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Observation and explanation of two-dimensional interconversion of oximes with multiple heart-cutting using comprehensive multidimensional gas chromatography

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ABSTRACT

Real-time interconversion processes produce unconventional peak broadening in gas chromatography (GC), and can be used to generate kinetic and thermodynamic data. In this study, an unusual separation situation in comprehensive two dimensional GC where two dimensional interconversion (*i.e.* a raised plateau in both first and second dimension, ^{1}D and ^{2}D) was observed in analysis of oxime isomers. This resulted in a characteristic and unusual rectangular peak shape in the two dimensional result. A related theoretical approach was introduced to explain the peak shape supported by simulation results which can be varied depending on concentration profiles and kinetics of the process. The simulated results were supported by experimental results obtained by a comprehensive heart-cut multidimensional GC (H/C MDGC) approach which was developed to clearly investigate isomerisation of *E/Z* oxime molecules in both ^{1}D and ^{2}D separations under different isothermal conditions. The carrier gas flow and oven temperature were selected according to initial results for 1D interconversion on a poly(ethyleneglycol) stationary phase, which was further used in both ^{1}D and ^{2}D separations to result in broad zones of oxime interconversion in both dimensions. The method involved repetitive injections of oxime sample, then sampling contiguous fractions of sample into a long ^{2}D column which is intended to promote considerable interconversion. Comprehensiveness arises from the fact that the whole sample is sampled from the ^{1}D to the ^{2}D column, with the long ^{2}D column replacing the short ^{2}D column used in classical comprehensive two-dimensional gas chromatography, where the latter will not promote sufficient interconversion. Data processing and presentation permits a 'rectangular' distribution corresponding to the separated compounds, characteristic of this experiment.

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1. Introduction

Separation in gas chromatography (GC) conventionally involves non-reactive and inert partition/adsorption processes, producing a single peak for each compound in the chromatogram. However, some configurationally labile molecules may undergo molecular transformation into different species on the separation time-scale, which can lead to unusual non-Gaussian peak shapes (strong peak broadening; overlapping or incompletely resolved peaks)[1,2]. This phenomenon may present difficulty in interpretation of GC data,

for instance of some aldehydes or unsymmetric ketones which undergo interconversion within the separation timeframe. In general, interconversion is a process where two isomers undergo mutual conversion into each other, *i.e.* A $\rightleftharpoons$ B (such as enantiomerisation or diastereomerisation processes) [3]. Interconversion in 1DGC has been studied in detail and is adequately understood. Model on-line reactions have been studied in GC, especially interconversion of *E/Z* oxime isomers. Proposed mechanisms for oxime isomerisation include 1) inversion, via *sp*-hybridisation of the nitrogen atom with the 180° C—N—O bond angle in the transition state [4,5] or 2) rotation around the C—N bond axis facilitated by polarisation of the C—N bond to result in *E/Z* isomerisation [4,5].

The overall separation/interconversion event defines the peak shape observed. Instead of two separate peaks of the *E* and *Z*

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isomers, on-line conversion of one isomer into the other causes observations somewhat like peak fronting or tailing in chromatograms. Interconversion on the separation timeframe may lead to a characteristic plateau between the two isomer peaks. The effect of temperature programming on interconversion has also been explored. Increasing the temperature of the system increases the magnitude of the observed plateau, due to increasing reaction rate at higher temperature [6]. The stationary phase used in the experiment also affects the extent of interconversion. Among different phases, it was found that oxime interconversion was more readily observed on a poly(ethyleneglycol) (PEG) phase [7].

Multidimensional GC (MDGC) conventionally employing two sequentially-arranged columns with different selectivity [8] has emerged as a high resolution technique, and proven useful for application to complex samples [9,10]. MDGC modes include single and multiple heart-cut (H/C) MDGC (GC–GC) [9,11]. A narrow H/C window will avoid sampling too many components into the ^{2}D column. A long ^{2}D column will improve ^{2}D separation, at the expense of longer analysis time. As a result, normally only a few target regions are sampled in conventional GC–GC. The second mode, comprehensive two-dimensional GC (GC×GC), subjects the total sample to 2D separation, with transfer of ^{1}D effluent zones usually less than the peak width of ^{1}D peaks, for further separation on a short ^{2}D column. A short narrow bore ^{2}D column provides fast analysis time, and maintains high resolution [9] although this does reduce the ^{2}D separation magnitude compared with the ^{2}D column in H/C MDGC analysis.

MDGC has been applied to interconverting compounds, to study the molecular behaviour of these processes [12]. Interconversion in ^{1}D of GC × GC has been studied where chiral phases were employed in both ^{1}D and ^{2}D [13], for a chiral oxime, to accomplish R/S separation and E/Z isomerisation. Although enantioseparation is difficult to achieve with a short column, the strategy employed a longer enantioselective column and allowed 'wrap-around' i.e. $^2t_R > P_M$, to provide sufficient resolution of the closely eluting enantiomers. In general, interconversion is not observed on short ^{2}D columns, due to insufficient time to promote interconversion. Characteristic interconversion profiles on ^{2}D in GC × GC were observed such as the small extent of interconversion of 2-phenylpropanaldehyde oxime on a ^{2}D IL111 column at 140 °C [12]. The interconversion in both ^{1}D and ^{2}D led to an unconventional overall distribution defining rectangular peak shapes that have not been previously explained or investigated in detail. This could affect purity of target peaks in ^{2}D, e.g. causing error in quantitative analysis. In order to investigate such phenomena and the resultant peak shape, an arrangement that has long columns in both ^{1}D and ^{2}D can be employed. Considering that a long ^{2}D column with suitable phase (PEG) is required to promote interconversion, an option is to use a comprehensive H/C GC–GC (comprehensive MDGC) approach, with a sequential sampling strategy which progressively shifts the ^{1}D H/C over the ^{1}D elution profile with multiple injections applied to the whole sampled region. This allows both long ^{1}D and long ^{2}D columns to be investigated to deliver interconversion in both dimensions of the separations. A strategy of using long ^{1}D and ^{2}D columns has been previously used in GC–GC with multiple injections for high resolution alternative fuel characterisation of trace oxidation products [14]. The final data can be reconstructed into what mimics a GC × GC data presentation format.

This study reports observation of 2D interconversion in practical analysis of enantio- and stereo-isomers of oximes with GC × GC. The related theoretical approaches explain characteristic peak shapes in 2D interconversion. A comprehensive H/C GC–GC technique was further developed to investigate interconversion in both ^{1}D and ^{2}D separations employing PEG columns, with multiple repetitive heart-cuts, each offset by the sampling period, and a Deans switch (DS). Data analysis and the presentation approach are illustrated, and investigation of effects of temperature programs on separation results support the simulation results.

2. Theoretical

The characteristic shapes of the ^{1}D and ^{2}D interconversion peaks arise from: 1) interconversion of the isomers on the ^{1}D column generating a broad zone of the isomers; 2) sampling of several regions along the interconversion zone resulting in injections of different initial concentration ratios of the two isomers (A_0 to B_0 ratio) to the ^{2}D column; 3) combination of all the ^{2}D profiles (injected with different isomer ratios) into an overall comprehensive 2D plot. Note that simulation of the ^{1}D separation profile is known and is not the focus here. The sampling profiles obtained by H/C of the ^{1}D interconversion zone with different concentration ratios were generated, and the unified equation [15] was applied for simulation of interconversion profiles (peak shapes) in the ^{2}D separation in this study. The equation describes peak shapes using the time-dependent Gaussian distribution functions $\Phi_A(t)$ and $\Phi_B(t)$ (concentration profiles) of the isomer A and B with retention times of $t_{R,A}$ and $t_{R,B}$, respectively, and the conversion density function (concentration profile of the interconverted species, $\bar{c}_{df}(t_{R,A} < t < t_{R,B})$) which can result in useful relationships for simulation of the interconversion profile according to

$$\Phi_A(t) = \frac{A_0 e^{-k_1^{ue} t_{R,A}}}{\sigma_A \sqrt{2\pi}} e^{-\frac{(t-t_{R,A})^2}{2\sigma_A^2}} \tag{1}$$

$$\Phi_B(t) = \frac{B_0 e^{-A_\infty/B_\infty k_1^{ue} t_{R,A}}}{\sigma_B \sqrt{2\pi}} e^{-\frac{(t-t_{R,B})^2}{2\sigma_B^2}} \tag{2}$$

$$\bar{c}_{df}(t_{R,A} < t < t_{R,B}) = -\frac{2(A_0 - A(t_{R,A}))}{(t_{R,B} - t_{R,A})^2}(t - t_{R,A})$$
$$+ \frac{2(A_0 - A(t_{R,A}))}{t_{R,B} - t_{R,A}} + \frac{2(B_0 - B(t_{R,B}))}{(t_{R,B} - t_{R,A})^2}(t - t_{R,A}) \tag{3}$$

where k_1^{ue} is the reaction rate constant. σ_i is the standard deviation of peak i (i = A or B) with width w_i and $\sigma_i = \frac{w_i}{\sqrt{8 \ln 2}}$. A_0 and B_0 are initial concentrations of A and B. These initial concentrations indicate the benefit of Eqs. (1)–(3) since these allow interpretation of the magnitude of the plateau for simulation of the A and B values resulting from interconversion in ^{1}D or ^{2}D. A_∞ and B_∞ are equilibrium concentrations of A and B. For simulation of the ^{2}D interconversion profiles, A_0 and B_0 will vary depending on the selected H/C regions, over the duration of the ^{1}D interconversion zone. All the simulated ^{2}D profiles were then combined into a comprehensive MDGC plot. The simulated MDGC results were performed with different inputs of the ^{1}D interconversion profiles and rate constants.

3. Experimental

3.1. Sample preparation

For 1DGC experiments, acetaldehyde oxime (acetaldoxime; 97%, Sigma-Aldrich, MO) was prepared in 1-hexanol with ethanol used as an internal standard. For GC–GC experiments, acetaldoxime was prepared in acetone. For GC × GC experiments, 2-phenylpropanaldehyde oxime (synthesis and characterisation procedures described elsewhere [13]) was prepared in HPLC grade n-hexane (Merck, Darmstadt, Germany).