arrangement. The process on the wax column is the same as for the achiral compound above (E/Z isomer separation, with interconversion). Now, when the chiral compound reaches the chiral column, each of the E enantiomers ((R)E and (S)E) and Z enantiomers will commence to resolve. This is shown in Fig. 5. The swapping of positions of E and Z is clear in this figure. The chiral 2-methyl butyraldehyde oxime molecule exhibits a small extent of interconversion on the wax column at 100 °C (Fig. 5(A)(i) and (B)(i)) and only a minor plateau is given even at 20 psi. The chiral column selectivity towards the E and Z isomers is readily seen to vary greatly from that of the SolGel column, as the isomers swap positions with the Z isomer now showing a large reduction in relative retention on the chiral phase. The relative resolution of the two enantiomers for E are also very much greater than that for the Z enantiomers. Thus for Fig. 5(A)(i) and (B)(i) R_s is from 3 to 4, whilst for (A)(ii) and (B)(ii) R_s of the (R,S)E enantiomers is from 7 to 8, and for the Z enantiomers, R_s is <1.0.

There appears to be a small shoulder (marked with the arrow (a) in Fig. 5(A)(ii)) preceding the Z isomers, which is taken to arise from the Z isomer zone at the interconversion region marked (a) in Fig. 5(A)(i), moving to now be earlier than the Z enantiomers of the unconverted compounds. Likewise the small shoulder after the E enantiomers will be the E enantiomers of the interconversion region moving to a later retention than the non-converted E enantiomers. This is better seen in Fig. 5(B)(ii).

3.3.2.2. Chiral–wax arrangement. The chiral compounds will be resolved into (R) and (S) species (here, each of E and Z will be racemic) so now two pairs of peaks (R,S)E and (R,S)Z will be obtained. Note that E and Z show markedly different chiral resolutions, and often (R,S)Z may partially overlap one enantiomer of E. Then on the wax column, (R)Z and (R)E will interconvert, and also (S)Z and (S)E, but again from a starting basis of an input distribution that has already resolved the E and Z isomers.

Thus it is necessary to identify and interpret (i) the peaks that interconvert, and (ii) the extent of interconversion and how it is displayed in the final chromatogram.

Following the use of the system shown in Fig. 2 (System A) for the non-modulated dual column–dual FID system, Fig. 2 (System B) arrangement will be tested, but with only a single detector at the outlet of the triple column arrangement.

To some extent, the experience with the achiral compound will inform the understanding of interpretation of such a process. It is

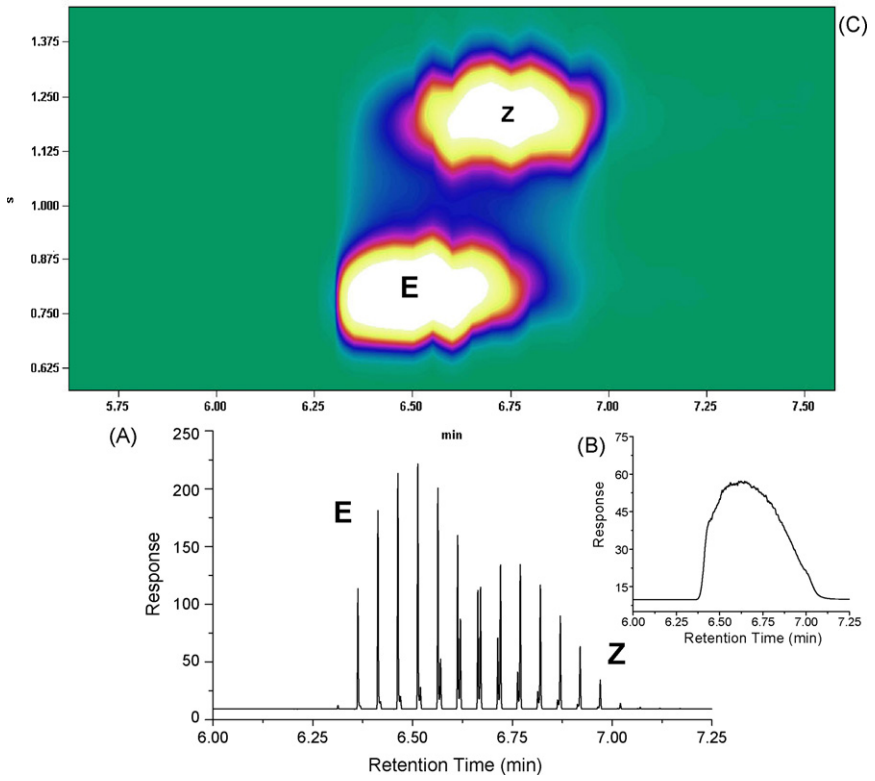

Fig. 7. Isothermal (140 °C) GC × GC heptanaldoxime analysis. P_M = 3 s; 5.0 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in A.

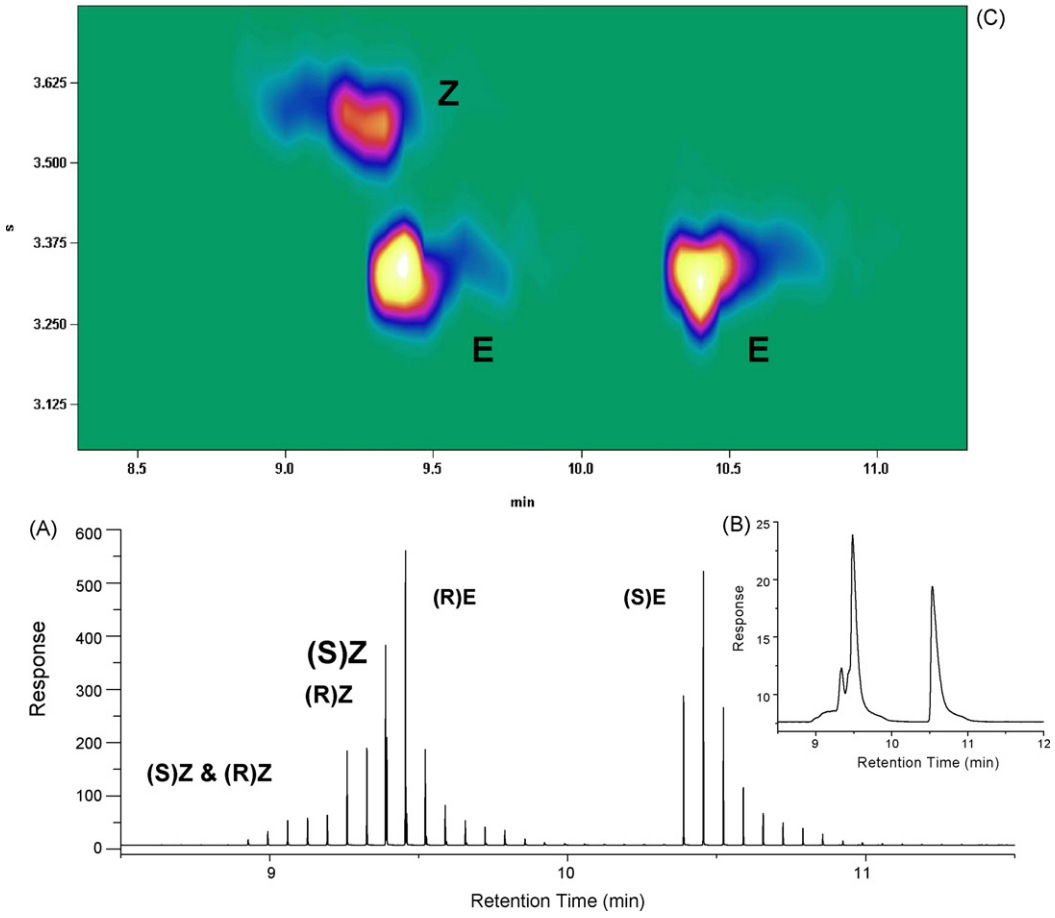

Fig. 8. Isothermal (100 °C) GC × GC 2-methylbutyraldehyde oxime analysis. P_M = 4 s. Chiral–wax column system. 1.5 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

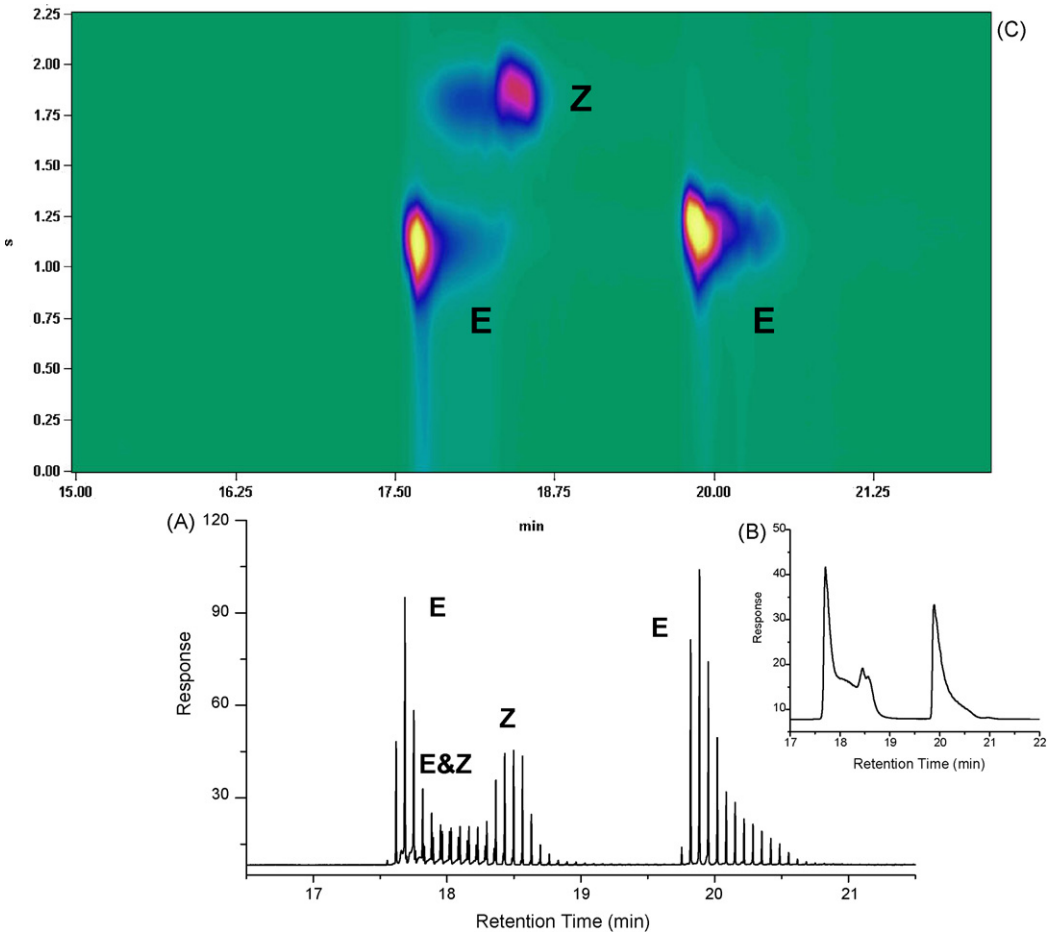

Fig. 9. Isothermal (110 °C) 2-methylpentanaldehyde oxime analysis. P_M = 4 s; 1.5 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

important to note that the interconversion could lead to a product that has either a greater or lesser retention on the second column, and this will lead to different presentations of the overall distribution. Since often the Z isomers are not well resolved from one of the E isomers, this will make the interconversion process difficult to distinguish with certainty when using 1D GC. But only one E isomer will be affected by overlap with the Z isomers. The Z isomers also exhibit small resolution on the chiral column, so this can also make it difficult to recognize the individual (R)Z and (E)Z isomers. Fig. 6 shows this. The Z isomers now overlap the earlier E enantiomer after passage through the wax column. At T = 90 °C, the Z peaks elute just before the E peak. At 110 °C, one of the Z peaks elutes just after the E enantiomer (see Section 3.4.2).

3.4. Comprehensive 2D GC

Previous reports have revealed interesting chromatographic data from the use of GC × GC for interconverting species [23–25]. Based on prior data, it was decided to choose a wax–chiral column arrangement for GC × GC experimentation to have first separation of isomers with interconversion, followed by chiral resolution, where applicable.

3.4.1. Achiral compound

In the present case, only a wax–chiral column set was employed for ($^1D_1 + {}^1D_2$), with a wax column as the ^2D column. Fig. 7 is the GC × GC result for the achiral heptanaldoxime compound, and so the 1D insert shown in Fig. 7(B) should be similar to the chromatographic result shown in Fig. 4(D)(ii). Given that a slightly higher oven temperature was used here, and that there is no chiral separation for the achiral compound, so only a broad peak is obtained. Fig. 7(A) shows that at 140 °C, the heptanaldoxime isomers show considerable interconversion having a broadened distribution on the ^1D column set. There is almost baseline resolution of E and Z on the ^2D column, with the first peak being the E isomer. The Z isomer elutes just after the E, as reported in Fig. 7(C); their retention difference on the ^2D column is about 0.4 s. This clearly demonstrates the ability of GC × GC to provide detailed chromatographic information on each compound compared with the apparent single broad 'hump' in Fig. 7(B). The broadened bands for both E and Z are indicative of peak broadening (coalescence) of the forth kind (according to Schurig [11]) which is a result of extensive interconversion between the isomers, but not so extensive that the peaks become rapidly interconverting on the GC time scale. Rapid interconversion would generate a peak of conventional peak width (as indicated by comparison with an internal standard). This will only happen if the rate of interconversion is extremely fast and leads to the two compounds effectively having the same chromatographic distribution constant. Whilst Schurig referred to 4 types of coalescence for enantioselective complexation GC, type 4 would appear to be generally applicable to any rapidly interconverting case.

3.4.2. Chiral compounds

Fig. 8 is the modulated (GC × GC) case for 2-methylbutyraldehyde oxime at 100 °C. Fig. 8(A) is the linear presentation of chromatographic data, i.e. the non-transformed

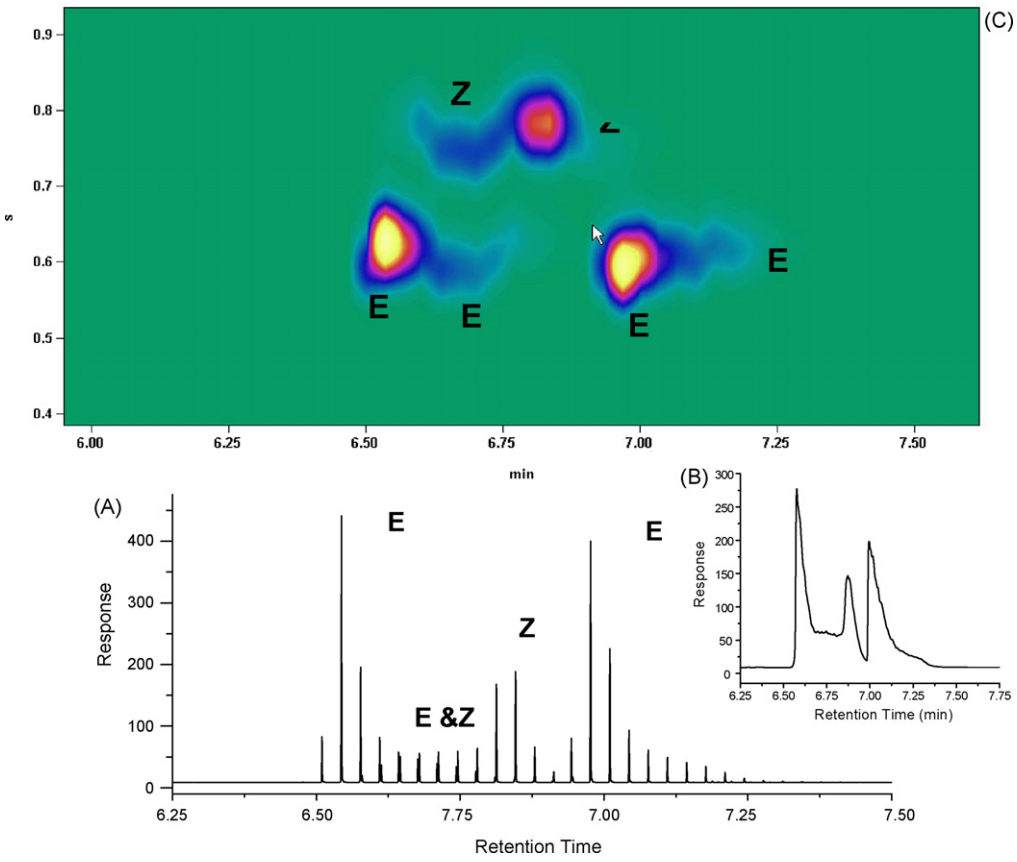

Fig. 10. Isothermal (120 °C) 2-methylpentanaldehyde oxime analysis. P_M = 2 s; 1.5 mL/min flow rate. (A) modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

detector response. The (R) and (S) enantiomers of E isomer are located at about 3.3 s on the second dimension, whilst the Z enantiomer is well resolved from E on the second column, and is located at about 3.6 s (Fig. 8(C)). Compared with the rather difficult to interpret 1D GC result in Fig. 8(B), the GC × GC reveals the individual overlapping compounds and the 'tails' that exist for each compound which should permit a rational model to be developed to describe the chromatographic result. Here it is easy to note the different effects on the E isomers and Z isomer. There appears to be a tail before the Z isomer, but the tail is after the E isomers. The tails appear to be of about the same magnitude in these two cases. The two isomers of (R,S)Z are not well resolved, and this is reflected in the apparent single peak for Z. Inset B shows the non-modulated result for the same compounds and conditions. The small preceding tail for Z (and the tail after the E isomer) is also seen in this inset, but it is not clear what species causes this tail. The 2D GC × GC plot indicates this preceding zone for Z to in fact be due to presence of the Z isomer. This result is interpreted as due to the Z isomer in the interconversion zone on the wax column eluting earlier than the unconverted Z isomer, on the chiral phase column.

Injection of 2-methylpentanaldehyde oxime at 110 °C under GC × GC operation leads to the result shown in Fig. 9. In contrast to 2-methylbutyraldehyde oxime, the Z isomer now elutes after the earlier E enantiomer. At this temperature, retention is relatively long. The Z isomer now also appears to show partial resolution into its enantiomers (see inset, Fig. 9(B)) although the transformed data in Fig. 9(C) does not seem to show this so clearly for isomer Z. However, this is confirmed by the pattern of modulation of the Z peak in Fig. 9(A) at 18.5 min, which does not exhibit the classical in-phase, 180° out-of-phase nor intermediate phase peak shape, but rather has a broader distribution that can be interpreted as emerging peak separation. The Z isomer elutes close to, but just after, the first eluting E isomer. The tailing on the peaks (earlier for Z, later for E) is still seen. There is now considerable resolution between the E enantiomers, and there is a wide baseline region between the Z isomers and later E enantiomer.

The above 2-methylpentanaldehyde oxime compound exhibits considerable shift in relative retentions of the Z and E isomers in 2D space with change in T. At 10 °C higher oven temperature (120 °C, Fig. 10) the Z enantiomers (now not well resolved on the chiral column) elute closer to the later eluting E enantiomer. The tails reported for Figs. 8 and 9 are now extended somewhat in Fig. 10. The vertical alignment of the tails (E and Z) in the 2D plot would classically be interpreted as an interconversion region between the first E enantiomer and the Z peak as shown in Fig. 10(B). The resolution provided by GC × GC operation clarifies the contribution of the E and Z isomers to this overlap region. The Z isomer here almost obeys 180° out-of-phase modulation (Fig. 10(A)), which suggests practically unresolved R and S enantiomers. Between the Z and later eluting E isomers, the response almost returns to baseline (Fig. 10(B)).

At 130 °C (Fig. 11) the (R,S)Z enantiomers, which are still unresolved, elute very close to the later E enantiomer. Now, there is no baseline resolution between the isomers on the ^{1}D column. The tails referred to above are still seen. Overall resolution of the E enantiomers has decreased significantly, as expected for higher temperature operation on a chiral column.

3.5. Development of a model for chiral interconversion in GC × GC

Based on the observations above, a schematic model of the process of wax–chiral sequential column separation of the chiral

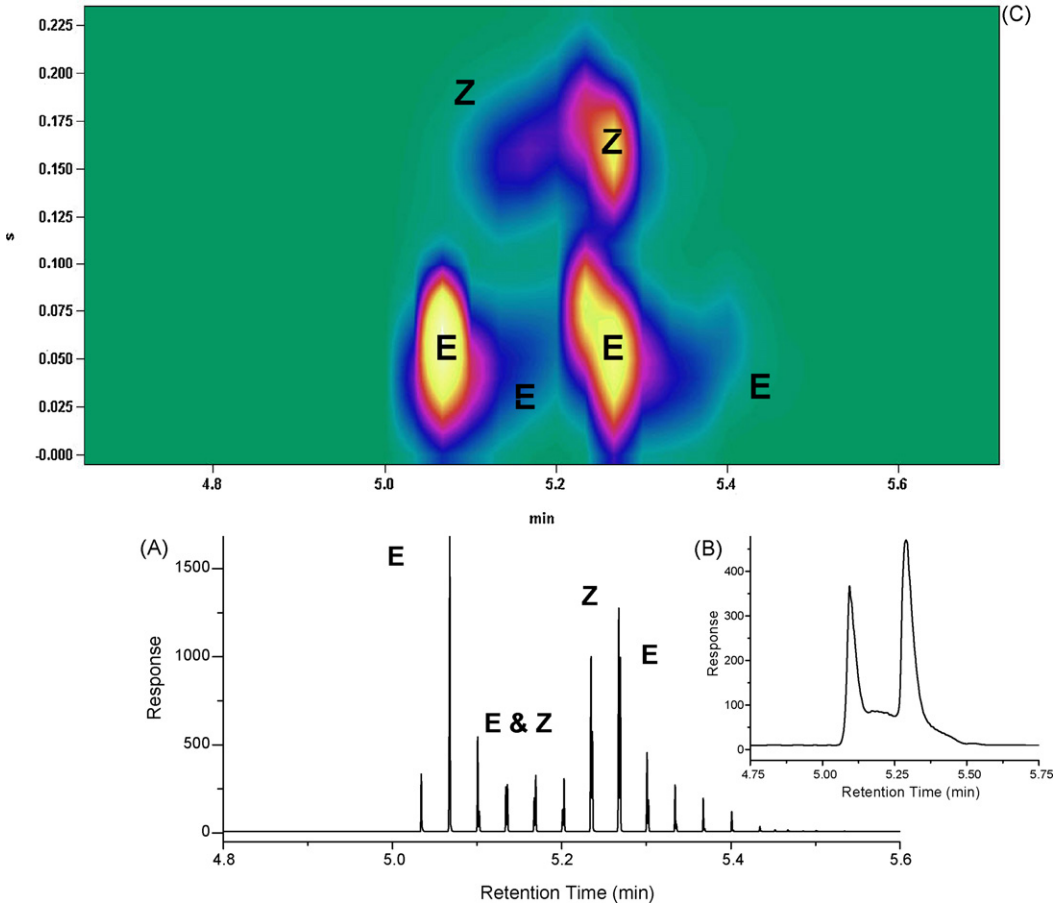

Fig. 11. Isothermal (130 °C) 2-methylpentanaldehyde oxime analysis. P_M = 2 s; 1.5 mL/min flow rate. (A) Modulated GC result; (B) Equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

compound is proposed in Fig. 12. Considering the separation on the wax column on $^1D_1$ in Fig. 12(A), the effect of chiral resolution on $^1D_2$ of each compound in turn (here taking the E and Z isomers separately) is shown in Fig. 12(B)(i) and (B)(ii). The interconversion zone is separately illustrated in Fig. 12(B)(iii), and this interconversion zone comprising (R,S)Z and (R,S)E now can be considered to move apart on the chiral phase (with Z isomers not so well resolved as stated earlier). The tailing of the Z isomer to earlier retention arises due to the interconversion zone of the Z isomer (Fig. 1), and is caused by this zone eluting earlier on the chiral phase than the original Z isomers. The tailing to longer retention of the E isomer arises from the interconversion zone of the E isomers eluting later on the chiral column. The two E enantiomers of this zone will move to locate just after their respective enantiomers. Here, the (R) enantiomer is shown to elute earlier than the (S), but this has not yet been confirmed. It is shown for demonstration purposes only. The overall distribution is then given by the summation of (B)(i)–(B)(iii), as presented in Fig. 12(C). Fig. 12(C) bears close resemblance to Fig. 8(B), and so represents but one chromatographic condition of relative retentions of E and Z isomers with the respective chiral resolution and extent of interconversion. Note that at this stage, development of a more rigorous model must await further experimentation and interpretation, and the present proposal is offered as a means to inform consideration of future, more complete description of the chromatographic results for the behaviour reported in this study.

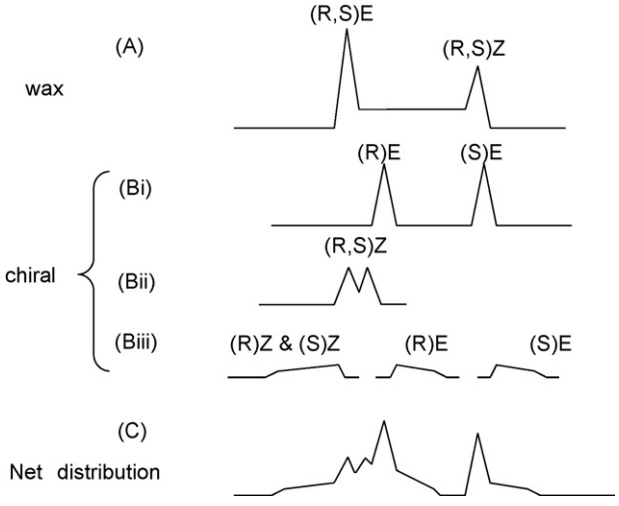

Fig. 12. Model of analysis of a chiral compound on a wax–chiral column set, in the case where there is interconversion of the compound. The two processes of what is believed to occur for the E and the Z enantiomers are isolated. (A) is the initial separation into E and Z forms on the wax phase; (B)(i) and (B)(ii) are the individual (R,S)E and (R,S)Z enantiomer separations, whilst (B)(iii) is the chiral resolution of the interconversion zone from the wax column. (C) is the net effect shown as a summation of each of the parts given in B(i)-(iii). Here, the (R) enantiomer is shown to elute before the (S) enantiomer, however this has not been proven at this stage.

4. Conclusion

A study of chiral oxime compounds which undergo E/Z isomerisation on polar (wax type) columns is reported. Since the wax column does not lead to chiral separation, a chiral (cyclodextrin) column was used to provide enantiomer separation. The chiral compounds undergo classical isomerisation on the wax column as has been seen in previous achiral studies, however the chiral column does not appear to provide isomerisation. The relative retentions and hence isomer separation of E and Z can swap on the two phases depending on the compound and conditions employed. In order to examine the effect of chiral resolution, and provide some measure of isomerisation, a dual column arrangement with either a wax–chiral or chiral–wax arrangement was implemented. In one method, an FID was located between the two columns to assist in understanding of the separate effect of the two columns. The resulting distribution exhibited a complex overlap of enantiomer separation and interconversion. The use of comprehensive 2D GC with a wax–chiral arrangement chosen, assisted in resolving the different effects that these compounds undergo.

A model is proposed to aid interpretation of the results of the process observed. At this stage, kinetic data for interconversion have not been derived for this system, however this should be possible. Since interconversion does not appear to arise on the chiral column, only kinetic data on the wax column will be obtainable. The use of a single column that simultaneously provides both chiral and E/Z resolution, along with E/Z interconversion, would be an interesting system to investigate.

References

[1] S.H. Langer, H.R. Melton, T.D. Griffith, J. Coca, J. Chromatogr. 122 (1976) 487.
[2] O. Trapp, S. Caccamese, C. Schmidt, V. Böhmer, V. Schurig, Tetrahedron: Asymmetry 12 (2001) 1395.
[3] O. Trapp, V. Schurig, J. Am. Chem. Soc. 122 (2000) 1424.
[4] O. Trapp, G. Schoetz, V. Schurig, J. Pharm. Biomed. Anal. 27 (2001) 497.
[5] O. Trapp, V. Schurig, Chem. Eur. J. 7 (2001) 1495.
[6] O. Trapp, G. Schoetz, V. Schurig, Chirality 13 (2001) 403.
[7] M. Jung, V. Schurig, J. Am. Chem. Soc. 114 (1992) 529.
[8] W. Bürkle, H. Karfunkel, V. Schurig, J. Chromatogr. 288 (1984) 1.
[9] V. Schurig, J. Chromatogr. A 906 (2001) 275.
[10] V. Schurig, TrAC, Trends Anal. Chem. 21 (2002) 647.
[11] V. Schurig, J. Chromatogr. A 965 (2002) 315.
[12] D.H. Hochmuth, W.A. Koenig, Liebigs Annalen (1996) 947.
[13] W.R. Melander, H.J. Lin, J. Jacobson, C. Horvath, J. Phys. Chem. 88 (1984) 4527.
[14] O. Trapp, V. Schurig, J. Chromatogr. A 911 (2001) 167.
[15] O. Trapp, V. Schurig, Comput. Chem. 25 (2001) 187.
[16] O. Trapp, Anal. Chem. 78 (2006) 189.
[17] J. Krupcik, P. Oswald, P. Májek, P. Sandra, D.W. Armstrong, J. Chromatogr. A 1000 (2003) 779.
[18] P.J. Marriott, Y.-H. Lai, J. Chromatogr. 447 (1988) 29.
[19] P.J. Marriott, Y.H. Lai, Inorg. Chem. 25 (2002) 3680.
[20] Y. Lai, P.J. Marriott, B. Tan, Aust J. Chem. 38 (1985) 307.
[21] S. Reich, O. Trapp, V. Schurig, J. Chromatogr. A 892 (2000) 487.
[22] J. Krupcík, J. Mydlová, P. Májek, P. Simon, D.W. Armstrong, J. Chromatogr. A 1186 (2008) 144.
[23] P. Marriott, O. Trapp, R. Shellie, V. Schurig, J. Chromatogr. A 919 (2001) 115.
[24] P. Marriott, K. Aryusuk, R. Shellie, D. Ryan, K. Krisnangkura, V. Schurig, O. Trapp, J. Chromatogr. A 1033 (2004) 135.
[25] O. Trapp, R. Shellie, P. Marriott, V. Schurig, Anal. Chem. 75 (2003) 4452.
[26] P.J. Marriott, T. Massil, H. Hügel, J. Sep. Sci. 27 (2004) 1273.
[27] J.-L. Boucher, M. Delaforge, D. Mansuy, Biochemistry 33 (2002) 7811.