Multidimensional gas chromatography investigation of concentration and temperature effects of oxime interconversion on ionic liquid and poly(ethylene glycol) stationary phases

Yada Nolvachai a,1, Chadin Kulsing a,b,1, Oliver Trapp c, and Philip J. Marriott a,*

a Australian Centre for Research on Separation Science, School of Chemistry, Monash University, Wellington Road, Clayton, VIC 3800, Australia.
b Chromatographic Separation and Flavor Chemistry Research Unit and Center of Molecular Sensory Science, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
c Department Chemie, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, Haus F, 81377 München, Germany.

Submitted to: Analytica Chimica Acta

* Corresponding author: Tel.: +61 3 99059630; fax +61 3 99058501;
Email: Philip.Marriott@monash.edu

† These authors contributed equally to this work.

Running Head: MDGC Investigation of Oxime Interconversion

Keywords

2D interconversion; Deans switch; isomer ratio; cryogenic modulation; multidimensional gas chromatography; on-line isomerisation.
Abstract

This study develops analytical approaches using comprehensive heart-cut multidimensional gas chromatographic (H/C MDGC) to experimentally investigate effects of concentration and temperature on peak shapes and on kinetic analysis of E/Z isomerisation of oximes which undergo various extents of interconversion on 1D and 2D separation columns. Different stationary phase combinations were investigated. An SLB-IL76 1D column results in good separation of the isomers but with negligible interconversion (1Dsep). Precise targeted heart-cuts (H/C; 3–15 s windows) and cryogenic trapping of selected sections eluting from this 1D column allows well controlled E/Z isomer ratios to be delivered to a 2D poly(ethyleneglycol) (PEG) phase column. This phase catalyses oxime interconversion, with separation; this is termed 2Dinter. This column set describes a 1Dsep × 2Dinter arrangement. The interconversion forward rate constant ($k_{1}^{UE}$, calculated by using DCXplorer) was found to be independent of the %E isomer (i.e. first order kinetics); $k_{1}^{UE}$ increased at higher temperature (increased Gibbs activation energies, $\Delta G_{app}$).

In addition, a long 1D SLB-IL111 column gave oxime separation with interconversion (1Dinter). Comprehensive multiple H/C of the zone performed on the 2D PEG (2Dinter) at 40, 60 and 80 °C resulted in a 2D presentation (1Dinter × 2Dinter) with a unique rectangular display of interconversion from each H/C pulse with varied isomer concentration ratios. Data for $k_{1}^{UE}$ from 1DGC analysis suggests underestimation of $k_{1}^{UE}$ on 1Dinter SLB-IL111 from the 2D result. The $k_{1}^{UE}$ value on 2Dinter PEG column is more reliably evaluated by summation of all the 2Dinter profiles rather than that obtained from the average value for the individual H/C pulses.
1. Introduction

Online molecular transformations in gas chromatography (GC) are known to cause distortion of peak shapes e.g. strong peak broadening, and overlapping or incompletely resolved peaks (peak coalescence) [1] which reduces separation efficiency.[2-4] Investigation of these phenomena by ‘dynamic chromatography’ [5-10] is useful for evaluation of kinetic and thermodynamic parameters for volatile compounds, such as enantiomerisation, E/Z isomerisation, rearrangement or dehydration of aldehydes, unsymmetrically substituted ketones and imines. [2, 3, 11, 12]

Interconversion of E/Z oxime isomers has been well investigated. Although unlikely for oximes to interconvert in the gas phase, the presence of a stationary phase such as poly(ethyleneglycol) (PEG) enhances the rate of interconversion, detected as separated isomer peaks connected with a characteristic plateau comprising molecules that have undergone an isomerisation event during the separation. [13] Oxime interconversion in the GC stationary phase via sp-hybridisation of the nitrogen atom with the C–N–O bond being ~180° in the transition state may arise, [14, 15] or rotation around the C–N bond axis facilitated by polarisation of the C–N bond to result in E/Z isomerisation. [14, 15] Several approaches have investigated on-line interconversion and reaction during separations to determine rate constants and the energy barrier for the reactions. [2, 11]

Effects of isomer concentration ratios on the interconversion rate have not been intensively investigated, most likely due to unavailability of individual E or Z isomers.

Multidimensional gas chromatography (MDGC) is a high resolution separation technique for identification of volatile analytes in multicomponent samples. [16] This study uses heart-cut (H/C) MDGC with two columns, providing different separation mechanisms, connected sequentially with a Deans switch (DS) that offers an effective H/C process. [17] The DS alters the 1D flow between a short deactivated fused silica capillary to an FID, and the 2D column. In this manner, both 1D and 2D separations may be examined. For multiple H/C analysis, the time between one H/C ‘window’ and the next window should be sufficiently long in order to allow complete elution of all H/C analytes without wraparound (mixing up the peaks in one H/C with the next H/C analysis). [18] Addition of a cryo-focusing device allows trapping of H/C pulses, reduces peak broadening during transfer of pulses from 1D to 2D columns, and enables multiple injection analysis, whilst improving limit of detection.
On-line interconversion in comprehensive two-dimensional gas chromatography (GC×GC) was observed where long 1D and short 2D columns were employed in order to perform 2D analysis in a single run. [2, 13, 19, 20] Whilst interconversion on 1D was readily observed, the study of interconversion on 2D has not been clearly observed due to the method requirement to use a short 2D column, with the separation timeframe being too short to clearly observe interconversion. However, this process occurs in practical separation [21] and needs further investigation to understand separation peak shape and establish reliable approach for kinetics evaluation. In order to promote interconversion in both dimensions, an option is to apply a long 2D column, but this will result in oversampling in GC×GC, with loss of detail and interconversion information for the 1D separation. Thus, multiple H/C 2DGC is preferred since this allows the use of both long 1D and 2D columns with minimum resolution loss. [18, 21]

This study develops comprehensive H/C MDGC for column selection, investigation of concentration effects on oxime interconversion and simultaneous investigation of interconversion in both 1D and 2D separations employing ionic liquid (IL) and PEG columns, respectively. Reliability in kinetic and thermodynamic calculation for interconversion in each dimension in the comprehensive MDGC result by using the unified equation based approach [22] was investigated, and the 2D interconversion results for different 1D and 2D temperature (T) is discussed.

2. Theoretical

Oxime interconversion in GC can be described by considering individual chromatographic and kinetic events occurring in each theoretical chromatographic plate. After injection, three main steps occur in each plate, over the length of the separation column, until analytes reach the detector. These steps are: distribution of the isomers A and B between mobile and stationary phases; relative isomerisation process in each phase; and shifting of the mobile phase to the next plate. These steps are captured by sets of distribution constants of each isomer (K_A and K_B governing retention of each isomer), and rate constants of interconversion in carrier gas and stationary phases (k_{M,A}, k_{M,B}, k_{S,A} and k_{S,B}). [23] (Note that these k values are not to be confused with retention factors). The separation/interconversion constants define the overall peak shape. Instead of two separated E/Z isomer peaks, online conversion of one isomer into the other at
some position in the column, causes different manifestation of the overall isomer elution pattern in the chromatogram. A slower moving isomer can be converted to a faster one, or vice versa, leading to apparent distortion, or peak fronting or peak tailing. These events within the timeframe of the chromatographic experiment can cause a typical characteristic plateau formed between the two peaks, or even collapse into an unresolved (broad) band.

Evaluation of the kinetic and thermodynamic parameters for \( E/Z \) interconversion can be performed by using several approaches. Classical kinetic studies determine each isomer concentration after eluting from the stationary phase, whilst comparison of experimental peak shapes and retention times with simulation of chromatographic results may be directly obtained by numerical or analytical solution of sets of differential equations based on rate of reaction, mass balance and diffusion principles. [11] Between these two extremes, the theoretical plate model, stochastic model, approximation function or the unified equation [22-24] can be applied, allowing approximation of kinetic parameters from the chromatographic results. The use of the unified equation can evaluate reaction rate constants of either reversible or irreversible (pseudo-)first-order reactions of processes occurring in dynamic chromatography and on-column reaction chromatography. This approach assumes the overall peak shape can be separated into the non-interconverted and interconverted parts which are treated differently by using time dependent Gaussian and probability density summation functions, respectively.

The solution of the unified equation allows direct calculation of the forward rate constant \( k_1^{\text{UE}} \) for reaction \( A \rightleftharpoons B \) from chromatographic parameters such as retention times of isomer A and B \( (t_{R,A} \text{ and } t_{R,B}, \text{ where } t_{R,B} \geq t_{R,A}) \), peak width at half height \( (w_h, \text{ captured here by peak standard deviations } \sigma_A \text{ and } \sigma_B) \) and relative plateau height \( (h_p) \) of the interconverted isomers according to the relationship [25]
\[
\begin{align*}
137 & \quad - \frac{1}{t_R} \ln \left( B_0 e^{\frac{\Delta_\omega k_1^u t_R}{\theta_0}} \left( \frac{100 e^{\frac{(t_B^R-t_R^A)^2}{\sigma_B^2}}}{\sigma_B^2} \frac{100 e^{\frac{(t_B^R-t_R^A)^2}{\sigma_B^2}}}{\sigma_B^2} - \frac{100}{t_R^B-t_R^A} + \frac{100B_0+A_0(100-h)_p(1+\frac{2}{\sqrt{\pi}})}{t_R^B-t_R^A} \right) \right) \\
138 & \quad \ln \left( A_0 \left( \frac{h_p-100 e^{\frac{(t_R^B-t_R^A)^2}{\sigma_A^2}}}{\sigma_A^2} + \frac{100-h_p(1+\frac{2}{\sqrt{\pi}})}{t_R^B-t_R^A} \right) \right) \right) \\
139 & \quad \left( 1 \right)
\end{align*}
\]

where \( A_0 \) and \( B_0 \) are initial concentrations and \( A_\omega \) and \( B_\omega \) are the equilibrium concentrations of the isomers A and B. Based on this equation, the software DCXplorer [25] was applied for calculation of \( k_1^{UE} \) from the chromatographic results. Application of DCXplorer is facilitated by a graphical user interface with fast simulation as applied for a range of on-line reaction studies in GC such as enantiomerisation of 1,2-dialkyl diaziridines [26] and benzothiadiazines [27] or epimerisation of tetramezines [28]. The Gibbs activation energies as a function of temperature, \( \Delta G^\ddagger(T) \), can then be calculated according to the Eyring equation

\[
\Delta G^\ddagger(T) = -RT \ln \left( \frac{k_1^{UE} h}{k_B T} \right)
\]

where \( k_B \) is the Boltzmann constant and \( h \) is Planck’s constant. By assuming that \( E/Z \) oxime interconversion is a reversible and degenerate process, the statistical factor \( \kappa \) was set in this study to be 0.5. This study applied DCXplorer for calculation of \( k_1^{UE} \), and \( \Delta G_{app} \) for oxime interconversion on SLB-IL111 (70 m) and PEG (30 m) columns at different temperatures. Example calculations of kinetics and thermodynamics parameters from the given set of chromatographic parameters are provided in the Supplementary Data Microsoft Excel file. The parameters at different ratios of isomers and temperature were also obtained on the PEG column. All chromatographic data were converted to .csv and edited with Microsoft Excel for data alignment and correction prior to construction of 2D plots for each column set generated by using Fortner Transform software (Fortner, Inc., Savoy, IL).

3. Experimental
3.1 Chemicals

Acetaldehyde oxime (97%, Sigma-Aldrich, MO) was prepared in hexane (Merck, Germany) with a concentration of 10% v/v as the sample for all experiments.

3.2 Instrumentation

Separations were performed on an Agilent 7890A GC (Agilent Technologies, Mulgrave, Australia) coupled with two flame ionisation detectors (FID). All experimental processes performed in this study are summarised in Fig. 1 with different column combinations for 1DGC, targeted H/C MDGC and comprehensive multiple H/C MDGC. Briefly, three column configurations were employed with a Deans switch (DS, Agilent Technologies) located at the end of the 1D chromatographic column, where the flow can be directed to either a short deactivated fused silica capillary (as a restrictor, required to balance the flow in H/C analysis) directly connected to FID1 or a 2D chromatographic column connected to FID2. The corresponding DS operations are ‘off’ and ‘on’, respectively. For all experiments, an inlet temperature of 230 °C and detector temperature of 250 °C were used. H2 was used as carrier gas.

3.2.1 1DGC

Isothermal 1DGC experiments were performed using three different columns at 60 °C: SLB-IL111 (70 m × 0.25 mm I.D. × 0.2 μm df; Supelco, Bellefonte, PA), SLB-IL76 (30 m × 0.25 mm I.D. × 0.2 μm df; Supelco) and Stabilwax (30 m × 0.25 mm I.D. × 0.25 μm df; Restek, Bellefonte, PA) for comparison with analysis using MDGC. In order to control the flow variation, the column configurations in 1DGC analysis were set to be the same as that in MDGC. Either SLB-IL111 or SLB-IL76 was connected as the 1D column in the H/C MDGC system as shown in Fig. 1D, with the Stabilwax 2D column. The 1D analysis for SLB-IL111 and SLB-IL76 columns can be obtained by turning off the DS, and diverting the flow to the deactivated fused silica restrictor. For 1D analysis on the Stabilwax column, the whole sample zone was trapped at the cryotrap (SGE, Ringwood, Australia) positioned after 1D separation on SLB-IL111 (Fig. 1). The sample was then released to effectively correspond to a single Stabilwax column separation. This was
performed as the benchmark 1DGC approach for the calculation of kinetics compared with the
2D analysis. The applied flow rates in $^1$D and $^2$D separations were 2 and 3 mL min$^{-1}$,
respectively, in all cases.

3.2.2 Targeted H/C MDGC

Targeted H/C analysis in the middle of the interconversion zone (Fig. 1A) obtained from the
$^1$D$_{\text{inter}}$ SLB-IL111 column (70 m × 0.25 mm I.D. × 0.2 μm $d_f$) was performed in order to select
the $^2$D$_{\text{sep}}$ column: BPX5 (30 m × 0.25 mm I.D. × 0.25 μm $d_f$; SGE), SLB-IL59, SLB-IL61, SLB-
IL76, SLB-IL82, SLB-IL100 or SLB-IL111 (30 m × 0.25 mm I.D. × 0.2 μm $d_f$; all from
Supelco), that offers adequate isomer separation without trapping, for quantification of the
isomer ratio in each H/C pulse from the $^1$D$_{\text{inter}}$ column. The applied flow rates in $^1$D and $^2$D
separations were 2 and 3 mL min$^{-1}$, respectively.

In addition, two targeted H/Cs sampled from different parts of each isomer (Fig. 1B) obtained
from $^1$D SLB-IL76 (30 m × 0.25 mm I.D. × 0.2 μm $d_f$) were performed with cryogenic trapping
prior to elution on $^2$D$_{\text{inter}}$ Stabilwax (30 m × 0.25 mm I.D. × 0.25 μm $d_f$) for investigation of the
isomer concentration ratio effect on interconversion; i.e. variable proportions of the first isomer
and some second isomer were collected together, then eluted on $^2$D. Initially, the applied flow
rates in $^1$D and $^2$D separations were 2 and 3 mL min$^{-1}$, respectively. After trapping, the $^1$D and
$^2$D flows were then adjusted to be 0.1 and 1.1 mL min$^{-1}$, respectively.

3.2.3 Comprehensive multiple H/C MDGC

Multiple H/C throughout the full interconversion zone (Fig. 1C) obtained from the $^1$D$_{\text{inter}}$ SLB-
IL111 column (70 m × 0.25 mm I.D. × 0.2 μm $d_f$) was performed both with and without trapping
at the inlet of the $^2$D column. Each H/C was further separated on $^2$D SLB-IL76 (30 m × 0.25 mm
I.D. × 0.2 μm $d_f$) for quantification of the isomer ratios in the H/C fraction ($^1$D$_{\text{inter}}$$^2$D$_{\text{sep}}$) and on
$^2$D$_{\text{inter}}$ Stabilwax (30 m × 0.25 mm I.D. × 0.25 μm $d_f$) for investigation of interconversion in both
$^1$D and $^2$D separation ($^1$D$_{\text{inter}}$$^2$D$_{\text{inter}}$). The applied flow rates in $^1$D and $^2$D separations were 2 and
3 mL min$^{-1}$, respectively, in all cases.

4. Results and discussion
GC and MDGC approaches to investigate concentration effects and to generate interconversion of E/Z oximes in both ¹D and ²D isothermal separations were developed (Fig. 1). Selection of flow rate, temperature and stationary phases were investigated in target H/C MDGC analysis. The studied MDGC systems included targeted ¹D_inter−²D_sep, ¹D_sep−²D_inter, ¹D_inter×²D_sep and ¹D_inter×²D_inter, where ¹D_inter and ¹D_sep correspond to performing separation, or interconversion with separation, on the iᵗʰ dimension, respectively. Forward rate constants (k¹ਜ) of interconversion on the ²D PEG column starting with different ratios of oxime concentrations were then calculated by using DCXplorer and the values compared. The results were processed and presented as contour plots at different T.

4.1 Column selection for separation and interconversion in ¹DGC and targeted H/C MDGC

The nonpolar column (5% phenyl methylpolysiloxane stationary phase BPX5) separates oxime isomers with insignificant interconversion in ¹DGC.[29] Some interconversion can be observed by using polysiloxane based stationary phases with lower methyl group content. Among the investigated phases, PEG was found to most effectively generate oxime interconversion in ¹DGC.[29] This column was thus selected as the focus for investigation of concentration effects on interconversion in this study, and was used as the ²D column in MDGC (²D_inter). Although the use of the Deans switch requires higher flow in the ²D column here being 3 mL min⁻¹ (compared to 2 mL min⁻¹ for ¹D flow), a T of 60 °C was found to promote some interconversion even under such high flow, as shown in Fig. 2C.

Apart from the PEG column, more highly polar IL stationary phases clearly show some oxime interconversion. However, interconversion was not observed on all the studied IL columns when a length of 30 m was used, even at low flow rate and high T. This is due to either increased barrier to interconversion on these IL phases or the elution time of the oximes is much ‘faster’ compared to the interconversion rate. These columns are thus suitable to be applied for separation with suppressed interconversion. The tricationic IL, SLB-IL76, showed the greatest isomer separation with negligible interconversion (Fig. 2B); of the IL phases, only ²D SLB-IL76 resulted in good separation of oxime isomers obtained from a targeted H/C pulse in the middle of
the interconversion zone eluted from the $^{1}$D$_{\text{inter}}$ column without cryogenic trapping as shown in Fig. 2F–K.

Separation of oxime isomers is governed by the relative phase selectivity towards the $E$ and $Z$ isomers, efficiency, and total retention. The higher polarity phases (SLB-IL82, SLB-IL100 and SLB-IL111) are expected to provide better selectivity towards $E/Z$ isomers which are cis-trans structures with different polarities (due to the different net dipole effect). For example, increasingly polar IL phases offer better selectivity towards cis-trans isomer separation of fatty acid methyl esters.[30-32] However, more polar IL phases have strong cohesion reducing accessibility of oximes into the phases (this decreases retention). As a result, isomer retention time is insufficient (<0.5 min), resulting in the observed co-elution on these higher polarity phases, Fig. 2I–K. On the other hand, less polar SLB-IL76 with weaker cohesion (and greater retention) offers better selectivity and results in better oxime isomer separation (Fig. 2H). SLB-IL76 was thus selected to be applied as either the $^{1}$D or $^{2}$D separation column in this study ($^{1}$D$_{\text{sep}}$ or $^{2}$D$_{\text{sep}}$).

Interestingly, oxime interconversion was observed on a 70 m SLB-IL111 column (but not on the 30 m column). A long column is required to increase both separation time and extent of interconversion, Fig. 2A. The 70 m column was thus selected for generation of the interconversion zone on $^{1}$D separation ($^{1}$D$_{\text{inter}}$) in the comprehensive H/C analysis employing a $^{2}$D PEG column for $^{1}$D$_{\text{inter}}$$^{2}$D$_{\text{inter}}$ analysis, or $^{2}$D SLB-IL76 column for $^{1}$D$_{\text{inter}}$$^{2}$D$_{\text{sep}}$ analysis.

4.2 Investigation of isomer concentration ratio effect on the interconversion on $^{2}$D PEG column with multiple targeted $^{1}$D$_{\text{sep}}$$^{2}$D$_{\text{inter}}$

The SLB-IL76 was used as $^{1}$D$_{\text{sep}}$ prior to interconversion on the $^{2}$D$_{\text{inter}}$ PEG column. In 1D analysis with the DS off, the $E$ to $Z$ isomer ratio of acetaldehyde oximes in the stock solution was approximately 5:9 (36.64%) based on the separated peak area ratios, Fig. 2B. Precise targeted H/C windows (with DS on for a certain period from 3–15 s) allowed sampling of two separated sections from each isomer peak in $^{1}$D$_{\text{sep}}$ to then be performed (targeted $^{1}$D$_{\text{sep}}$$^{2}$D$_{\text{inter}}$). The two sections are H/C by choosing different sampling times, to allow different amounts of $E$ and $Z$ to
be sampled, then combined at the cryogenic trap, prior to elution on $^2$D$_{\text{inter}}$. By dividing each isomer peak area into equal sections (as illustrated in Fig. 1B), a given ratio of isomers in each trapped/combined pulse can be determined and the total concentration kept constant for all the investigated pulses. Each original isomer peak was therefore equally divided into five sections resulting in 10 sections in total with the same area. As a result, the $E$ to $Z$ isomer ratios were varied as 5:0, 4:1, 3:2, 1:1, 2:3, 1:4 and 0:5 (100, 80, 60, 50, 40, 20 and 0% $E$, respectively). The $^1$D$_{\text{sep}}$ results illustrating the H/C sections were detected by FID1 in Fig. 3A–H (left; $^{1}t_{R}$) with the corresponding $^2$D$_{\text{inter}}$ results (for the H/C pulses with different isomer ratios) detected by the FID2 in Fig. 3A–H (right; $^{2}t_{R}$).

The $k_{1}^{\text{UE}}$ values for oxime interconversion on the $^2$D$_{\text{inter}}$ PEG column at different %$E$ were calculated by using DCXplorer. $k_{1}^{\text{UE}}$ values negligibly depended on %$E$ at all the investigated temperatures, see Fig. 3I. Variation from 20 to 100%$E$ also caused insignificant errors in calculation of thermodynamic parameters as shown in the plot of $\Delta G_{\text{app}}$ vs temperature in Fig. 3J. This proves that interconversion of acetaldehyde oxime on PEG phase can be considered (pseudo-)first-order reactions.

### 4.3 Quantification of isomer ratios for each H/C pulse from $^1$D SLB-IL111 by using $^2$D SLB-IL76 ($^1$D$_{\text{inter}}$×$^2$D$_{\text{sep}}$)

Comprehensive MDGC analysis including 30 H/C was performed without cryogenic trapping over the whole sample zone eluting from a $^1$D$_{\text{inter}}$ SLB-IL111 column (70 m). Each H/C pulse (with 6 s window at 60 °C) was further separated on a $^2$D$_{\text{sep}}$ SLB-IL76 column (30 m). The overall analysis can be represented by a 2D contour plot.

Analysis performed without trapping, with direct presentation of chromatograms for all the pulses without correction of $^{1}t_{R}$ and $^{2}t_{R}$, resulted in the overlaid chromatograms and the 2D plot shown in Fig. 4A. For each H/C pulse, the corrected $^{1}t_{R}$ ($x$ axis) is the H/C time; whilst, the $^{2}t_{R}$ values ($y$ axis) are total analysis times with the H/C time subtracted. The corrected $^{1}t_{R}$ vs $^{2}t_{R}$ plot is shown in Fig. 4B. The %$E$ data for each H/C pulse are shown in Table S-1, Supplementary data (quantification based on the isomer peak areas) and further used to experimentally obtain the initial concentrations ($A_{0}$ and $B_{0}$) for the kinetic calculation on a $^1$D$_{\text{inter}}$×$^2$D$_{\text{inter}}$ system. Note
that the overall 2D plot (Fig. 4B) is similar to the previously investigated oxime interconversion
in GC×GC results employing conventional ¹D PEG×²D nonpolar columns;[2] however, the ¹D
IL column with greater ²D resolution was investigated here.

4.4 Investigation of interconversion on both ¹D SLB-IL111 and ²D PEG columns

(¹D_{inter}×²D_{inter})

SLB-IL111 (70 m) and PEG (30 m) columns were employed. The entire interconversion zone
from ¹D_{inter} at 60 °C was H/C over the 6 s window without cryogenic trapping. Each pulse with
different isomer concentration ratio (Table S-2, Supplementary data) further underwent
interconversion on ²D_{inter} and revealed different plateau characteristics.

The overall ¹D_{inter}×²D_{inter} analysis resulted in a rectangular display with the four vertic es (if they
are present) representing the isomers without interconversion, and the four sides (or plateaus)
represent molecules undergoing at least one isomerisation event in ¹D_{inter} (horizontal direction)
and ²D_{inter} (vertical direction). The corresponding 2D plot with corrected ¹t_{R} and ²t_{R} is shown in
Fig. 4C. Cryogenic trapping of the H/C pulses was also performed in order to minimize peak
broadening during transfer of broad zones from ¹D to ²D columns [18, 33] with the resulting 2D
plot shown in Fig. 4D. By projection of the overall intensity profile onto ¹t_{R} and ²t_{R} axes, \( k_{1}^{UE} \) for
oxime interconversion on SLB-IL111 and PEG phases can be calculated. Although the
calculated values on the ¹D SLB-IL111 phase slightly varied outside the error bars, e.g. of
±0.3×10^{-4} s^{-1} for the repeated analysis at 60 °C, calculation on the ²D PEG phase with trapping
showed significantly higher \( k_{1}^{UE} \) value (by 1.9×10^{-4} s^{-1}) since analysis without trapping resulted
in a broader peak shape for the ²D separation, as seen by the broader peaks in the vertical
direction in Fig. 4C compared to that in Fig. 4D.

Analysis with trapping was then performed for the experiments below. A single H/C zone
resulted in small fluctuation of \( k_{1}^{UE} \) values, as shown by the plot of \( k_{1}^{UE} \) values calculated for
each H/C fraction obtained from ¹D_{inter} SLB-IL59 in Fig. 4E–I. By using \( k_{1}^{UE} \) data obtained from
¹DGC analysis as the benchmark, underestimation of \( k_{1}^{UE} \) on ¹D_{inter} SLB-IL111 from the 2D
result was observed. The corresponding \( k_{1}^{UE} \) values obtained from ¹D and ²D analysis were
9.4×10^{-4} and 4.9×10^{-4} s^{-1}, respectively. In addition, the $k_{1}^{UE}$ value on $^{2}$D_{inter} PEG obtained from the summation of all the $^{2}$D_{inter} profiles (overall projection onto the $^{2}$D axis, $k_{1}^{UE} = 9.3×10^{-4} s^{-1}$) was closer to the benchmark value (8.5×10^{-4} s^{-1}) than that obtained from the average value for the individual H/C pulse (6.6×10^{-4} ± 1.1×10^{-4} s^{-1}).

An increase in $T$ both increased the interconversion rate (due to more molecules possessing enough energy to overcome the interconversion barrier) and decreased separation space between the two isomer peaks (due to less relative interaction with the stationary phase). As a result, the rectangular area in the 2D plot was reduced or compressed at higher $T$ with higher signals (more interconversion) on the four sides of the rectangular peak shapes, Fig. 5A–I. With the use of cryogenic trapping, the rectangle dimensions can be controlled by independently increasing or decreasing interconversion $T$ in each dimension. As a result, the peak dimensions can be horizontally or vertically lengthened by increasing the interconversion temperature on $^{1}$D_{inter} (at $^{2}$D temperature being 40, 60 and 80 °C, see Fig. 5A–C, D–F and G–I, respectively) or $^{2}$D_{inter} (at $^{1}$D temperature being 40, 60 and 80 °C, see Fig. 5(A, D, G), (B, E, H) and (C, F, I), respectively).

According to the overall $^{1}$D and $^{2}$D projection approach, the associated errors in kinetics calculation for each column at a particular temperature caused by temperature variations of the other column were within 1.0×10^{-4} s^{-1} with the exception of that at 80 °C where the values were unexpectedly low due to the ambiguous determination of chromatographic parameters caused by peak co-elution. The relatively small errors suggest that, for a given 2D result, the calculated kinetics values on one column were not strongly affected by the variation of the elution profiles on the other column (e.g. variation of $^{2}$D_{inter} temperature affecting <10% of the calculated $k_{1}^{UE}$ values on the $^{2}$D PEG column at 40 °C).

5. Conclusions

A multiple targeted H/C MDGC approach was successfully applied to generate H/C pulses with a well-controlled amount of each isomer for the investigation of the isomer ratio effect on interconversion of oximes. This approach allows generation of samples with any concentration ratio from the injected equilibrium mixture, and thus does not require preparation of the pure isomer. On-line oxime interconversion on an ionic liquid stationary phase has also been demonstrated via the use of a long 70 m SLB-IL111 column counterbalancing the effect of low
retention which reduces interconversion on the 30 m IL column. An IL column has not previously been reported to promote interconversion of oximes. Investigation of a variety of different IL molecular structures or design of novel IL that might enhance interconversion would be informative.

With the developed H/C MDGC techniques, the long IL column was coupled with 2D PEG to investigate on-line interconversion in both 1D and 2D separations. These provide interesting 2D displays of the overall process; different shapes in each H/C event may be explained according to the effect of isomer concentration. The method allows different proportions of the E and Z isomers to be selected from the 1D column, and introduced to 2D, and different oven T settings permit different extents of interconversion according to the chosen stationary phase. In addition, for a given 2D interconversion result, the overall projection approach was found to be reliable for approximation of kinetic parameters on the 2D analysis. Providing that all the chromatographic parameters are clearly identified (e.g. without significant co-elution), the calculated kinetics values were slightly affected by the variation of 1D elution profiles.

Acknowledgements

We acknowledge funding from the ARC Linkage program grant LP150100465. The authors thank Supelco for provision of IL columns.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.xxxx.xx.xxx.

References


FIGURE 1.
FIGURE 2

A  

$^1D \text{IL111 (70 m)} \times ^2D \text{XX (30 m)}$

B

$^1t_R$ (min)

C

$k_{1\text{IL}(\text{IL111})} = 9.4 \times 10^{-4} \text{ s}^{-1}$  

($\pm 0.4 \times 10^{-4} \text{ s}^{-1}$)

D  

E  

F  

G  

H

I

J

K

$^2t_R$ (min)

$20 \text{ pA}$

$\text{BPX5}$

$\text{WAX}$

$\text{IL59}$

$\text{IL61}$

$\text{IL76}$

$\text{IL82}$

$\text{IL100}$

$\text{IL111}$

$0.5 \text{ to } 4.5$
FIGURE 3.
FIGURE 4

A

FID Response (pA)

0  200  400  600  800

16 17 18 19 20 21 22

$1_t_R + 2_t_R$ (min)

B

FID Response (pA)

0  200  400  600  800

2 3 4 5 6 7 8

$2_t_R$ (min)

C

$2_t_R$ (min)

WAX $k_{1}^{WAX} = 7.4 \times 10^{-4}$ s$^{-1}$

H111 $k_{1}^{H111} = 4.3 \times 10^{-4}$ s$^{-1}$

D

$2_t_R$ (min)

WAX $k_{3}^{WAX} = 9.3 \times 10^{-4}$ s$^{-1}$

H111 $k_{3}^{H111} = 4.9 \times 10^{-4}$ s$^{-1}$

E

Log(FID Response)

0  1  2  3  4

1.5 2.5 3.5 4.5 5.5

$2_t_R$ (min)

WAX $k_{1}^{WAX} = 6.0 \times 10^{-4}$ s$^{-1}$

$6.3 \times 10^{-4}$ s$^{-1}$

$6.8 \times 10^{-4}$ s$^{-1}$

$5.6 \times 10^{-4}$ s$^{-1}$

F

G

H

I

$9.3 \times 10^{-4}$ s$^{-1}$

$8.5 \times 10^{-4}$ s$^{-1}$
FIGURE 5.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>40 °C</th>
<th>60 °C</th>
<th>80 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T:</strong> 40 °C</td>
<td><img src="image1.png" alt="Image A" /></td>
<td><img src="image2.png" alt="Image B" /></td>
<td><img src="image3.png" alt="Image C" /></td>
</tr>
<tr>
<td><strong>T:</strong> 60 °C</td>
<td><img src="image4.png" alt="Image D" /></td>
<td><img src="image5.png" alt="Image E" /></td>
<td><img src="image6.png" alt="Image F" /></td>
</tr>
<tr>
<td><strong>T:</strong> 80 °C</td>
<td><img src="image7.png" alt="Image G" /></td>
<td><img src="image8.png" alt="Image H" /></td>
<td><img src="image9.png" alt="Image I" /></td>
</tr>
</tbody>
</table>

**Time: 10 seconds**

**WAX k**:
- A: 2.3 x 10^{-4} s^{-1}
- B: 4.3 x 10^{-4} s^{-1}
- C: 2.5 x 10^{-4} s^{-1}
- D: 7.1 x 10^{-4} s^{-1}
- E: 1.1 x 10^{-4} s^{-1}
- F: 6.3 x 10^{-4} s^{-1}
- G: 13.7 x 10^{-4} s^{-1}
- H: 13.6 x 10^{-4} s^{-1}
- I: 8.6 x 10^{-4} s^{-1}

**Rt (min):**
- A: 0.2 x 10^{-4} s^{-1}
- B: 4.1 x 10^{-4} s^{-1}
- C: 10.2 x 10^{-4} s^{-1}
- D: 1.0 x 10^{-4} s^{-1}
- E: 4.9 x 10^{-4} s^{-1}
- F: 10.5 x 10^{-4} s^{-1}
- G: 0.1 x 10^{-4} s^{-1}
- H: 4.0 x 10^{-4} s^{-1}
- I: 0.9 x 10^{-4} s^{-1}
LIST OF FIGURE CAPTIONS

Fig. 1. Diagram illustrating the various MDGC approaches performed in this study.

Fig. 2. Column selection experiments: isothermal 1D separation of E/Z oxime isomers at 60 °C on (A) 70 m SLB-IL111 and (B) 30 m SLB-IL76 using carrier gas flow of 2 mL min\(^{-1}\) and (C) 30 m PEG column using a flow of 3 mL min\(^{-1}\), with corresponding \(k_1^{\text{UE}}\) values calculated by using DCXplorer. The middle H/C pulse obtained from the 70 m SLB-IL111 column at 60 °C shown in A was further separated on different 30 m 2D columns using a flow of 3 mL min\(^{-1}\): (D) BPX5, (E) Stabilwax, (F) SLB-IL59, (G) SLB-IL61, (H) SLB-IL76, (I) SLB-IL82, (J) SLB-IL100 and (K) SLB-IL111.

Fig. 3. Multiple targeted H/C analysis of E/Z oxime isomers at 60 °C on \(^1\)D\(_{\text{sep}}\)  SLB-IL76–\(^2\)D\(_{\text{inter}}\) PEG with different H/C isomer ratios, A–H: (left) \(^1\)D\(_{\text{sep}}\) results at FID1 and (right) \(^2\)D\(_{\text{inter}}\) results at FID2 with plots of (I) \(k_1^{\text{UE}}\) calculated by using DCXplorer vs \(E\) content at different isothermal \(T\) being 40 (*), 60 (–) and 80 °C (×), and (J) \(\Delta G_{\text{app}}\) vs \(T\) at different %\(E\) being 20 (○), 40 (*), 50 (Δ), 60 (–), 80 (×) and 100% (o). The kinetic and thermodynamic data for all the investigated experiments with three replicates (a total of 108 \(^1\)D\(_{\text{sep}}\)–\(^2\)D\(_{\text{inter}}\) experiments) at different concentration and \(T\) are provided in Tables S-1 and S-2, Supplementary data.

Fig. 4. Comprehensive two dimensional H/C analysis of E/Z oxime isomers at 60 °C on \(^1\)D\(_{\text{inter}}\) SLB-IL111×\(^2\)D\(_{\text{sep}}\)  SLB-IL76 obtained for 30 H/C experiments without cryogenic trapping: (A) direct presentation of chromatograms for all the pulses without correction of \(1_t R\) and \(2_t R\) and (B) the corrected alignment of chromatograms with the corresponding transformed 2D plots shown as the inset to the right of each figure. The analysis on \(^1\)D\(_{\text{inter}}\) SLB-IL111×\(^2\)D\(_{\text{inter}}\) PEG obtained from (C) 30 H/C experiments without cryogenic trapping and (D) 30 H/C experiments with cryogenic trapping prior to the \(^2\)D\(_{\text{sep}}\) are also provided with the forward rate constant \((k_1^{\text{UE}})\) values on each column calculated by using DCXplorer according to overall projection approach. \(^2\)D\(_{\text{inter}}\) results and the corresponding \(k_1^{\text{UE}}\) values for individual H/C analyses obtained from H/C sections (E)–(H) in D are shown in E–H with the detailed plot of \(1_t R\) vs \(k_1^{\text{UE}}\) shown in I. The two
dashed lines in I indicate the $k_{1}^{\text{UE}}$ calculated according to the overall projection approach 
\((9.3 \times 10^{-4} \text{ s}^{-1})\) and the 1DGC benchmark approach \((8.5 \times 10^{-4} \text{ s}^{-1})\).

Fig. 5. A–I: Comprehensive two dimensional H/C analysis of E/Z oxime isomers at 40, 60 or 80
\(^\circ\text{C}\) in different separation dimensions (\(^1T\) and \(^2T\)) on \(^1\text{D}_{\text{inter}}\) SLB-IL111\(^2\text{D}_{\text{inter}}\) PEG obtained with the $k_{1}^{\text{UE}}$ values for each column calculated by using DCXplorer according to overall projection approach. The dimensions of $x$ and $y$ axes are the same in all Figures. The analysis was obtained from a total 270 H/C experiments (30 H/C for each Figure) with cryogenic trapping.
Figure 2

Click here to download high resolution image

$^{1}D\ IL111\ (70\ m) \times ^{2}D\ XX\ (30\ m)$

$\frac{k_{1}^{UE}(IL111)}{k_{1}^{UE}(WAX)} = \frac{9.4 \times 10^{-4}}{8.5 \times 10^{-4}}\ s^{-1}$

$\pm 0.4 \times 10^{-4}\ s^{-1}$

$20\ pA$

$BPX5$

$WAX$

$IL59$

$IL61$

$IL76$

$IL82$

$IL100$

$IL111$