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Fast throughput, highly sensitive determination of allergenic disperse dyes in textile products by use of sample composition

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ABSTRACT

A simple, highly sensitive and fast procedure for the control of allergenic disperse dyes in textile products was optimized. The method is based on ultrasound assisted extraction of textile samples with 20 mL of methanol under controlled conditions (15 min, 70 °C) followed by separation and analysis by LC–MS–MS. The sample preparation process was optimized by means of a surface response experimental design and provided quantitative recoveries of dyes, much better than the poor recoveries provided by current standard procedures. The chromatographic separation was optimized by means of computer-assisted method development by use of a special chemometric tool developed specifically for LC–MS systems, as previously reported by the authors. The result is a rapid chromatographic procedure that enables accurate quantification, at very low concentrations, of all 23 allergenic and/or carcinogenic disperse dyes considered. Matrix effects in the LC–MS procedure were studied. Under the experimental conditions, both conventional and strategic sample composition are proposed as efficient procedures that reduce the costs and work involved in the control of allergenic dyes in finished textile products. The benefits of strategic sample composition are demonstrated by means of an example case study, and the pros and cons of preparing the composite samples from sample extracts or directly from textile products are discussed.

1. Introduction

Disperse dyes are low molecular weight organic dyes that are derivatives of azo, anthraquinone and other compounds. Essentially planar and non-ionic with attached polar functional groups, these dyes have the capacity to slide between the tightly packed polymer chains in polyurethane and other synthetic fabrics, while the polar groups improve their solubility in water; the dipolar bonding between the dye and the polymer also affects the color of the dye [1]. Initially developed for dyeing cellulose acetate fibers, the main application of disperse dyes is now in dyeing polyester, although they may also used for nylon, polyacrylonitrile and many of the newer synthetic hydrophobic fibers, and can be found in a vast variety of consumer products including textiles, toys, paper, etc. Regrettably, a number of these dyes are contact dermatitis sensitizing agents [2–6]. Moreover, some of the dyes that contain azo groups in their structure can be reduced by azoreductases present in intestinal bacteria, liver enzymes and skin-surface micro-flora, thus forming potentially or known carcinogenic aromatic amines [7,8]. According to Hatch and Maibach [9], 49 dyes have been identified as contact allergens and two thirds of these are disperse dyes, although they represent a very small fraction of the total of about 8000 commercially used dyes.

Increased awareness of the potential risk to consumer health associated with exposure to such dyes led to German legislation coming into force in 1996; this legislation restricts the use of several allergenic disperse dyes for dyeing textile products that may come into direct and prolonged contact with human skin [10]. This awareness also led to the development and issue of the DIN 54231 standard procedure [11] for the analysis of 9 disperse dyes in textile products, which appears to be a routine procedure in many analytical laboratories.

A number of papers have been published as regards the determination of disperse dye residues in wastewaters [12–14], food and toys [15,16], and in the context of forensic studies [17–19]. Generally, solid phase or solvent extraction processes are applied for sample preparation. Capillary electrophoresis and liquid chromatography with UV or mass spectrometric detectors are used to separate and quantify extracted dyes. However, studies on textiles (other than for forensic studies) and related materials are scarce [20]. It should be stressed that only a few dyes are considered in the DIN 54231 standard procedure, in relation to the number of allergenic disperse dyes actually identified. Currently, at least 20 allergenic dyes are considered in commercial consumer care protocols issued by textile retailers worldwide. Furthermore, the detection limits are rather high with thin layer chromatogra-

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Table 1
Allergenic disperse dyes considered in the study.

Number	Color index denomination [25]	CAS number	Chemical class	Notes
1	CI Disperse Blue 1	2475-45-8	Anthraquinone	a,b
2	CI Disperse Blue 102	69766-79-6	Monoazo (heterocyclic)	
3	CI Disperse Blue 106	12223-01-7	Monoazo (heterocyclic)	a
4	CI Disperse Blue 124	61951-51-7	Monoazo (heterocyclic)	a
5	CI Disperse Blue 26	3860-63-7	Anthraquinone	
6	CI Disperse Blue 3	2475-46-9	Anthraquinone	a
7	CI Disperse Blue 35	56524-77-7	Anthraquinone	a
8	CI Disperse Blue 7	3179-90-6	Anthraquinone	
9	CI Disperse Brown 1	23355-64-8	Monoazo	
10	CI Disperse Orange 1	2581-69-3	Monoazo	
11	CI Disperse Orange 3	730-40-5	Monoazo	a
12	CI Disperse Orange 37/76/59	13301-61-6	Monoazo	a
13	CI Disperse Red 1	2872-52-8	Monoazo	a
14	CI Disperse Red 11	2872-48-2	Anthraquinone	
15	CI Disperse Red 17	3179-89-3	Monoazo	
16	CI Disperse Yellow 1	119-15-3	Nitrodiphenylamine	
17	CI Disperse Yellow 3	2832-40-8	Monoazo	a,b
18	CI Disperse Yellow 39	12236-29-2	Indigoid	
19	CI Disperse Yellow 49	54824-37-2	Indigoid	
20	CI Disperse Yellow 9	6373-73-5	Nitrodiphenylamine	
21	CI Disperse Orange 11	82-28-0	Anthraquinone	c
22	CI Disperse Yellow 23	6250-23-3	Diazo	c
23	CI Disperse Yellow 7	6300-37-4	Diazo	

[a] Dyes included in German l Law LMGB (1/1/96) and in the DIN 54231 standard.
[b] Potentially carcinogenic.
[c] Releasing carcinogenic aromatic amines by reductive decomposition.

phy (TLC) or diode array-high performance liquid chromatography (HPLC-DAD), both of which are accepted in the DIN 54231 standard procedure, as well as the LC–MS procedure. In general, laboratories using the DIN procedure accept 5 mg L^{-1} as the detection limit in extracts, which for 0.5 g samples (as recommended) means a practical detection limit of 75 μg g^{-1} in consumer products. Moreover, the available results on international proficiency tests for laboratories, many of which use the DIN 54231 standard procedure, have indicated poor recoveries and reproducibility [21].

The globalized economy has dramatically changed many quality control practices because retailers are now selling goods produced outside their area of control. This means that in many cases quality and safety control tests must be carried out on finished goods, involving huge additional costs. Although the TLC approach described in the DIN 54231 standard procedure can be used as a screening process to enable detection of controlled disperse dyes so that only the positive samples are analyzed further (densitometry in the case of TLC or the complete analysis by HPLC-DAD or LC–MS), the time and handling involved are considerable.

A highly sensitive procedure for the analysis of 23 disperse dyes is presented here. The procedure uses LC–MS–MS to separate and quantify the dyes extracted from textile samples. The extraction process was optimized by means of factorial designs and the chromatographic separation was developed by means of computer assisted method development, which uses a specific tool for LC–MS separations [22]. Because the final objective is to reduce the cost and to speed up the routine analytical processes for finished consumer products, sample composition by application of the principles of strategic sample composition [23,24] is proposed.

2. Experimental

2.1. Reagents, standards and special samples

The mixture of dyes considered included the 23 disperse dyes listed in Table 1. Dyes number 2, 3, 4, 9, 11, 12 and 18 were supplied by Dr. Ehrenstorfer GMbH (Augsburg, Germany). Dyes number 5, 7, 8, 14, 15, 16, 19 and 21 were supplied by the Institute for Engineering of Polymer Materials and Dyes (Zgierz, Poland). The remaining dyes in Table 1 were supplied by Sigma–Aldrich (Steimheim, Germany). Individual stock and diluted solutions and mixtures of dyes were prepared in acetonitrile:water (60:40), filtered through 0.22 μm Durapore syringe filters (Millipore) and stored at −18 °C when not in use.

HPLC gradient grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid was supplied by Sigma–Aldrich (Madrid, Spain). Ultrapure water was produced in the laboratory with a Milli-Q gradient system from Millipore (Bedford, MA, USA).

A few dyed polyester textiles were prepared in-house to have available fully controlled real samples containing disperse dyes of known nature and origin (CI Disperse Blue 106 and CI Disperse Yellow 23). The material containing the CI Disperse Yellow 23 was used to optimize the sample extraction process whereas the one containing the CI Disperse Blue 106 was used to control the sample composition process. The remaining samples considered in this study were commercial products obtained from local stores.

2.2. Apparatus

Samples were ground and homogenized in a Retsch SM100 cutting mill (Haan, Germany), sieved (2 mm) and stored in polypropylene bags at room temperature until analysis. Grinding samples is only necessary for developing sample composition schemes based on raw textile products, but is not necessary for application of the described procedure to individual samples.

Extraction of dyes from samples was carried out with a Branson Sonifier S-450D (400 W output power), with a temperature control system (Danbury, CT, USA).

Two chromatographic systems were used in the study. A Waters Alliance 2695 quaternary solvent module (Milford, MA, USA) equipped with a photodiode array detector (Waters 2996) was used to develop the retention model for the set of dyes under study. This equipment has a dwell volume of 0.85 mL, and an extra column volume of 0.12 mL. It was controlled by Empower 2 software (Waters). In general, standard HPLC equipment can be used to develop retention models without blocking LC–MS instrumentation, with the only condition that the same column is used in both

apparatuses. The high-pressure mixing instrument used to run the LC–MS experiments was based on two Varian ProStar 210 solvent delivery modules (Walnut Creek, CA, USA), equipped with an autosampler (Varian ProStar 410) and a triple quadrupole MS detector (Varian MS 1200L) operated by the Varian MS Workstation V 6.9.1. This instrument has a dwell volume of 0.59 mL and negligible extra column volume. A Phenomenex Gemini C18 analytical column of 50 mm length, 2.0 mm internal diameter and 5.0 μm particle size (Torrance, CA, USA), was used in all experiments. In addition, a guard cartridge (Gemini C18 ODS, 4 mm length, 2.0 mm i.d.) was used to protect the analytical column.

2.3. Sample extraction

The sample (0.5 g) was divided into small pieces, or in the case of raw composite samples was ground, then placed in an extraction vial with 20 mL of methanol. The vial was sealed with an aluminum cap furnished with PTFE-faced septum then placed in a water bath maintained at 70 °C and extracted by ultrasound during 15 min by operating the ultrasound probe immersed in the water bath at 25% of full capacity. The extracts were cooled to room temperature, filtered through 0.22 μm membrane filters, and the solid-sample residue discarded.

2.4. Chromatographic separation

Chromatographic conditions for the LC–MS–MS separation of dyes were developed by means of the PREGA-LC–MS module [22], a freeware chemometric tool for the development and optimization of reversed-phase liquid chromatography developed in the author's laboratory. This software creates a retention model for the peaks to be separated by running a series of isocratic elutions. The initial retention model is iteratively calibrated against some (usually two or three) gradient elutions of different shape and with different operational conditions. The final retention model is used for the computer assisted unattended optimization of the separation. For this purpose, the mass spectra of all peaks and backgrounds are entered and the four most intense peaks selected as quantification and qualification ions. This information is used to calculate the so-called selectivity-matrix and used together with the runtime as the objective function in the multi-objective Pareto optimization [26] process, which is based on an evolutionary algorithm. Full details of this chemometric tool are reported elsewhere [22], and are available at http://www.usc.es/gcqprega, where the application can be downloaded free of charge.

The final optimized chromatographic conditions are summarized in Table 2.

2.5. Sample composition

Composite samples were prepared by following the principles of strategic sample composition [23,24] (SSC). In conventional sample composition, if a composite sample produces a positive result, all the individual samples involved must be individually re-analyzed in order to evaluate which are responsible for the positive result, and to estimate the values for the analytes in each individual sample. SSC on the contrary, involves the preparation and analysis of some additional composite samples made from combinations of the individual sample specimens considered. These samples are prepared according a particular composition-design matrix and the results elaborated by special regression techniques to provide an estimate of the concentrations of analytes in the individual samples. Because the composition-design matrices used in SSC are supersaturated matrices, the final number of analytical determinations is lower than in the conventional sample composition approach in case of positive samples. Full details of the SSC technique are reported elsewhere, as applied to a variety of practical situations [27–32]. The practical implementation of SSC requires the use of GAMICH [23], a chemometric tool developed in the author's laboratory and which can be accessed free of charge on demand.

Table 2
HPLC optimal chromatographic conditions for determination of disperse dyes by LC–MS obtained with the LC–MS module of PREGA software.

Column	Phenomenex Gemini C_{18} (50 mm × 2.0 mm × 5 μm)
Guard column	Phenomenex Gemini C_{18} Guard Cartridge (2 mm × 4 mm)
Mobile phase	A: Milli-Q (0.1% formic acid) B: AcN (0.1% formic acid)
Temperature	40 °C
Injection volume	10 μL

Elution program

Time (min)	Flow (mL/min)	%Modifier (B)
0	0.4	30
11	0.4	94

MS detector parameters

Drying gas API	Nitrogen, 1.45 bar at 300 °C
Nebulizer gas API	Nitrogen, 3.45 bar
Shield voltage	±600
Needle voltage	±5500
Colision gas	Argon, 2.1 mTorr

3. Results and discussion

3.1. Development of the LC–MS–MS procedure

The optimal MS parameters for detection and analysis of the disperse dyes considered are summarized in Table 3. These conditions were obtained by continuous infusion of the individual standard solutions by altering the voltage and collision energy controls on the mass spectrometer. Other common parameters in the MS detector are summarized in Table 2.

The retention model for peaks in the mixture of dyes was developed with the tools available in the PREGA v. 6 software. The retention model is the critical step in any computer assisted method development tool, and PREGA applies a two stage approach consisting of the development of a raw retention model based on the isocratic data available for peaks. This retention model does not usually enable extrapolation from the experimentally acquired values, therefore in order to obtain a powerful retention model that enables management of gradients outside the retention area covered by the raw model, the model was iteratively re-calibrated against some gradients of different shape and conditions. In the case of dyes, the raw retention model was developed by injecting the standards, in isocratic mode, in the range 30–80% of modifier (acetonitrile with 0.1% formic acid). At least three data points are needed to build the retention model for each peak. The raw model was calibrated against three gradient elutions (a linear gradient from 30 to 95% of modifier in 15 min at flow 0.5 mL min^{-1}; a curved gradient (curve 5 in the Waters solvent module programmer) also from 30 to 95% of modifier in 30 min at 0.5 mL min^{-1}, and finally a curved gradient (curve 8 in the programmer) from 30 to 85% in 25 min at 0.2 mL min^{-1}. All injections were carried out at 40 ± 1 °C. After recalibration, the retention model provided errors in retention times for peaks ranging from 0.1 to 1.3 min (average 0.57 min), and was considered reliable and robust for starting the optimization procedure. Modifier proportions in mobile phase below 30% were never attempted because of the low solubility of disperse dyes in aqueous solutions.

The optimization process in PREGA involves simulation of separations under isocratic conditions and any type and shape of gradients, and the evaluation of these simulated chromatograms

Table 3
Optimal MS parameters conditions for determination of disperse dyes. Q1 and Q2 are qualification ions. Quant is the quantification ion. CE are collision energies in multiple ions monitoring mode.

No.	Compound	ESI mode	Precursor ion, *m/z*	Quant, *m/z*	CE (V)	Capillary voltage (V)	Q1, *m/z*	CE 1 (V)	Q2, *m/z*	CE 2 (V)	Q1/quant (%)
1	CI Disperse Blue 1	(+)	268	252	−24.5	60	–	–	–	–	–
2	CI Disperse Blue 102	(+)	366	208	−17	52	147	−30.5	–	–	24.8
3	CI Disperse Blue 106	(+)	336	178	−15.5	40	147	−30.5	206	−11	14.2
4	CI Disperse Blue 124	(+)	378	220	−16	60	–	–	–	–	–
5	CI Disperse Blue 26	(+)	299	284	−20	72	266	−30.5	–	–	36.2
6	CI Disperse Blue 3	(+)	297	252	−17.5	44	235	−33	282	−15.5	28.1
7	CI Disperse Blue 35	(+)	285	270	−23	60	196	43.5	–	–	11.0
8	CI Disperse Blue 7	(+)	359	283	−31.5	52	314	−17.5	328	−18.5	63.4
9	CI Disperse Brown 1	(−)	477	431	9	−40	401	12	413	11.5	44.1
10	CI Disperse Orange 1	(+)	319	122	−20	56	169	−21	–	–	92.0
11	CI Disperse Orange 3	(+)	243	122	−15.5	44	226	−11	92	−22.5	80.4
12	CI Disperse Orange 37/76/59	(+)	392	351	−17.5	56	305	−31.5	–	–	5.0
13	CI Disperse Red 1	(+)	319	122	−20	56	169	−20.5	–	–	90.3
14	CI Disperse Red 11	(+)	269	254	−19.5	56	226	−28	–	–	58.7
15	CI Disperse Red 17	(+)	345	164	−23	36	177	−27.5	–	–	46.2
16	CI Disperse Yellow 1	(−)	274	166	12	−56	–	–	–	–	–
17	CI Disperse Yellow 3	(−)	268	134	18	−60	–	–	–	–	–
18	CI Disperse Yellow 39	(+)	291	130	−25.5	72	245	−23.5	–	–	84.1
19	CI Disperse Yellow 49	(+)	375	238	−13	40	223	−22.5	208	−36.5	9.0
20	CI Disperse Yellow 9	(+)	275	240	−11	32	194	−21	182	−31	27.14
21	Disperse Orange 11	(+)	238	223	−23	56	–	–	–	–	–
22	Disperse Yellow 23	(−)	301	196	26	−72	–	–	–	–	–
23	Disperse Yellow 7	(−)	315	210	26	−80	106	37	–	–	71.9

by means of an objective function. The process is based on an evolutionary algorithm and the optimal elution program is finally offered to the user. Although PREGA software was developed for optimizing chromatographic separations with conventional photometric detectors, the LC–MS module has been developed to deal specifically with chromatographic systems fitted with mass spectrometers as detectors. The main difference between the LC–MS module and the conventional PREGA modules is the objective function applied. Any of the published chromatographic response functions [33] or Pareto multi-objective optimality [34] may be used with conventional UV detectors, but a special multi-objective function has been developed for LC–MS, and is called the "selectivity matrix". The selectivity matrix accounts for potential spectral interferences between quantification and qualification ions used for the analytes. If the selected ions for any peak do not interfere with those selected for another peak, this means that the two peaks do not necessarily need to be separated in the column because the selectivity provided by the detector will enable accurate quantification of both peaks even if fully overlapped. On the contrary, if one or more ions are common to both peaks, these peaks must be separated. The required resolution between interfering peaks depends on the number of ions considered and the class of interfering ions (e.g. if there is no interference in the quantification ion, lower resolution is needed than in cases where peaks from the other analyte interfere with the quantification ions). In this way, a square selectivity matrix is built up, indicating the required resolution for each peak in the mixture in relation to the other peaks. For example, if two peaks exhibit different clean quantification and qualification fragments, those peaks can overlap without problem in the final optimized separation. Thus, the associated value in the selectivity matrix for that peaks may be zero or a convenient small value. On the contrary, for peaks showing more or less critical selectivity conflicts, the resolution values imposed by the selectivity matrix are large (1.5–2.0 usually). Thus, this process is equivalent to weigh each peak in terms of the resolution needed for that peak. The closer a simulated separation adheres to these requirements, the better the value assigned to the chromatogram in the optimization process. Obviously, the selectivity matrix is a multi-objective function with as many dimensions as peaks in the mixture to be separated. Additionally, the runtime is incorporated as an independent dimension to this objective function. It should be noticed, that using the selectivity matrix as defined here, the optimization process although apparently having a unique global optimum, may frequently derive in somewhat different experimental elution conditions because the complex multimodal character inherent to the response surfaces in chromatography. A detailed discussion of the selectivity matrix and the essential aspects of the LC–MS module approach to the computer assisted optimization of chromatographic separation are reported elsewhere [22]. Optimal elution conditions for the mixture of dyes are shown in Table 2 and the chromatographic traces for dyes under these optimal elution conditions are shown in Fig. 1.

The quality parameters of the chromatographic method developed were studied in terms of linearity, linear range, repeatability and reproducibility. Linearity was tested in the range 0.05–5 $\mu g\,mL^{-1}$ by injecting replicate standards at different concentrations. The commonly accepted detection limit in the DIN 54231 standard procedure is 5 $\mu g\,mL^{-1}$, although it is clearly stated in this procedure that lower detection limits can be obtained with mass spectrometry as the detection system (a detection limit of 0.7 $\mu g\,mL^{-1}$ is mentioned for C.I. Disperse Blue 1). Acceptable linearity was found for all compounds, with correlation coefficients of at least 0.99. Relative standard deviations for consecutive injections of standards at different concentration levels ranged from 0.3 to 5.9% and were maintained below 10% for injections on successive days. Individual instrumental detection limits, based on a signal-to-noise ratio of 3, for concentrations below 0.003 $\mu g\,mL^{-1}$ were obtained for all disperse dyes considered.

3.2. Extraction of dyes from samples

In order to control the parameters of the process, study of dye extraction from real samples was developed using a specially prepared textile material containing only one of the disperse dyes (CI Disperse Yellow 23). Preliminary experiments were developed following the conditions proposed in the DIN 54231 standard procedure [11] (0.5 g of sample, extracted during 30 min with 7.5 mL of methanol), in an attempt to extract the dye exhaustively from the sample by successive re-extractions. Normalized results for the polyester material containing the CI Disperse Yellow 23 are

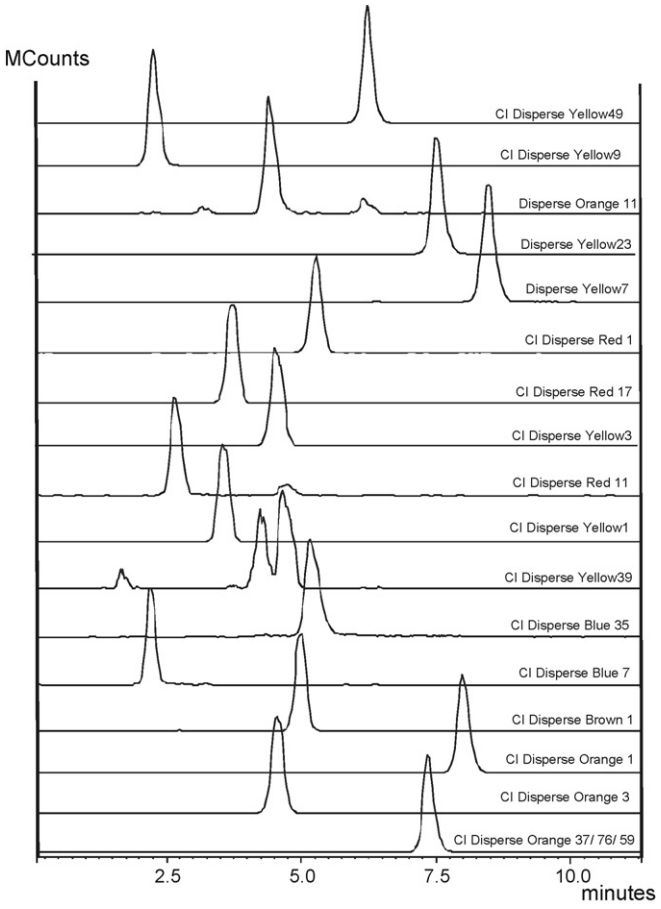

Fig. 1. Typical appearance of the LC–MS–MS chromatogram for the 23 allergenic disperse dyes obtained in MRM mode (1 μg mL^{-1} standard injected).

shown in Fig. 2. The procedure extracted only about 57% of the dye from the sample in a single extraction stage. Furthermore, changes in the extraction process parameters (the amount of solvent and the time of ultrasound-assisted extraction) affected the extraction results, although the solvent volume appeared to be more significant. Recoveries obtained with the same amount of solvent were comparable, despite the differences in time (5 and 30 min), whereas the extraction carried out with only 5 mL of methanol rather than 7.5 mL provided recoveries of less than 40% in the first single extraction.

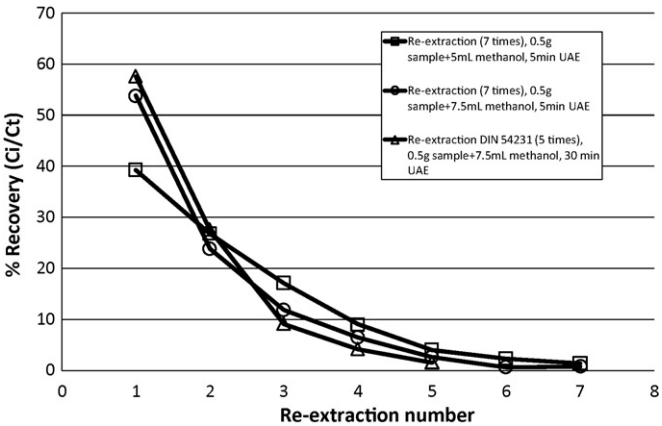

Fig. 2. Comparison of recoveries for CI Disperse Yellow 23 as a function of the volume of the extraction solvent and the ultrasound assisted extraction time.

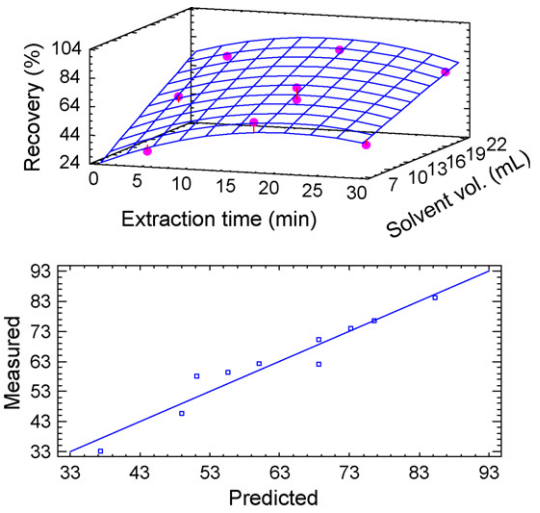

Fig. 3. Response surface and experimental data (a) in the optimization of extraction of disperse dyes from textile materials. Goodness of fit plot (b) for the quadratic model used to fit the experimental data.

In light of these data, we decided to study the extraction process in detail by means of an orthogonal response surface experimental design involving 10 experiments. This experimental design enabled estimation of the main factors as well as the interaction effects and quadratic effects, leaving four degrees of freedom for error estimation. In this study, the main factors considered were the amount of solvent (methanol) and the time of ultrasound-assisted extraction; the extraction temperature was maintained at the value suggested in the DIN 54231 standard procedure. The recovery of the dye in textile material was used as the objective function. Fig. 3 shows the response surface (a) fitted for the experiment data (also plotted in graph), and the goodness of fit of the quadratic model (b). The Pareto graph in Fig. 4 shows the factor effects in the extraction of the disperse dye in the tested material. In this graph each effect is represented by a bar and the length of each bar is proportional to the absolute value of the calculated standardized effect (standardized effects are obtained by dividing the estimated effect of each factor or interaction by its standard error). The vertical line represents the statistically significant boundary at the 95% confidence level. Only effects surpassing this boundary are statistically significant.

The volume of extraction solvent was the only statistically significant factor in the system having a positive coefficient, which indicated that higher volumes of methanol would allow better recoveries in a single extraction stage. In addition, although not statistically significant, the quadratic effect of the extraction time apparently affected the extraction. Because the extraction time was not a significant factor, the curvature imposed by the quadratic term in the response surface could be attributed to degradation

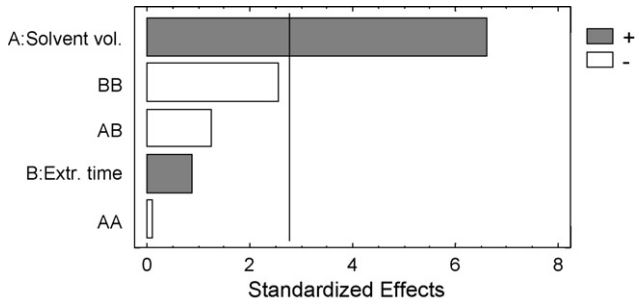

Fig. 4. Pareto chart in the analysis of results for CI Disperse Yellow 23 extraction from polyester textiles.

of the dye over time as a result of the effect of extraction temperature. Some of the dyes considered are labile in solution (e.g. CI Disperse Blue 124 decomposed easily, and thus produced false negative values in the analysis and false positive values for CI Disperse Blue 106, derived from degradation of this dye). Thus, the extraction time was limited to intermediate values in the final optimal extraction conditions (extraction for 15 min at 70 °C with 20 mL of methanol). Under these conditions, a single extraction stage provided recoveries of 92.5 ± 4.4% for CI Disperse Yellow 23. Although the final volume of extracts produced was greater than proposed in the DIN 54231 standard procedure, the enhanced sensitivity of the mass spectrometric detection enabled efficient determination of disperse dyes in textile products.

3.3. Evaluation of matrix effects

It is well known that in LC–MS–MS the most common matrix effects are not of spectral origin but produced by differences in desolvation of molecules of analytes by the co-eluted molecules of the sample matrix extract and ionization processes at the electrospray interface. The matrix effects lead to both suppression and enhancement of ionic effects that bias the analytical results.

Although disperse dyes are not commonly used in leather products, a blank leather material (not containing disperse dyes) was used to test for matrix effects after spiking with the 23 disperse dyes considered at the 2.0–10.0 $\mu g\,g^{-1}$ level. The corresponding sample was submitted to the optimized extraction and determination procedure and the results compared with those obtained by injecting the standard mixture of dyes at the equivalent concentration. The same comparison was carried out with spiked blank materials made from polyester and cotton. Duplicate determinations were performed for each spiking level. The results of these experiments clearly indicated that matrix effects occur in the case of the leather sample but not in the case of polyester or cotton. Some examples of matrix effects observed in the leather sample are shown in Fig. 5. For some dyes (e.g. CI Disperse Orange 1) signal magnification was observed. In other cases (e.g. CI Disperse Yellow 7), the signal was suppressed and finally, some dyes were not affected by the matrix components (e.g. CI Disperse Yellow 23). Clearly when matrix interference occurs, the standard addition procedure must be applied to produce quantitative results. Fortunately, for polyester, which is the most common matrix in which the dyes must be tested, no matrix effects were observed and external calibration can therefore be applied.

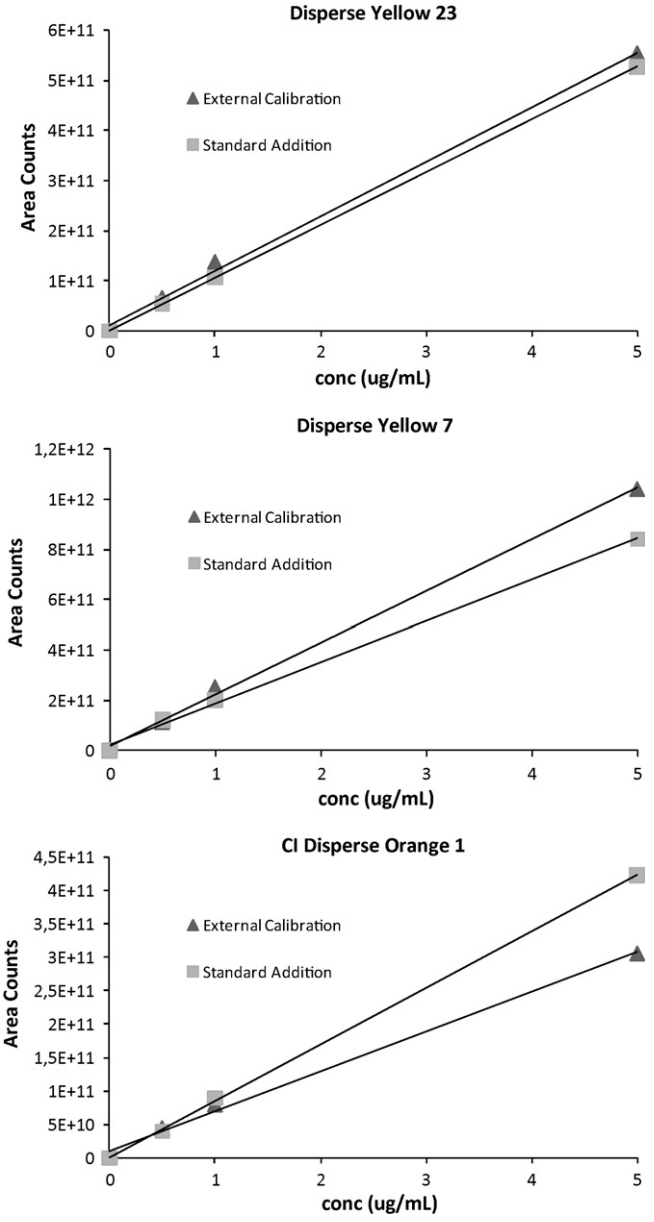

Fig. 5. Comparison of external calibration graphs and standard addition plots for some disperse dyes extracted from a leather sample.

3.4. Sample composition

Sample composition can be used to reduce the costs of analytical control when: (a) the property to be measured is additive (no interference due to combining sample specimens into a composite sample), (b) the analytical determination technique to be applied is sensitive enough to detect the presence of at least one contaminated sample specimen in the composite sample (CS), and (c) the probability of the presence of positive samples in the original sample specimens (OSS) is very low. In conventional sample composition, a number of OSSs are mixed to form the CS. Although not strictly necessary, for practical reasons it is convenient to mix the same amount of each OSS to form the CS, so that CS can be diluted in proportion to the number of OSSs used. In all further discussion this condition will be assumed.

In the case of textile products, composite samples can be prepared by mixing and extracting equal amounts (e.g. 0.5 g each) of the individual OSSs or by mixing equal volumes of extracts obtained from the individual OSSs. We will refer to the first type of CSs as *raw material* CSs and the second as *extract* CSs. In conventional sample composition, raw material CS is clearly convenient because only single extraction and determination processes are used to characterize the positive/negative status of the ensemble of OSSs. If the CS produces a positive result, then all OSSs must be extracted and analyzed. On the contrary, use of extracts to prepare the CS involves considerably more work, even though the extracts are available for re-testing in cases of positive results. Thus, the type of CS that is most appropriate will depend on the probability of the presence of positive samples and the costs and/or difficulties associated with sample preparation in relation to the analytical measurement and other limitations imposed by the analytical procedure. Furthermore, sample composition can be easily automated when OSS extracts are used [30].

The condition for avoiding false negative results in the analysis of the CS prepared by mixing extracts of the OSSs is that the quantification limit of the analytical technique to be applied is well below the total concentration of analytes in the final CS according

to the formula:

$$\mathrm{LOQ_{method}} \leq \alpha \frac{\sum_1^n \mathrm{Analyte_{mass}}}{V_{CS}} \quad (1)$$

where $\sum_1^n \mathrm{Analyte_{mass}}$, accounts for the additive character of the masses of analyte contributed by each OSS to the composite sample, V_{CS} is the final volume of the composite sample extract and α is a security factor (usually between 0.7 and 0.5) used to prevent the experimental variability compromising decisions about positive samples.

When the whole sample preparation process is taken into account, Eq. (1) can be expressed as:

$$\mathrm{LOQ_{method}} \leq \alpha \frac{\sum_1^n \mathrm{Analyte_{mass}}}{V_{CS}} \leq \alpha \frac{\sum_1^n (C_i m_i v_{ai}/V_i)}{\sum_1^n v_{ai}} \quad (2)$$

for extract CSs and:

$$\mathrm{LOQ_{method}} \leq \alpha \frac{\sum_1^n \mathrm{Analyte_{mass}}}{V_{CS}} \leq \alpha \frac{\sum_1^n C_i m_i}{V_{CS}} \quad (3)$$

for raw CSs, where, C_i is the concentration of analyte in each OSS, m_i is the mass of each OSS taken in the extraction process; V_i is the final volume of the obtained extract and v_{ai} the volume of the aliquot of extract used in the composition process. In practice, V_i is usually constant because it is imposed by the sample preparation procedure and also the same aliquot volume (v_{ai}) is taken for all OSS extracts, and thus Eq. (2) is considerably simplified. Similarly, Eq. (3) is considerably simplified if the same mass (m_i) is used for all OSSs. However, the same amount of OSS is not always used for extraction. In such cases, the expressions in Eqs. (2) and (3) cannot be deconvoluted to evaluate the contribution of each OSS in the analyses of the composite samples, and the minimum amount used must be considered in calculations. This would favor the appearance of false positive results when the contaminated samples are not those included in minimal amounts. However, Eq. (2) shows that the differences in the mass of each OSS can be compensated at the time of preparing the composite sample by proportionally adjusting the volumes of extract aliquots. In this way, the influence of such differences is removed and the calculations enable estimation of the contribution of each OSS, thereby minimizing the risk of false positive results.

As regards the decision taken about the CS analysis, if the result obtained for the composite sample, corrected for dilution, is negative (meaning that the analyte is not detected or appears under a pre-established acceptable level), control of all the OSS involved is halted and the samples are all declared negative. On the contrary, if the CS result is positive, we know that at least one of the OSSs must be positive although we do not know how many or which OSSs are positive. In this type of conventional sample composition mode, all OSSs considered must be re-tested individually to evaluate which are responsible for the positive results. It is clear that the costs of the analytical control are only reduced if the probability of the presence of positive samples is very low. Otherwise, more and more individual OSSs must be tested, thus cancelling out the advantages of sample composition.

Advanced sample composition modes avoid the need to re-test all the individual OSSs in cases of positive samples but retain all the advantages of conventional sample composition in the case of negative results. One such advanced techniques is the strategic sample composition (SSC) developed in this laboratory some years ago [23,24,27–32]. In SSC, a conventional composite sample is prepared (from the raw material or from extracts) and analyzed. However, in case positive results are obtained for this CS, the SSC process goes further by preparing some additional CSs, as dictated by a composition design matrix. To make this process clear, in the following we will refer to an example of SSC developed for the control of disperse dyes in 10 OSSs. Composite samples were formed by sampling 9 different commercial textile products and the specially prepared polyester materials containing the CI Disperse Blue 106 dye. In this way, we were sure that there was at least one positive specimen in the composite sample. One raw material composite sample and one extract composite sample were prepared and analyzed. As expected, positive results were obtained for Disperse Blue 106 and negative results for the remaining controlled dyes. From this point, instead of re-testing the 10 OSSs, five more composite samples were prepared (solutions to prepare these additional composite samples are readily available in the case of CS prepared from extracts), following the design shown in Table 4. In this table the conventional CS is represented as the first row in the composition design matrix. This matrix is self-explanatory, because code 1 in a particular cell indicates that the corresponding individual OSS (column) must be mixed in that (row) composite sample. A zero code indicates the opposite. Thus, the first row in this composition design matrix identifies the initially prepared conventional composite sample (all cells with code 1 status) that produced positive results. Clearly, the design matrix is a supersaturated matrix because the number of columns is larger than the number of rows. Thus, special regression procedures must be applied to resolve the equation system [23]. All of these calculation facilities as well as data handling and reporting are provided by the GAMICH software developed in the author's laboratory. In the columns for concentration of Disperse Blue 106, readings that differentiate dilution in composite samples prepared have not been corrected. All composite samples can be diluted to the same final volume, although this is not necessary and the differences due to dilution can easily be corrected during calculations. In fact, it is clear that the most diluted composite sample is the conventional CS (row 1) thus, if this CS was prepared taking the adequate precautions to avoid false negative results (Eq. (1)) it is clear that this risk is practically nil in the analysis of the remaining composite samples prepared during the SSC process.

The concentrations of Disperse Blue 106 in the original sample specimens can be estimated from the data in Table 4. The estimates derived from the raw material composite sample and from the extract composite sample were respectively 2447 $\mu g\,g^{-1}$ and 2216 $\mu g\,g^{-1}$ for original sample specimen number 10, and were negligible for the remaining specimens. In order to check these estimates, the original sample specimens were analyzed individually. Only the specially prepared polyester material produced a positive result, with a concentration of 2161 $\mu g\,g^{-1}$, so that the agreement between estimated results and the real quantitative measurement of individual sample specimens was excellent in the case of extract CS and quite acceptable (because the conclusions were the same), in the case of raw material CS. It should be stressed that the raw material sample composition with its inherent advantages, also imposed some limitations as regards the representativeness of the formed composite samples. In general, it is slightly more complicated to weigh equal amounts of raw textile materials for mixing than to measure equal volumes of extracts. Moreover, differences in the OSS sample weights can be compensated at the time of mixing aliquots of extracts by taking proportionally different volumes. This provides a more accurate estimate of the concentrations in the OSSs. Furthermore, two approaches can be adopted for preparing a raw material composite sample of textile products. The first is to take an amount of each OSS that is $1/n$ of the recommended sample weight in the analytical procedure (e.g. in the disperse dyes case, $0.5/n$ g, where n is the number of OSSs involved in the composite sample). Obviously when n increases, which is convenient in terms of cost reduction, the representativeness of the aliquots may be easily compromised, especially in complex textile products with different components and dyes. The other possibility is to take large enough amounts of each OSS to grant sample representative-

Table 4
SSC composition matrix and results for CI Disperse Blue 106 in the composite samples obtained.

Composite sample	Individual original sample specimens (OSSs)									Measured CI Disperse Blue 106 ($\mu g\,mL^{-1}$)	
	1	2	3	4	5	6	7	8	9	Raw materials CS	Extracts CS
CS_1	1	1	1	1	1	1	1	1	1	9.49	7.50
CS_2	1	1	1	1	0	0	0	0	0	0.04	<LOD
CS_3	1	0	0	0	1	1	1	0	0	0.16	0.04
CS_4	0	1	0	0	1	0	0	1	1	17.3	14.16
CS_5	0	0	1	0	0	1	0	1	0	0.26	0.05
CS_6	0	0	0	1	0	0	1	0	1	18.23	16.60

ness, then mix and grind all these aliquots in a suitable cutting mill (the previously mentioned mill has produced satisfactory results in most cases tested in our laboratory), to provide a homogeneously ground material that comprises the composite sample, and then to take an appropriate weight of the ground material for proceeding with sample preparation and analytical measurement. Although this process may be developed quite accurately, the possibilities of producing material that is not sufficiently homogeneous are not negligible, which may explain the observed differences in the estimates of the final columns in Table 4. Additionally, further manipulation of samples is required, and the cost and time involved, as well as the probabilities of cross-contamination in the grinding process, are also increased. Here we see that the raw material composite sample provided consistently higher values than the extract composite sample, possibly because more material corresponding to OSS 10 (which was the OSS corresponding to the special polyester material containing the Disperse Blue 106 dye) has entered in the sample composition process during grinding, due to differences in the nature of the textile samples.

Finally, it should be stressed that a large number of OSS can be combined for disperse dye analysis with the proposed analytical procedure. If the value suggested as the LOD in the DIN 54231 standard procedure ($75\,\mu g\,g^{-1}$) is taken as the practical limit in consumer safety protocols, theoretically more than 75 OSSs can be mixed (with a safety factor of $\alpha = 0.6$ in the composition), since the proposed procedure provides a quantification limit better than $0.4\,\mu g\,g^{-1}$. In practice, 20 OSSs can be composed with negligible risk of false negative results, thus providing a manageable process and proportionally reducing the costs and work involved in the control of disperse dyes in multi-supplier textile products.

4. Conclusions

A simple, highly sensitive and fast procedure for the control of allergenic disperse dyes in textile products was optimized. The method uses ultrasound assisted-extraction of 0.5 g of textile samples with 20 mL of methanol during 15 min at 70 °C, followed by separation and analysis by LC–MS–MS. Under optimal conditions, disperse dyes are quantitatively recovered from samples in a single extraction stage. This result compares favorably with the recoveries obtained in the method recommended in the DIN 54231 standard procedure, which is commonly used by analytical laboratories undertaking this type of analysis. The optimized separation program enables accurate detection and measurement of all the 23 allergenic dyes considered, within 15 min, at very low quantification limits (lower than $0.4\,\mu g\,g^{-1}$). Matrix effects in the LC–MS procedure were evaluated and appeared significant for matrices such as leather, although the most common matrices to be analyzed for disperse dyes (polyester) can be processed by external calibration. In such conditions, sample composition - either conventional or strategic sample composition - is proposed as an efficient procedure for reducing the costs and work involved in the control of allergenic dyes in finished textile products. The benefits of strategic sample composition are demonstrated by means of an example case study. Composite samples can be prepared from extracts or directly from the textile products. Both composition modes have advantages and disadvantages, so that in selecting the most appropriate method, analysts should consider the number of original sample specimens as well as the limitations imposed by the analytical procedure, and the costs of sample preparation stages relative to the costs of analytical measurement stages. In the case of disperse dyes, the optimized conditions described reduce the costs of analytical control by up to twenty times.

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