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DOI

[10.1016/j.chroma.2016.01.070](https://doi.org/10.1016/j.chroma.2016.01.070)

Publication date

2016

Document Version

Final published version

Published in

Journal of Chromatography A

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[Link to publication](#)

Citation for published version (APA):

Pirok, B. W. J., Knip, J., van Bommel, M. R., & Schoenmakers, P. J. (2016). Characterization of synthetic dyes by comprehensive two-dimensional liquid chromatography combining ion-exchange chromatography and fast ion-pair reversed-phase chromatography. *Journal of Chromatography A*, 1436, 141-146. <https://doi.org/10.1016/j.chroma.2016.01.070>

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Characterization of synthetic dyes by comprehensive two-dimensional liquid chromatography combining ion-exchange chromatography and fast ion-pair reversed-phase chromatography



Bob W.J. Pirok^{a,b,*}, Jitske Knip^a, Maarten R. van Bommel^{a,c}, Peter J. Schoenmakers^a

^a University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical-Chemistry Group, Science Park 904, 1098 XH Amsterdam, The Netherlands

^b TI-COAST, Science Park 904, 1098 XH Amsterdam, The Netherlands

^c University of Amsterdam, Faculty of Humanities, Conservation and Restoration of Cultural Heritage, Johannes Vermeerplein 1, 1071 DV, Amsterdam, The Netherlands

ARTICLE INFO

Article history:

Received 24 November 2015

Received in revised form 21 January 2016

Accepted 24 January 2016

Available online 30 January 2016

Keywords:

Early synthetic dyes

Art conservation

Comprehensive two-dimensional liquid chromatography

LC × LC

Fast ion-pair LC

ABSTRACT

In the late 19th century, newly invented synthetic dyes rapidly replaced the natural dyes on the market. The characterization of mixtures of these so-called early synthetic dyes is complicated through the occurrence of many impurities and degradation products. Conventional one-dimensional liquid chromatography does not suffice to obtain fingerprints with sufficient resolution and baseline integrity. Comprehensive two-dimensional liquid chromatography (LC × LC) is employed in this study, with ion-exchange chromatography in the first dimension and fast ion-pair liquid chromatography in the second. Retention in the first dimension is largely determined by the number of charges, while the selection of a small ion-pair reagent (tetramethylammonium hydroxide) in the second dimension causes retention to be largely determined by the molecular structure of the dye. As a result, there is a high degree of orthogonality of the two dimensions, similar to the values typically encountered in GC × GC. The proposed LC × LC method shows a theoretical peak capacity of about 2000 in an analysis time of about three hours. Clear, informative fingerprints are obtained that open a way to a more efficient characterization of dyes used in objects of cultural heritage.

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1. Introduction

The invention of the first commercially successful synthetic dye (mauveine) in 1856, sparked a completely new branch of chemistry. Until 1850 natural dyes were used to achieve all important colors. Within fifty years after the invention of mauveine more than five hundred new colorants were patented and brought onto the market. The main reason for the success of these so-called early synthetic dyes was that they were much cheaper to produce. The starting material was coal tar, which was a waste product of the oil industry. Dyeing became much easier and faster and the range of different colors expanded enormously. Within just a few decades the natural-dye industry in Europe collapsed, as the early synthetic dyes replaced natural dyes on the market. The early synthetic dyes can be divided in four groups: acidic, basic, mordant or direct.

While the first two groups comprise the well-known water-soluble anionic and cationic dyes, the latter two may require clarification. A mordant dye requires a mediating compound (the “mordant”) to bind to the fiber material, for which it otherwise has little or no affinity. As most modern and early synthetic mordant dyes are dichromates and chromium complexes, mordant dyes are often also referred to as chrome dyes. Direct dyes (or substantive dyes) are water-soluble dyes, which are directly applied to fibres (e.g., cotton) as cellulose from an aqueous solution.

Analysis of dyes from cultural-heritage objects, such as textiles, furniture and paintings, is highly relevant to gain knowledge about the history and creation of objects and information on their original appearance. Such knowledge is essential to develop conservation and preservation strategies. However, the characterization of early synthetic dyes is a great challenge, because of (i) the sheer number of dyes that were synthesized, produced and used following their discovery, (ii) the impurity of the dyes, which were prepared so as to yield brilliant colors, rather than pure materials, and (iii) the chemical instability of many synthetic dyes. In a sample obtained from a historical object (while minimally affecting the material integrity of

* Corresponding author at: University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical-Chemistry Group, Science Park 904, 1098 XH Amsterdam, The Netherlands.

E-mail address: B.W.J.Pirok@uva.nl (B.W.J. Pirok).

valuable cultural heritage), we may expect (i) a number of different dyes from a wide range of possibilities, consisting of (ii) a number of closely related chemical compounds and, potentially, (iii) a variety of degradation products from these compounds. Every synthetic dye may give rise to a complex set of analytes in a real sample. A single art (e.g., tapestry) object may easily give rise to some 10–20 different samples, each containing 10–20 dye-related compounds, often from several of the above classes. A universally applicable analytical method should, therefore, be capable of separating hundreds of dyes.

The complexity, limited chemical stability and non-volatility of synthetic dyes renders high-performance liquid chromatography (HPLC) an obvious choice for analysis. Although hardened paint layers often consist of cross-linked polymers, dyes are soluble and/or extractable.

Both in art science [1,2] and in food science [3–5] researchers have utilized the strong, often distinctive absorption bands in the UV–vis spectrum intrinsic to many natural and synthetic dyes for detection after HPLC separation. Chromatographic methods employing UV or diode-array detectors (DAD) provided excellent robustness in combination with great sensitivity (low detection limits) and selectivity.

Mass spectrometry (MS) has been another popular technique for dye analysis since 1993 [6], because of its high sensitivity and ability to obtain structural information on unknown compounds. However, MS by itself cannot be used for the qualitative and quantitative analysis of complex mixtures, due to various adverse effects (e.g., ionization suppression, discrimination) in the ionization chamber. The coupling (or “hyphenation”) of HPLC with MS [7,8] in many ways represents the best of both worlds.

Regardless of the detection technique, a strong limiting factor for hyphenated techniques is the limited chromatographic peak capacity. This implies that unresolved compounds in divergent concentrations may be introduced into the MS at the same time, challenging the dynamic range of the technique and jeopardizing quantitation through matrix effects. Complex mixtures, such as samples containing synthetic dyes demand a separation system with higher peak capacities.

In comprehensive two-dimensional liquid chromatography (LC \times LC), two very different (“orthogonal”) separation dimensions are combined. The total peak capacity of the separation system approaches the product of the peak capacities of the individual separation dimensions. Despite this clear need, to our knowledge LC \times LC has not yet been applied for the analysis of synthetic dyes.

In this work, we present a comprehensive two-dimensional liquid-chromatography separation system utilizing a strong ion-exchange system operated under gradient conditions in the first dimension and a very fast ion-pair reversed-phase gradient-elution system in the second dimension. We discuss various considerations that affect method development of the two individual dimensions and we demonstrate the separation of a complex, degraded mixture of synthetic dyes.

2. Experimental

2.1. Instrumental

An Agilent 1290 Infinity 2D-LC system (Agilent, Waldbronn, Germany) was used for all experiments in this study. The system was comprised of two Infinity 1290 binary pumps (G4220A), an Infinity 1290 diode-array detector (G4212A) equipped with an Agilent Max-Light Cartridge Cell (G4212-6008, $V_0 = 1.0 \mu\text{L}$), an Infinity 1290 autosampler as injector and two Infinity 1290 thermostated column compartments (G1316C). In the column compartment

for the second dimension a 2-position 8-port valve (G4236A) with 60- μL loops was installed.

To protect the first-dimension column, an Agilent 1290 Infinity in-line filter was utilized in front of the column. For anion-exchange chromatography experiments, an Agilent PL-SAX column (PL1951-3802, 150 \times 2.1 mm i.d., 8- μm particles, 1000- \AA pore size) was used. For (ion-pair) reversed-phase chromatography, the utilized column was an Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (959941-902, 50 \times 4.6 mm, 1.8- μm particles) column. The needle was set to draw and eject at a speed of 10 $\mu\text{L}/\text{min}$ and to allow 2 s of equilibration time. The dwell volumes for the first and second dimension were approximately 65 μL and 55 μL , respectively. Data was recorded at several wavelengths throughout the study at 40 Hz. The system was controlled by a computer with Agilent OpenLAB CDS Chemstation Edition (Rev. C.01.04 [35]) software.

Data was processed and analysed by software written in house in a MATLAB 2013a (Mathworks, Woodshole, MA, USA) environment.

For one-dimensional LC experiments, the flow delivered by the first-dimension pump was guided to the autosampler and the first-dimension column through the in-line filter. This column was then coupled directly to the DAD detector.

2.2. Chemicals

All solutions were prepared with deionised water (Arium 611UV, Sartorius, $R = 18.2 \text{ M}\Omega \text{ cm}$, Germany). Acetonitrile (ACN, LC-MS grade) was obtained from Avantor Performance Chemicals (Deventer, The Netherlands). Methanol (ULC/MS grade) was obtained from Biosolve (Valkenswaard, The Netherlands). Ammonium sulphate (BioXtra, $\geq 99\%$), formic acid (reagent grade, $\geq 95\%$), tetramethylammonium-hydroxide solution (TMA, 25% in water) and tetrabutylammonium-hydroxide solution (TBA, 40% in water) were obtained from Sigma-Aldrich (Darmstadt, Germany).

54 samples of authentic dyestuff from the period 1850–1920 were obtained from the reference collection of the Cultural Heritage Agency of the Netherlands (RCE, Amsterdam, The Netherlands). Table S1 (Supplementary material) gives an indication of some of the possible dye structures. The actual samples are considerably more complex, because possible impurities and degradation products are not listed in the table.

2.3. Analytical conditions

2.3.1. Sample preparation

For each dyestuff an approximately 5000-ppm solution (by weight) was prepared in water/methanol 1:1 (v/v). Next, 100 μL of each dyestuff solution were combined to form a solution of all dyestuffs at individual concentrations of approximately 100 ppm.

2.3.2. Methods

For the development of an anion-exchange-chromatography method, the Agilent PL-SAX column (see Section 2.1) was used. The flow rate was set to 0.5 mL/min. The mobile phase consisted of water/acetonitrile 1:1 [v/v] (Mobile Phase A), and 100 mM ammonium sulphate in water/acetonitrile [v/v] 1:1 (Mobile Phase B). The column oven was set to maintain a temperature of 25 $^{\circ}\text{C}$. The injection volume was 1.0 μL . The binary pump was set to gradient-elution mode with a time program of 16 min: 0 to 0.5 min, isocratic at 100% A; 0.5–10.5 min, linear gradient to 100% B; maintained at 100% B for 4 min; 14.5–15.0 min, linear gradient to 100% A; maintained at 100% A for 1 min.

The ion-pair reversed-phase gradient method was developed on the Agilent ZORBAX Eclipse Plus C18 RRHT column (see Section 2.1). The flow rate was set to 1.0 mL/min. Buffers were prepared containing 10 mM of TMA or TBA in water, brought to pH 3.0 with formic

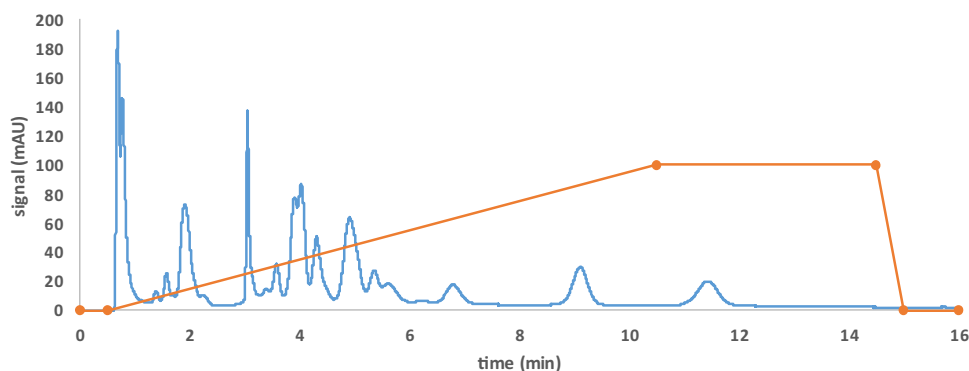


Fig 1. Overlay of UV chromatograms of the separation of a mixture of 54 synthetic dyes by strong anion-exchange chromatography with ammonium sulphate in the mobile phase. Gradient analysis, mobile phase (A) water/acetonitrile (1:1), (B) 100 mM ammonium sulphate in water/acetonitrile (1:1). UV detection at 254 nm. Flow: 0.5 mL/min, Column: Agilent PL-SAX 150 × 2.1 mm i.d., 8- μ m particles. Injection volume: 1.0 μ L.

acid. Mobile phase A consisted of buffer/acetonitrile 95:5 (v/v) and mobile phase B was acetonitrile/buffer 95:5 (v/v). The injection volume was set to 1.0 μ L. The binary pump was set to gradient-elution mode with a time program of 15 min: 0–0.5 min, isocratic at 100% A; 0.5–12.5 min, linear gradient to 100% B, maintained at 100% B for 0.5 min; 13.0 to 15.0 min, linear gradient to 100% A.

For the LC \times LC method, the above IEC and ion-pair RPLC methods were combined. The injection volume was set to 20 μ L. The temperature in both column ovens was set to 25 °C. The modulation time was 2 min. In the first dimension, the Agilent PL-SAX column was used and the mobile phase consisted of water/acetonitrile 1:1 [v/v] (Mobile Phase A), and 100 mM ammonium sulphate in water/acetonitrile [v/v] 1:1 (Mobile Phase B). The flow rate was set to 10 μ L/min. The binary pump was set to gradient-elution mode with the following time program: 0 to 10 min isocratic at 100% A; 10 to 190 min, linear gradient to 100% B; 190–200 min, linear gradient to 100% A; maintained at 100% A for 40 min. In the second dimension, the Agilent ZORBAX Eclipse Plus C18 RRHT column was used and the mobile phase consisted of buffer/acetonitrile 95:5 (v/v), brought to pH 3.0 with formic acid as mobile phase A and acetonitrile/buffer 95:5 (v/v) as mobile phase B. The flow rate was set to 2.4 mL/min. The binary pump was set to execute a gradient program: 0 to 1.5 min, linear gradient from 100% A to 100% B; 1.5 to 1.6 min, linear gradient to 100% A; maintained at 100% A for 0.4 min, until the next modulation.

2.4. Compound identification

For tentative identification, UV–vis spectra were recorded at all apices in the LC \times LC data from 210 to 640 nm with steps of 2 nm. The spectra and retention times in the RPLC separation were compared with the in-house database of the Dutch Cultural Heritage Agency (RCE). In some cases more than one chemical structure is associated with the same dye. Such isomers and/or homologs result in several peaks in the chromatogram. For example, for marten yellow more than two peaks can be expected. All extracted UV–vis spectra that were tentatively identified are provided in the Supplementary materials (Table S2).

3. Results & discussion

To make full use of the very high peak capacity and selectivity of an LC \times LC method, it is imperative that the two individual separation mechanisms are as different (“orthogonal”) as possible. Taking into account the concept of sample dimensionality [9], the family of early synthetic dyes can be described as mainly featuring variation in (i) the presence and number of ionic moieties and (ii) hydrophobicity. Ion-exchange chromatography (IEC) and ion-pair

reversed-phase chromatography (IP-RPLC) were explored as possible separation mechanisms to cover these two respective sample dimensions as much as possible.

3.1. Strong anion-exchange chromatography as first dimension

Ion-exchange chromatography was an obvious option for the separation of the early synthetic dyes, because they consist of organic molecules with divergent charged states. Because most of the analytes are known to be acids, the separation of anionic species was targeted in this dimension. Stationary phases used in IEC are notorious for their long equilibration times. With the second dimension very time-restricted, IEC was deemed to be only acceptable as first dimension.

In strong anion-exchange, the stationary phase features cationic groups (typically a quaternary ammonium) which are insensitive to pH changes. A high-ionic strength (salt or buffer) solution is required to facilitate elution from the stationary phase. Such mobile phases are often undesirable from a detection perspective. Initial experiments on a strong anion-exchange (SAX) column yielded relatively large retention factors for multi-valent anions. One possible solution may be to replace the SAX column by a weak anion-exchange column that features ionisable groups, the charge of which can be adjusted by varying the pH. Because the population of synthetic dyes respond differently to pH, which increases the complexity of the separation, and because synthetic dyes allow the use of a UV detector, a strong anion-exchange was chosen.

Depending on the nature of the stationary phase, the overall retention obtained also involves hydrophobic interactions between the analytes and the stationary phase [10]. To minimize these, the mobile phases contained 50% acetonitrile at all times. Among the salts tested only ammonium sulphate allowed elution of di-valent and tri-valent synthetic dyes within a reasonable decent time using gradient elution.

Fig. 1 displays the chromatogram for the separation of 54 synthetic dyestuffs using the Agilent PL-SAX column at a flow rate of 0.5 mL/min. The large convoluted peak around 0.8 min represents the dead time, at which all neutral and cationic dyes co-elute. Between $t=1$ and $t=3$ min, the mono-valent anionic dyes were found to elute, followed by the di-valent anionic dyes (up to $t=7$ min). The peaks observed after $t=8$ min were found to be the tri-valent anionic dyes. Overall, the separation performance of this column was deemed good, with the acids and bases separated from one another and decent resolution between the group of mono and di-valent anions.

However, salt concentrations above 75 mM ammonium sulphate were found to be required for the elution of the tri-valent anionic compounds. Such concentrations are somewhat unattractive

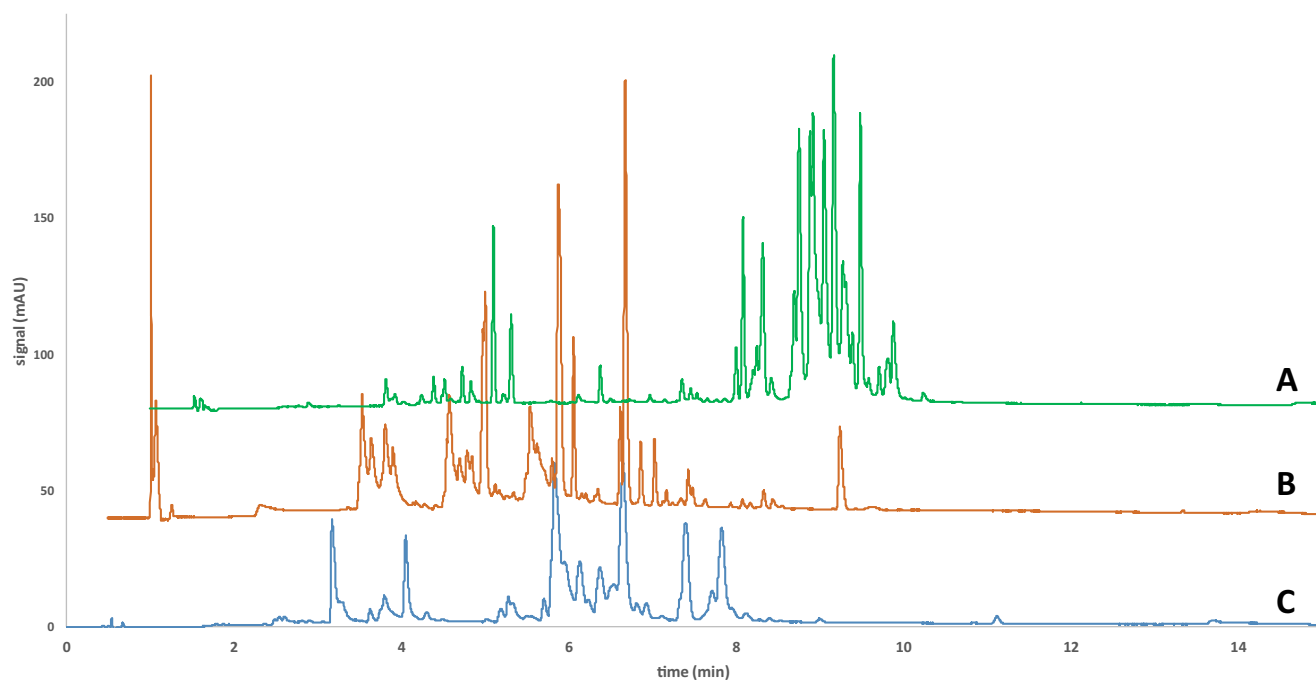


Fig. 2. UV chromatograms of the separation of a mixture of 54 synthetic dyes by (ion-pair) RPLC with (a) 10 mM TBA (b) 10 mM TMA, and (c) 10 mM NaOH (no-ion pair) in the mobile phase. Gradient analysis, mobile phase (A) 10 mM ion-pair in water/acetonitrile 95:5 [v/v], pH 3 (B) acetonitrile/water 95:5 [v/v], pH 3. UV detection at 450 nm. Flow: 1.0 mL/min, Column: Agilent ZORBAX Eclipse Plus RRHT 50 × 4.6 mm i.d. 1.8- μ m particles. Injection volume: 1.0 μ L.

tive first-dimension pumping system (necessitating thorough rinsing before system shutdown), but the detection is not impaired, because the first-dimension effluent is significantly diluted during the second-dimension separation.

3.2. Fast ion-pair reversed-phase chromatography as second dimension

One particular drawback of previously developed HPLC methods for the separation of early synthetic dyes was their limitation to one subgroup (e.g., only acidic or basic dyes). Due to the fact that acidic dyes comprise the most important class of the early synthetic dyes, a great deal of effort was invested in their separation using methods that often involved ion-pair chromatography with an alkyl-ammonium-based ion-pair reagent [2,4,7,11]. Our objective in developing a second-dimension LC method is to cover the broadest possible range of dyes in a fast (gradient-elution) chromatogram.

Initially, RPLC experiments were performed with gradients running from aqueous buffers to acetonitrile at several different pH values. Some of the possible structures (Supplementary materials; Table S1) contain carboxylic-acid functions, which are protonated at low pH. As a result, more retained peaks were observed in chromatograms at pH 3 than at pH 7 (comparison not shown). A representative chromatogram of the complete dye mixture obtained at pH 3 using a formic-acid buffer (aqueous solution of 10 mM NaOH brought to pH 3 with formic acid) as solvent A and acetonitrile as solvent B is shown as Fig. 2c. Fast one-dimensional separations of the complex mixture often showed significant baseline drifts at a “universal” detection wavelength of 254 nm. The one-dimensional chromatograms shown in Fig. 2 were, therefore, recorded at 450 nm. This problem was not encountered in LC × LC separations. Tentatively, the material in the sample that caused the baseline drift in one-dimensional LC was divided across many different fractions in the LC × LC experiments, reducing the intensity. These were recorded at 254 nm and did not show significant baseline disturbances (see Fig. 3, below).

Next, the possible effects of ion-pair reagents were investigated. The effect of the ion-pair concentration was investigated using 5 or 10 mM TBA in the mobile phase (Supplementary material Fig. S1). Better peak shapes were observed at 10 mM TBA. An ion-pair concentration of 10 mM was selected for further experiments.

The dye mixture contains many compounds with sulphonate groups. These contribute to low retention in RPLC, irrespective of the pH. To enhance retention of sulphonate-containing dyes positively charged ion-pair agents are typically used. Their actions are to (i) neutralize any anionic moieties and (ii) to enhance retention based on hydrophobicity through the alkyl chains on the ammonium ion. In Fig. 2b and a one-dimensional chromatograms are shown for mobile-phases containing 10 mM tetramethylammonium (TMA) and 10 mM tetrabutylammonium (TBA), respectively, at pH 3 (prepared from the hydroxides, analogously to the mobile phase of Fig. 2c). When comparing Fig. 2c and b, we observe that the addition of TMA leads to a better spreading of peaks throughout the chromatogram. Also, many more peaks are observed in Fig. 2b than in Fig. 2c. When comparing Fig. 2b and a, we observe a shift towards higher retention and a bunching of peaks in the latter half of the chromatogram. Clearly, the best spread of peaks is observed in Fig. 2b. A strong effect of the ion-pair on retention is undesirable for a second reason. If retention is largely determined by the number of anionic groups we may expect a strong correlation between the retention in ion-pair LC and in anion-exchange chromatography. In other words, retention in the two dimensions will be highly non-orthogonal. Relative to TBA, the carbon atoms of TMA induce little additional hydrophobic retention and the separation selectivity will be largely determined by the hydrocarbon skeleton of the dye molecules. For all these reasons, TMA was selected as ion-pair reagent for the LC × LC separations.

By strongly decreasing the ion-pair concentration and simultaneously increasing the acetonitrile concentration in the second-dimension gradient, we envisaged rapid elution of all compounds within the time allocated for the second-dimension separation. Ion-pairing is expected to be most significant at the beginning of the

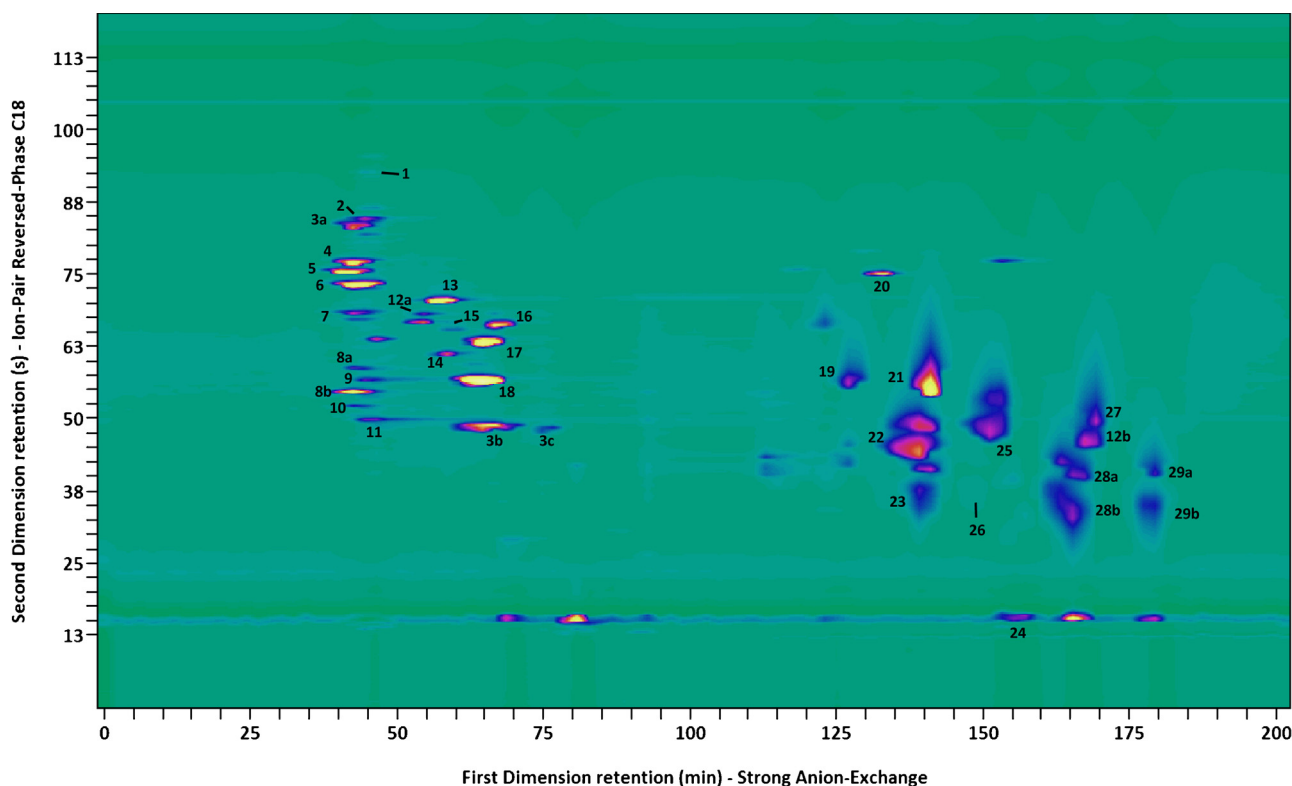


Fig. 3. UV chromatogram of the LC \times LC separation of a mixture of 54 synthetic dyes. First dimension: strong-anion-exchange chromatography with ammonium sulphate in the mobile phase, gradient analysis, flow rate: 10 μ L/min. Second dimension: ion-pair RPLC with TMA as ion-pair, gradient analysis, 2.4 mL/min, flow rate. loop size: 60 μ L, Modulation time: 2 min, injection volume: 20 μ L. UV detection at 254 nm. Tentative identification: 1. Auramine (trace); 2. Diamond green G; 3. Quinoline yellow; 4. Crystal violet; 5. Rhodamine G; 6. Rhodamine B; 7. Diamond green B; 8. Safranin T; 9. Methyl violet; 10. Fuchsin; 11. Methylene blue; 12. Martius yellow; 13. Pitric acid; 14. Flavazine L; 15. Naphtol yellow S; 16. Fast red AV; 17. Metanil yellow; 18. Crocein orange G; 19. Victoria blue B; 20. Eosine; 21. Wool red B; 22. Ponceau RR; 23. Fast acid magenta B; 24. Indigo carmine; 25. Crystal ponceau; 26. Patent blue V (trace); 27. Amaranth; 28. Orange GG; 29. Amido naphthol red G. (See Supplementary material Table S2 for extracted UV-vis spectra of all peaks). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

gradient, whereas the organic modifier concentration is the dominant factor in the latter half of the gradient.

3.3. LC \times LC analysis of synthetic dye mixtures

Based on the one-dimensional chromatograms (Figs. 1 and 2b), ion-exchange chromatography was combined with TMA-based ion-pair RPLC in LC \times LC experiments. A typical result is shown in Fig. 3.

Clearly, there is much more separation in LC \times LC than in any of the one-dimensional chromatograms. This allows using of a universal detection wavelength (254 nm) without any baseline problems. A rough estimate of the attained peak capacity can be obtained by dividing the duration of the gradient in each dimension (t_G) by the typical peak width (w), i.e., ${}^2Dn \approx {}^1n \times {}^2n \approx ({}^1t_G/{}^1w) \times ({}^2t_G/{}^2w) \approx (180/7) \times (1.5/0.017) = 2269$ (based on the peak eluting at ${}^1t_R = 127$ min and ${}^2t_R = 75$ s). The attained theoretical peak capacity allows the LC \times LC method to provide high-resolution fingerprints of extracted dyes in art-conservation studies and valuable insight in dye degradation and possible conservation strategies. For this peak chromatographic efficiency was estimated based on the estimated retention factor at the moment of elution ($\sqrt{N} = t_0(1 + k_e)/\sigma_t$). This yielded estimates of ${}^1N \approx 2200$ (reduced plate height ${}^1h \approx 3$) and ${}^2N \approx 4500$ (${}^2h \approx 6$).

In some cases, several compounds are observed for one dye. This is also reflected in the tentative identification in Fig. 3, where, for example, quinoline yellow is found multiple times due to the different number of sulfonate groups found for different homologues. Unexpected retention times in the first-dimension were observed for eosine, pitric acid and one component of martius yellow which

both eluted rather late. We have no explanation for these long retention times. Possibly, the large number of halogen groups in eosine and of nitro groups in pitric acid and in the martius yellow component affect the retention time in ion-exchange chromatography.

The orthogonality of the separation was addressed based on all (34) major peaks, using the asterisk approach [12] as illustrated in Fig. S2 (Supplementary material). A value of 63% was obtained, which is similar to values typically encountered in GC \times GC separations of oil samples [12].

One particular concern is the significant breakthrough [13] in the second dimension for several fractions. This is primarily the result of the constant use of 50% acetonitrile throughout the first-dimension gradient to enhance the orthogonality of the LC \times LC separation system by the prevention of retention based on hydrophobicity. A compromise had to be struck between hydrophobic retention in the first dimension and breakthrough in the second dimension. An alternative solution may be to use a weak anion-exchange (WAX) column that is not based on a polymeric stationary phase. This would allow (i) a lower fraction of organic modifier to be present in the first dimension mobile phase to mitigate breakthrough in the second dimension and (ii) a more efficient gradient system for the multi-valent synthetic dye species by utilizing a pH gradient to neutralize the stationary phase instead of using undesirably high salt concentrations. However, this route was not explored during the course of the present study. We deemed the degree of observed breakthrough to be acceptable, as the main goal of the project was to develop an LC \times LC method for qualitative analysis. Further improvements can be made by making full use of the potential of UHPLC in the second dimension. Finally, the applica-

tion of an exponential gradient instead of a linear one may improve the band spreading, reduce the band broadening observed for the multivalent synthetic dyes, and significantly reduce the analysis time.

4. Concluding remarks

We have demonstrated the separation of a highly complex mixture of degraded synthetic dyes using comprehensive two-dimensional liquid chromatography with gradient elution in both dimensions. Strong anion-exchange chromatography was used in the first dimension. Because of a constant presence of 50% acetonitrile throughout the gradient, hydrophobic retention was minimized and separation of the dyes was based on their ionic character (charge and effective size). In the second dimension reversed-phase LC was used. By using a small ion-pair cation (tetramethylammonium) the retention was mainly determined by the molecular structure of the dye. As a consequence, good orthogonality between the two separations was observed. The peak capacity of the developed LC \times LC method is in the order of 2000, which constitutes a great step forward in studies into the composition and the state of degradation of synthetic dyes. The total analysis time was about three hours. Given the scarcity and value of art-derived samples and the efforts needed to obtain useful extracts, such an analysis time is not prohibitive in the field. The new fingerprinting method will provide insights in the dyes used to create various art objects and in their current extent of degradation. Ultimately, these insights may result in guidelines for the conservation of cultural-heritage objects.

Acknowledgments

The MANIAC project is funded by the Netherlands Organisation for Scientific Research (NWO) in the framework of the Programmatic Technology Area PTA-COAST3 of the Fund New Chemical Innovations (project C.2322.0291). Agilent Technologies is acknowledged for supporting this work. The Dutch Institute for Cultural Heritage (RCE) is kindly acknowledged for providing the aged synthetic dye powder samples.

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.chroma.2016.01.070>.

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