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In-silico optimisation of two-dimensional high performance liquid chromatography for the determination of Australian methamphetamine seizure samples

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ABSTRACT

In-silico optimisation of a two-dimensional high performance liquid chromatography (2D-HPLC) separation protocol has been developed for the interrogation of methamphetamine samples including model, real world seizure, and laboratory synthesised samples. The protocol used Drylab® software to rapidly identify the optimum separation conditions from a library of chromatography columns. The optimum separation space was provided by the Phenomonex Kinetex PFP column (first dimension) and an Agilent Poroshell 120 EC-C18 column (second dimension). To facilitate a rapid 2D-HPLC analysis the particle packed C18 column was replaced with a Phenomenex Onyx Monolithic C18 withought sacrificing separation performance. The Drylab® optimised and experimental separations matched very closely, highlighting the robust nature of HPLC simulations. The chemical information gained from an intermediate methamphetamine sample was significant and complimented that generated from a pure seizure sample. The influence of the two-dimensional separation on the analytical figures of merit was also investigated. The limits of detection for key analytes in the second dimension determined for methamphetamine (4.59×10^{-4} M), pseudoephedrine (4.03×10^{-4} M), caffeine (5.16×10^{-4} M), aspirin (9.32×10^{-4} M), paracetamol (5.93×10^{-4} M) and procaine (2.02×10^{-3} M).

1. Introduction

The advent of two-dimensional high performance liquid chromatography (2D-HPLC) has enhanced an analyst's ability to develop separation procedures for the analysis of components in complex forensic [1], industrial [2], biomedical [3] and pharmaceutical [4] samples. Comprehensive two-dimensional gas chromatography with time of flight mass spectrometry has been used by forensic chemists to profile complex drug samples [5,6]. The use of chemometric data handling [7] is critical for the in-depth analysis of these complex samples. In forensic field studies multi-dimensional gas chromatography has been used to monitor volatile compounds of interest such as molecules from decaying bodies [8]; however, this study was limited by the inability of the separation to deal with the immensely complex sample background. Despite the complex nature of samples in forensic science, two-dimensional chromatography has not had a large uptake due to the time limits traditionally associated with sample analysis and the complexity of data handling [9]. Beyond forensic science however two dimensional chromatography has been used to elucidate cannabinoids in hemp [10] and alkaloids in Chinese medicine [11]. A targeted form of 2D-HPLC has been used to isolate ephedrine and pseudoephedrine from methamphetamine excipient standard mixtures [1], however the comprehensive chemical information on a seizure sample has not been determined.

In order for 2D-HPLC to be fully exploited for detailed forensic investigations fundamental aspects of the separation process need to be optimised. This is driven by the fact that conventional 1D-HPLC is limited by the number of theoretical plates (resolving

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power) available, the heterogeneity of the column material and the disruption of analytes due to viscous fingering [12]. The use of two chromatography columns in 2D-HPLC dramatically increases the resolving power of complex samples when different separation mechanisms are used in each dimension [13,14]. There is a range of experimental factors that influence a chromatographic separation (solvent type, temperature, flow rate etc.), the most important being the stationary phase and should be the first aspect addressed in any optimisation process [15].

Several techniques have been explored to optimise the utilisation of 2D-HPLC separation space including the analysis of relative retention profiles of a library of HPLC columns. However, due to the complexity of samples requiring a multidimensional separation this method proved to be problematic as representative standards cannot be easily found [16]; also, these types of approaches are typically very time consuming [17].

The importance of this aspect has been highlighted by Stevenson et al. [13] who have developed a fundamental study highlighting the significance of C18 and C1 stationary phases, for the separation of PAH's in a model system. This stresses the importance of selectivity for complex real world samples such as those in the forensic sciences. Gilar et al. [18] developed a robust method for calculating the orthogonality and separation space utilisation of two columns in a 2D-HPLC analysis. This method was used to calculate the $f_{coverage}$, also known as orthogonality (O) for the 2D-HPLC peak capacity equation [19] by dividing the separation space by a number of bins equal to the number of separated components to provide the fractional surface coverage. Peak capacity is directly proportional to orthogonality (O) and the utilisation space of a separation, which can be calculated by Eq. (1): where $\sum$bins is the number of occupied bins and P_{max} is the total number of bins.

$$O = \frac{\sum \text{bins} - \sqrt{P_{max}}}{0.63 \times P_{max}} \quad (1)$$

The mobile phase associated with a two-dimensional separation needs to be carefully considered to avoid solvent mismatch which may lead to issues such as viscous fingering as identified by Shalliker and Guiochon [20]. The selection of appropriate stationary phases, mobile phases, and analysis time need to be considered in the optimisation of 2D-HPLC experiments. Traditionally, 2D-HPLC method development has taken considerable time and there exists a real need for a simplified optimisation approach.

Recent work carried out by Andrighetto et al. [1] described the optimisation of complex samples using DryLab® simulation software, where ideal conditions were obtained for a 1D-HPLC separation by running every individual standard at two different gradients. The work highlighted many challenges in the HPLC method development that can be reduced or removed with the use of in-silico simulation. This saves time due to a reduction in the number of separations required that significantly reduces the use of laboratory resources, making it environmentally friendly; a key aspect of any modern laboratory [21]. Ideally, analyses of forensic significance could be enhanced by developing an optimised separation space in-silico before a time consuming multidimensional separation.

Characteristic to the synthetic process, the relative concentrations of by-products in any reaction pathway are variable. This volatility, when coupled with the extensive range of cutting agents commonly used in the production of illicit methamphetamine, allows for the formation of a chemical fingerprint that can be used by law enforcement agencies to individualise seizure samples on a batch to batch basis [22]. This paper highlights for the first time the use of a fast 2D-HPLC optimisation procedure for the interrogation of methamphetamine seizure samples.

2. Experimental

2.1. Chemicals

Milli-Q water was obtained in-house (Continental Water Systems, Victoria, Australia). HPLC grade acetonitrile (ACN) was purchased from Sigma–Aldrich Pty., Ltd. (Castle Hill, NSW, Australia).

2.2. Standards and samples

Paracetamol, caffeine, benzaldehyde, aspirin, creatine, procaine, *N*-methylalanine, and diphenylacetone were obtained from Sigma–Aldrich. Methamphetamine, pseudoephedrine, ephedrine and phenyl-2-propanone (P2P) were obtained from and the National Measurement Institute, Australian Government (Port Melbourne, Vic., Australia). Stock solutions of all standards were prepared by dissolving in a solution of 5% aqueous ACN at a concentration of 1 mg mL^{-1} and were diluted 10-fold with a 5% ACN in water solution and mixed prior to injection. All methamphetamine seizure samples were provided by Victoria Police Forensic Services Department (Macleod, Victoria, Australia).

2.3. Chromatography columns

Seventeen HPLC columns were trialled for the selectivity study of a model seizure sample (Table 1)

	Brand	Type	Size	Dimensions	Part number
Column 1	Agilent	Poroshell 120 EC-C8	2.7 μm	4.6 × 100 mm	695975-906
Column 2	Agilent	Poroshell 120 EC-CN	2.7 μm	4.6 × 100 mm	695975-905
Column 3	Phenomenex	Kinetex PFP 100 Å	2.6 μm	4.6 × 100 mm	00D-4477-E0
Column 4	Phenomenex	Kinetex Phenyl-Hexyl 100 Å	2.6 μm	4.6 × 100 mm	00D-4495-E0
Column 5	Phenomenex	Synergi Fusion-RP 80 Å	4 μm	4.6 × 150 mm	00F-4424-E0
Column 6	Phenomenex	Synergi Hydro-RP 80 Å	4 μm	4.6 × 150 mm	00F-4375-E0
Column 7	Phenomenex	Luna C5 100 Å	5 μm	4.6 × 50 mm	00B-4043-E0
Column 8	Phenomenex	Luna NH2 100 Å	5 μm	4.6 × 100 mm	00D-4378-E0
Column 9	Phenomenex	Luna HILIC 200 Å	5 μm	4.6 × 150 mm	00F-4450-E0
Column 10	Cosmosil	πNAP	2.5 μm	4.6 × 100 mm	08084-51
Column 11	Cosmosil	5PBB-R	5 μm	4.6 × 150 mm	05697-21
Column 12	Cosmosil	5NPE	5 μm	4.6 × 150 mm	37904-01
Column 13	Agilent	Poroshell 120 EC-C18	2.7 μm	4.6 × 100 mm	695975-902
Column 14	Agilent	Poroshell HPH-C18	2.7 μm	4.6 × 100 mm	695975-702
Column 15	Agilent	Poroshell 120,SB-C18	2.7 μm	4.6 × 100 mm	685975-902
Column 16	Agilent	Poroshell 120 Bonus RP	2.7 μm	4.6 × 100 mm	695968-901
Column 17	Agilent	Pursuit XRs 3 Diphenyl	3 μm	4.6 × 100 mm	A6021100X046

Table 1
Molecular weights detected in methamphetamine (MA) seizure samples including pseudoephedrine (PE).

Sample	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*
VPFSD-1	MA	144.9872	168.0204	–	–
VPFSD-2	MA	144.9872	168.0204	–	–
VPFSD-3	MA	179.0111	335.2257	–	–
VPFSD-4	MA	165.0756	–	–	–
VPFSD-5	MA	PE	148.1124	198.0980	383.1165
VPFSD-6	MA	PE	148.1128	198.0980	335.2248
VPFSD-7	MA	PE	335.2255	–	–
VPFSD-8	MA	335.2280	–	–	–
VPFSD-9	MA	335.2273	–	–	–
VPFSD-10	MA	335.2256	–	–	–
VPFSD-11	MA	PE	133.0875	148.1118	198.0966
VPFSD-12	MA	PE	198.0961	226.1589	383.1153

2.4. 2D-HPLC

Chromatographic analysis was performed with an Agilent 1260 system (Agilent Technologies, Mulgrave, Victoria, Australia), incorporating a quaternary pump with solvent degasser, an auto-sampler and a DAD module which monitored the absorbance at 254 nm. Chromatographic data was obtained and processed with Agilent ChemStation software. All injections were 60 μL and carried out in triplicate. The first dimension column was a Phenomonex Kinetex PFP 100 Å (4.6 × 100 mm, 2.6 μm particle diameter) and the second dimension a Phenomonex Onyx Monolithic C18 (4.6 × 100 mm). The first dimension gradient was completed at a flow rate of 0.1 mL min^{-1} with an initial mobile phase of 5% aqueous ACN that increased to 30% aqueous ACN over 100 min and then increased to 100% ACN for a further 10 min. The second dimension gradient was completed at 5 mL min^{-1} with an initial mobile phase of 5% aqueous ACN that increased to 80% aqueous ACN for 1 min. The comprehensive two-dimensional separation had an overall completion time of 110 min. Two-dimensional HPLC was completed in on-line comprehensive mode with a modulation time of 1.5 min, whereby a fraction volume of 75 μL was transferred to the second dimension via a sample loop and an 8 port, 2 position switching valve; valve timing was controlled with by the HPLC control software.

Measurements for bins, plate heights, plate numbers and peak variances were performed with Wolfram Mathematica 10.3 (distributed by Hearn Scientific, South Yarra, Victoria, Australia) using algorithms written in-house.

2.5. LC–MS

High resolution mass spectrometry (HRMS) was performed using an Agilent 6210 MSD TOF mass spectrometer with the following settings: gas temperature (350 °C), vaporizer (28 °C), capillary voltage (3.0 kV), cone voltage (40 V), nitrogen flow rate (0.5 mL/min), nebuliser (15 psi).

3. Results and discussion

A model seizure sample was prepared containing known concentrations of methamphetamine and 11 other common precursors and cutting agents: pseudoephedrine, ephedrine, phenyl-2-propanone (P2P), paracetamol, caffeine, benzaldehyde, aspirin, creatine, procaine, *N*-methylalanine, and diphenylacetone.

After a column selectivity study was performed a combination of the PFP and EC-C18 stationary phases was found to provide the greatest surface space utilisation with an $f_{coverage}$ of 0.18 (see Fig. 1). A bins plot is developed here in order to determine the orthogonality of the separation system, the use of the separation space is directly proportional to the orthogonality. This leads to a greater capacity for fully resolving components in complex samples. To facilitate a rapid separation towards the goal of 'fit-for-purpose' analysis the monolithic [23] C18 column was trialled in place of the particle packed column; an $f_{coverage}$ of 0.18 was maintained. The first dimension was completed at a flow rate of 0.1 mL min^{-1} to allow for a modulation frequency of 3 transfers of each first dimensional peak to the second dimension. Therefore, the second dimension must be performed very fast so that the second analysis can be done in a time equal to the period of fraction collection minus the fraction transfer time [24] a speed that can only be afforded by the monolithic column.

With this in mind, a fully comprehensive two-dimensional separation was completed with a total analysis time of 115 min, the separation is illustrated in Fig. 2. The retention times predicted by DryLab® are shown by the white circles, which have been overlaid on the actual two dimensional separation (Fig. 2).

A screenshot of Drylab's gradient optimisation window is illustrated in ESI1. After training the software with data from separations occurring over 2 different gradient durations the simulation is able to extrapolate 2 variables (i.e. *S* and k_w) for each peak that are required to predict the retention time with varying gradient durations. Drylab is able to simulate the retention profile of a complex separation matrix with a single step (linear) gradient, or by adding multiple steps; a 4 step gradient is presented here. When this process is done to optimise each retention dimension individually a 2D-HPLC retention map can be extrapolated and the final multidimensional simulation is predicted.

This is the first time in-silico software has been used to predict a two dimensional separation of methamphetamine samples and indeed forensically relevant samples at large. This is of great significance for the development of 2D-HPLC protocols, a process that historically takes considerable time. The total method development time for separation presented in Fig. 2 was less

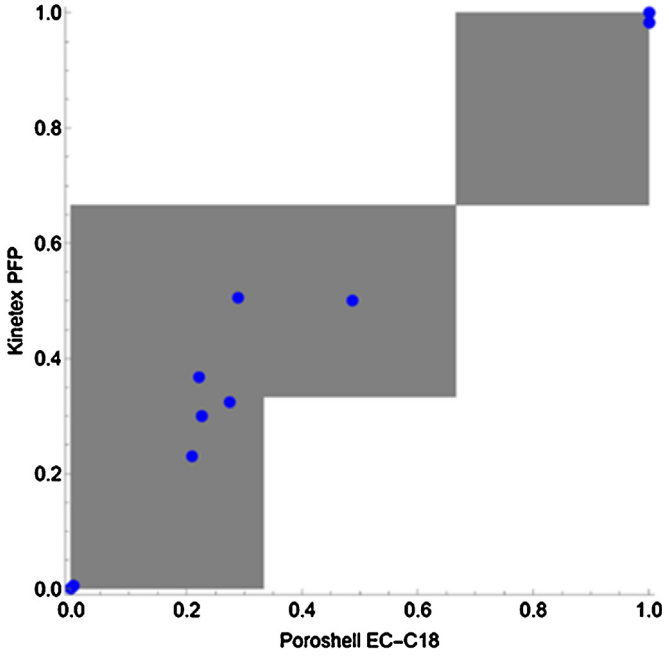

Fig. 1. Bins plot (Poroshell C18 × Kinetex PFP) of two dimensional HPLC separation of the model methamphetamine seizure sample.

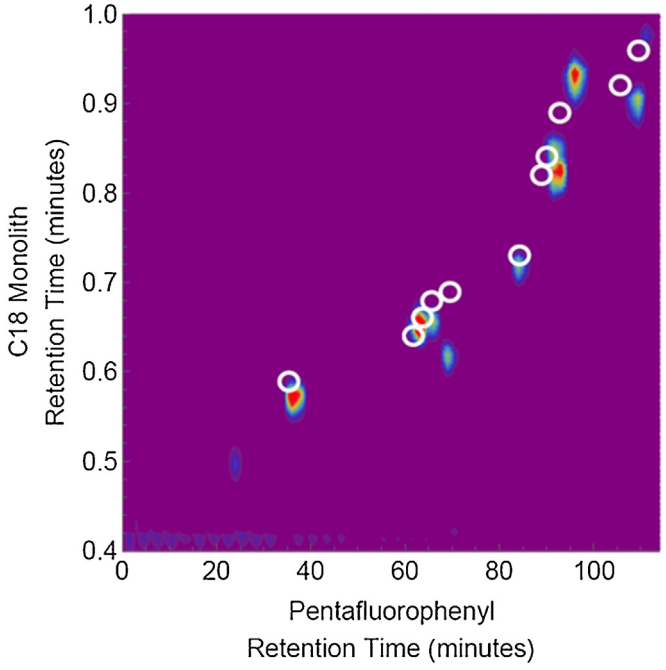

Fig. 2. Overlaid DryLab® and actual two dimensional separation of the model methamphetamine seizure sample.

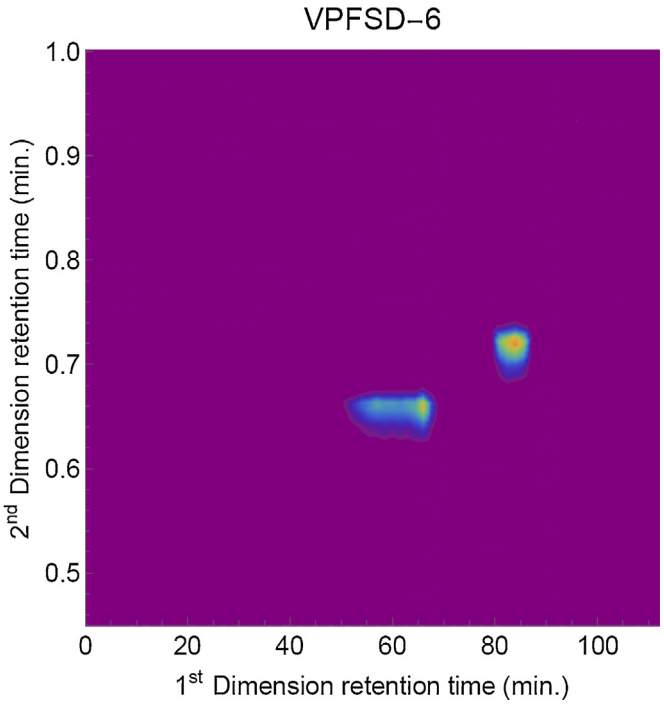

Fig. 3. Methamphetamine seizure sample.

than two hours with in-silico aided optimisation. This is significantly less than the time required for typical two dimensional separation optimisation that normally requires many individual sample injections [1]. The results of 2D-HPLC separation prediction was impressive as all components of interest were closely matched. This is not a trivial outcome due to the complex nature of the separation mechanisms involved allowing the user to have high confidence in the in-silico prediction.

In order to determine the effectiveness of this process twelve real methamphetamine seizure samples were analysed using the developed method. Several components were observed in all of the seizure samples; a characteristic chromatogram is presented in Fig. 3. The two major components in Fig. 3 are methamphetamine (first dimension retention time ($R_{t,1}$) of 83.4 min, second dimension retention time ($R_{t,2}$) of 0.72 min) and pseudoephedrine ($R_{t,1}$ = 68.1 min, $R_{t,2}$ = 0.66 min) that were determined by comparing the retention times against known standards. With only two detected component peaks this sample is particularly clean; this is not uncommon in crystal methamphetamine samples. Care was taken to see if trace amounts of excipients were present however none was observed in this or any of the other eleven samples.

In order to confirm 2D-HPLC findings, liquid chromatography/mass spectrometry (LC/MS) was used to analyse all twelve seizure samples, the major components of which are described in Table 1 and confirmed the simplicity of these random seizure samples.

The determination of excipients is of particular importance for chemical fingerprinting and is useful when analysing intermediate synthetic samples found in clandestine laboratories. To assess the level of chemical complexity that can be assessed with 2D-HPLC an intermediate sample from a methamphetamine synthesis in the laboratory was analysed and is presented in Fig. 4.

More than forty components were detected in the intermediate sample, which are highlighted by white dots in Fig. 4, thus emphasising the forensic significance of fingerprinting intermediate seizure samples to build a comprehensive database of the chemical pathways in a clandestine laboratory. The distribution of the components in the model system and the real intermediate sample generally appear in the top right hand quadrant of the two dimensional separation, due to the non-polar nature of the components present. While the other three quadrants are not exploited here, it is important for any analyst to use the full separation procedure in case some unknown polar excipients are present. While this will add to the overall separation time, it is of greater importance to the forensic community to have a comprehensive analysis. An $f_{coverage}$ of 0.49 was found when the surface space usage of the intermediate separation was completed,

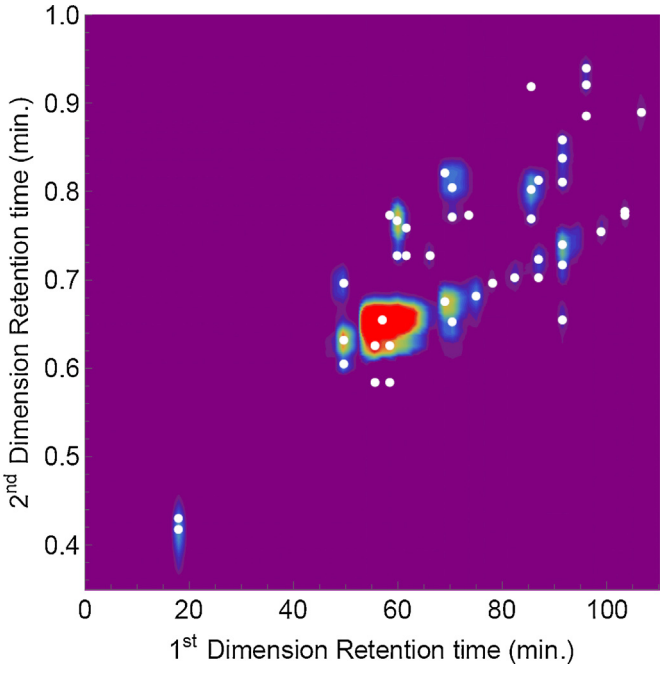

Fig. 4. Intermediate methamphetamine sample.

Table 2
2D-HPLC limit of detections.

Excipient	R_t (min)	R^2	Calibration equation	$LOD_{1D\text{-}HPLC}$ (M)	$LOD_{2D\text{-}HPLC}$ (M)
Methamphetamine	2.46	0.9999	$y = 1 \times 10^6 x - 8.8125$	2.66×10^{-5}	4.59×10^{-4}
Pseudoephedrine	1.55	0.9999	$y = 1 \times 10^6 x + 8.1786$	2.34×10^{-5}	4.03×10^{-4}
Aspirin	4.26	0.9998	$y = 918{,}340\,x - 29.056$	5.41×10^{-5}	9.32×10^{-4}
Caffeine	2.07	0.9985	$y = 2 \times 10^6 x - 12.829$	2.99×10^{-5}	5.16×10^{-4}
Paracetamol	0.76	0.9988	$y = 755{,}343\,x - 0.6191$	3.44×10^{-5}	5.93×10^{-4}
Procaine	1.23	0.9997	$y = 220{,}286x + 10.573$	1.17×10^{-4}	2.02×10^{-3}

far exceeding that of the standard mixture ($f_{coverage}$ of 0.18). While every attempt was made to create a standard mixture representative of a typical seizure sample the failure of the optimisation mixture to accurately reflect the separation space utilisation of the intermediate highlights the issues faced when selecting columns for 2D-HPLC.

It is important to consider the analytical figures of merit for this type of two dimensional separation, in order to determine if transferring peaks between dimensions has any influence on the detection limit. To achieve this several key excipients (paracetamol, caffeine, methamphetamine, pseudoephedrine, procaine and aspirin) were selected and the limits of detection (LOD) in the second dimension ($LOD_{2D\text{-}HPLC}$) was calculated and recorded in Table 2. The limit of detection were calculated multiplying the slope of the calibration by 3.3 and dividing by the standard error. The LOD of these compounds was in the range of 3×10^{-5} M, which was comparable to literature values [25–27].

The ideal modulation frequency when transferring analytes between dimensions in 2D-HPLC is 3–4 fractions per peak [28]. To ensure this frequency is maintained for peaks in adjacent fractions, the amount of transferred analyte must at least match the second dimension's limit of detection ($LOD_{2nd\text{-}dimension}$). In this work a modulation frequency of 3 fractions per peak was maintained whereby a minimum of 5.8% of the first dimension peak (fractions 1 and 3—derived by dividing the area of a Gaussian peak with an area of 1 into 3 evenly spaced segments) were transferred, with the assumption that the peak maxima is centred in the 4σ peak width. To maintain detected 3 fractions per peak the minimum amount of transferred material was 5.8% of the total peak area with a concentration at least equal to the $LOD_{2nd\text{-}dimension}$ after separation. Thus, the total concentration of compound injected (i.e. the $LOD_{2D\text{-}HPLC}$) is calculated by Eq. (2), which is transposed in two steps below:

$$\begin{aligned} 5.8\%\mathrm{LOD}_{2D-HPLC} &= 100\%\mathrm{LOD}_{2nd-Dimension} \\ \mathrm{LOD}_{2D-HPLC} &= 100\%/5.8\%\mathrm{LOD}_{2nd-Dimension} \\ \mathrm{LOD}_{2D-HPLC} &= 17.2 \times \mathrm{LOD}_{2nd-Dimension} \end{aligned} \quad (2)$$

As such, the $LOD_{1D\text{-}HPLC}$ (single dimension) must be multiplied by 17.2. Following the same procedure the effect will become significantly more pronounced as the modulation frequency increases, for example if a modulation frequency of 4 fractions per peak was used the $LOD_{2D\text{-}HPLC}$ is equal to $107.9 \times LOD_{2nd\text{-}dimension}$. Table 2 lists the LODs (one and two-dimensions) of standards for 6 common excipients in the manufacture of methamphetamine and is in the order of 5×10^{-4} M.

4. Conclusions

The use of in-silico optimisation of two-dimensional separations for the interrogation of methamphetamine seizure samples has been shown to have a significant impact on the experimental development time. The greatest challenge in 2D-HPLC method development has typically been the time required to identify the columns required to provide the greatest separation space utilisation. This was overcome here with Drylab® optimisation software. This improvement in analysis time also reduces the organic solvent load produced and directly impacts the laboratory waste generated. Importantly the Drylab® optimised in-silico separations matched closely with the model and synthetic samples allowing the analyst to have confidence in the predicted separation parameters despite the complex nature of the separation mechanisms involved. A 2D-HPLC separation was completed that isolated more than 40 individual chemical compounds that are structurally related from an intermediate sample from a methamphetamine synthesis. This knowledge can be used by the forensic analyst to create a chemical fingerprint of the seizure, which can theoretically be linked back to a clandestine laboratory.

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.forsciint.2016.07.016.

References

[1] L.M. Andrighetto, P.G. Stevenson, J.R. Pearson, L.C. Henderson, X.A. Conlan, DryLab® optimised two-dimensional high performance liquid chromatography for differentiation of ephedrine and pseudoephedrine based methamphetamine samples, Forensic Sci. Int. 244 (2014) 302–305.
[2] S.K.D. Pravadali-Cekic, P.G. Stevenson, R.A. Shalliker, Outlining a multidimensional approach for the analysis of coffee using HPLC, J. Chromatogr. Sep. Tech. 6 (2015) 284–293.
[3] R.J. Vonk, A.F.G. Gargano, E. Davydova, H.L. Dekker, S. Eeltink, L.J. de Koning, P.J. Schoenmakers, Comprehensive two-dimensional liquid chromatography with stationary-phase-assisted modulation coupled to high-resolution mass spectrometry applied to proteome analysis of saccharomyces cerevisiae, Anal. Chem. 87 (2015) 5387–5394.
[4] E.L. Regalado, J.A. Schariter, C.J. Welch, Investigation of two-dimensional high performance liquid chromatography approaches for reversed phase resolution of warfarin and hydroxywarfarin isomers, J. Chromatogr. A 1363 (2014) 200–206.
[5] S.M. Song, P. Marriott, A. Kotsos, O.H. Drummer, P. Wynne, Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC × GC-TOFMS) for drug screening and confirmation, Forensic Sci. Int. 143 (2004) 87–101.
[6] R.H. Lowe, E.L. Karschner, E.W. Schwilke, A.J. Barnes, M.A. Huestis, Simultaneous quantification of 9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in human plasma using two-dimensional gas chromatography, cryofocusing, and electron impact-mass spectrometry, J. Chromatogr. A 1163 (2007) 318–327.
[7] T. Gröger, M. Schäffer, M. Pütz, B. Ahrens, K. Drew, M. Eschner, R. Zimmermann, Application of two-dimensional gas chromatography combined with pixel-based chemometric processing for the chemical profiling of illicit drug samples, J. Chromatogr. A 1200 (2008) 8–16.
[8] A. Agapiou, E. Zorba, K. Mikedi, L. McGregor, C. Spiliopoulou, M. Statheropoulos, Analysis of volatile organic compounds released from the decay of surrogate human models simulating victims of collapsed buildings by thermal desorption–comprehensive two-dimensional gas chromatography–time of flight mass spectrometry, Anal. Chim. Acta 883 (2015) 99–108.
[9] A. Sampat, M. Lopatka, M. Sjerps, G. Vivo-Truyols, P. Schoenmakers, A. van Asten, The forensic potential of comprehensive two-dimensional gas chromatography, Trends Anal. Chem. 80 (2016) 345–363.
[10] J. Pandohee, B.J. Holland, B. Li, T. Tsuzuki, P.G. Stevenson, N.W. Barnett, J.R. Pearson, O.A. Jones, X.A. Conlan, Screening of cannabinoids in industrial-grade hemp using two-dimensional liquid chromatography coupled with acidic potassium permanganate chemiluminescence detection, J. Sep. Sci. 38 (2015) 2024–2032.
[11] P. Zou, S. Wang, Z. Zhang, X. Wu, Analysis of ephedrine alkaloids in Chinese medicine by 2D HPLC, Fenxi Ceshi Xuebao 26 (2007) 120–124.
[12] G. Guiochon, The limits of the separation power of unidimensional column liquid chromatography, J. Chromatogr. A 1126 (2006) 6–49.

[13] P.G. Stevenson, M. Mnatsakanyan, A.R. Francis, R.A. Shalliker, A discussion on the process of defining 2-D separation selectivity, J. Sep. Sci. 33 (2010) 1405–1413.
[14] M. Gilar, P. Olivova, A.E. Daly, J.C. Gebler, Orthogonality of separation in two-dimensional liquid chromatography, Anal. Chem. 77 (2005) 6426–6434.
[15] U.D. Neue, J.E. O'Gara, A. Méndez, Selectivity in reversed-phase separations: influence of the stationary phase, J. Chromatogr. A 1127 (2006) 161–174.
[16] D.N. Bassanese, B.J. Holland, X.A. Conlan, P.S. Francis, N.W. Barnett, P.G. Stevenson, Protocols for finding the most orthogonal dimensions for two-dimensional high performance liquid chromatography, Talanta 134 (2015) 402–408.
[17] T. Murahashi, F. Tsuruga, S. Sasaki, An automatic method for the determination of carcinogenic 1-nitropyrene in extracts from automobile exhaust particulate matter, Analyst 128 (2003) 1346–1351.
[18] M. Gilar, J. Fridrich, M.R. Schure, A. Jaworski, Comparison of orthogonality estimation methods for the two-dimensional separations of peptides, Anal. Chem. 84 (2012) 8722–8732.
[19] X. Li, D.R. Stoll, P.W. Carr, A simple and accurate equation for peak capacity estimation in two dimensional liquid chromatography, Anal. Chem. 81 (2009) 845–850.
[20] R.A. Shalliker, G. Guichon, How to improve your implementation of two-dimensional preparative HPLC: solvent viscosity considerations, BioProcess Int. 6 (2008) 52–60.
[21] J.H. Clark, Catalysis for green chemistry, Pure Appl. Chem. 73 (2001) 103–111.
[22] N. Stojanovska, S. Fu, M. Tahtouh, T. Kelly, A. Beavis, K.P. Kirkbride, A review of impurity profiling and synthetic route of manufacture of methylamphetamine, 3,4-methylenedioxymethylamphetamine, amphetamine, dimethylamphetamine and *p*-methoxyamphetamine, Forensic Sci. Int. 224 (2013) 8–26.
[23] P. Dugo, F. Cacciola, T. Kumm, G. Dugo, L. Mondello, Comprehensive multidimensional liquid chromatography: theory and applications, J. Chromatogr. A 1184 (2008) 353–368.
[24] G. Guiochon, N. Marchetti, K. Mriziq, R.A. Shalliker, Implementations of two-dimensional liquid chromatography, J. Chromatogr. A 1189 (2008) 109–168.
[25] D.M. Sultan, Simultaneous HPLC determination and validation of amphetamine, methamphetamine, caffeine, paracetamol, and theophylline in illicit seized tablets, Pharm. Sci. 6 (2014) 294–298.
[26] D. Deng, H. Deng, L. Zhang, Y. Su, Determination of ephedrine and pseudoephedrine by field-amplified sample injection capillary electrophoresis, J. Chromatogr. Sci. 52 (2014) 357–362.
[27] B.B. Patel, B.B. Shah, K.N. Gohil, P.M. Patel, Development and validation of spectrophotometric method for simultaneous estimation of rosuvastatin calcium and aspirin in bulk and pharmaceutical dosage form, Int. J. Res. Pharm. Sci. 2 (2012) 115–122.
[28] R.E. Murphy, M.R. Schure, J.P. Foley, Effect of sampling rate on resolution in comprehensive two-dimensional liquid chromatography, Anal. Chem. 70 (1998) 1585–1594.