Introduction

Single fibers collected at a crime scene are important physical trace evidence for proving contact between crime suspects and victims through comparative analysis of their clothes.1,2 Many studies have been conducted to analyze the dyes in colored fibers using microspectrophotometry,3–10 TLC,11–19 HPLC,20–25 and LC/MS,26–30 because the dyes can provide significant information in forensic analyses.

We previously reported a procedure to identify disperse dyes in their coexisting solutions using liquid chromatography linear ion trap tandem mass spectrometry (LC/LIT-MSn) and constructed a database.31 Dye-extractions are performed by heating colored fibers in various solvents and solvent mixtures,11–30 including the recommended pyridine/water (4:3, v/v) mixture by the SWGMAT guidelines.32 We reported a method for extracting a residual surfactant from threads using centrifugal filtration of a methanol solution extracted into a glass capillary.33 In this study, LC/LIT-MSn was applied to analyze the disperse dyes extracted from colored polyester fibers via extraction and centrifugal filtration because polyester fibers, which are commonly polyethylene-terephthalate (PET) fibers, are the most widely used synthetic fibers in clothes, carpets, sofas, etc.34 Dimethylformamide (DMF) was shown to be an effective solvent for dye extraction.

Experimental

Materials

Methanol (LC/MS grade), DMF (HPLC grade), and 1 M ammonium acetate (HPLC grade) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Water with a resistivity of 18.2 MΩ cm−1 was prepared using a Millipore-Q system (Merck Millipore Co., Ltd., Tokyo, Japan). Dispersed dyes and dyed polyester textile fibers were supplied as commonly used ones from dyestuff manufacturers (Nippon Kayaku Co., Ltd., Tokyo, Japan). The properties of the samples (textile fibers and dyes) used in this experiment are summarized in Table 1.

Stock solutions of standard dyestuffs were prepared in mixed solvents of methanol/DMF (1:1, v/v) at concentrations of 50 μg/mL. The solutions were suitably diluted with methanol and used for analysis.

Equipment and measurement conditions

The LC/MS apparatus used in this study comprised a Shimadzu Prominence ultra-fast liquid chromatograph (UFLC; Shimadzu Corporation, Kyoto, Japan) combined with an LTQ-IT-MS (LXQ/LIT-MS, Thermo Fisher Scientific Corporation, Yokohama, Japan). Separation by HPLC was conducted using an L-column 2 ODS semi-micro column (1.5 mm i.d. × 150 mm) with a particle diameter of 5 μm (Chemicals Evaluation and Research Institute, Tokyo, Japan). The flow rate of the mobile phase was 0.20 mL/min, and the column oven was maintained at 40°C. The composition of the mobile phase was changed from 40% A (5% methanol containing 10 mM
ammonium acetate) and 60% B (95% methanol containing
10 mM ammonium acetate) to 100% B in 15 min by steadily
increasing the percentage of B. Subsequently, the composition
was maintained at 100% B till 25 min and then changed back
to the starting composition for 10 min. Signals in the visible
region (380 - 760 nm) were collected using a diode array
detector (DAD). The sample injection volume was 5 μL, and
all of the solvents were passed through a 0.45-μm filter prior to
use.

LC/LIT-MS² was operated in positive electrospray ionization
mode using a previously described data-dependent MS³ method.¹¹
The electrospray voltage was 5 kV. Helium was used as the
collision gas, and the relative collision energy was set at
45%. The capillary temperature was set at 275°C, and the
sheath gas flow was 30 arbitrary units. An isolation width of
4 Da was used with a 30-ms activation time for the MSn
experiments.

The limit of detection (LOD) of the dyes was determined in
the selected reaction monitoring (SRM) mode using the
monitoring ions identified in Table 2 for each disperse dye
examined. The LODs of nine disperse dyes were determined by
estimating the dye concentration needed for the detection of
each dye. The LOD (based on peak height) was defined as the
minimum dye concentration needed to achieve a signal/noise
ratio of three in the chromatograms obtained by DAD and SRM
in LC/LIT-MS².

Analysis of dyes extracted from threads

The dye extractions were first performed for polyester threads
(7 mm in length) that were pulled from manufacturer-supplied
standard textiles colored with disperse dyes (Table 1). These
extractions were performed so as to determine the most effective
dye extraction solvent among an acetoniitrile/water (4:3, v/v)
mixture, a methanol/water (1:1, v/v) mixture, and DMF. The
acetoniitrile/water (4:3, v/v) mixture was recently recommended
as an extraction solvent for LC/MS,³⁰ replacing the pyridine/
water (4:3, v/v) mixture, because they have approximately the
same extraction ability for dyes.³⁰ For the extraction, 30 μL of
the solvent was placed with a textile thread in a glass capillary
tube [Microcaps, for 100 μL (length: 70 mm, i.d.: 1 mm),
Drummond Scientific Company, Pennsylvania, USA] and heated
at 100°C for 30 min in a heat block. After extraction, one end
of the sealed capillary tube was cut and connected to a syringe
filter unit (GL chromatik disc 4P, pore diameter: 0.45 μm,
Kurabo Industries Ltd., Osaka, Japan), which was then
connected to a glass vial (12 × 32 mm Macroivial Target Snap,
fixed insert, Thermo Fisher Scientific Co., Ltd., Kanagawa,
Japan) in a test tube (length: ~85 mm, i.d.: ~13 mm). The
extracted solution was filtered by centrifugation (5000 rpm for
5 min) after which the sample was ready for LC/LIT-MS²
analysis. The extraction procedure is schematically summarized
in Fig. S1.

A 30-μL internal standard solution [Disperse Red 146
dissolved in DMF/methanol (1:1, v/v) at a concentration of
50 μg/mL] was added to the extracted dye solutions for
LC/LIT-MS² analysis to quantify the extracted dye amounts by
the peak area ratio relative to the internal standard (Disperse
Red 146) based on the DAD data.

Analysis of dyes extracted from single fibers

Using the extraction procedure mentioned above (Fig. S1),
dyes were extracted from nine different polyester single fibers
(5 mm in length) obtained from the corresponding standard
textile threads colored with the dyes listed in Table 1. LC/LIT-
MS² was employed to analyze the extracted dyes, and
automatic dye detection was conducted using software (ToxID³⁵)
and our custom-built disperse dye search database.³¹

Results and Discussion

Detection limits of disperse dyes

Petrick et al. analyzed dyes in single fibers (5 mm in length)
using LC/MS,³⁰ while referring to previous reports that a single
2 - 10-mm-long dye fiber contains 2 - 200 ng of dye, and that
the LODs were 80 - 1280 pg for 5 μL of the extracted disperse
dyes injected into an HPLC (DAD) system.²⁹,²⁶

As shown in Table 2, the LODs identified by DAD based on
the peak heights for the nine disperse dyes were 500 - 1750 pg
for a 5-μL injection, but those determined from the SRM
chromatograms were 1 - 15 pg. These results indicate that LC/LIT-MSn has sufficient sensitivity for dye analysis of single fibers (5 mm in length).

Analysis of dyes extracted from threads
Among an acetonitrile/water (4:3, v/v) mixture, a methanol/water (1:1, v/v) mixture, and DMF, DMF was found to be the most effective solvent for dye extraction from the threads, as evident from the DAD data presented in Fig. 1. The superior performance of DMF may be attributed to its ability to strongly solvate most organic compounds and effectively separate disperse dyes from dyed fibers.

LC/LIT-MSn analysis of the dyes extracted from the standard polyester textile threads using DMF as the extracting solvent (Fig. S1) detected all dyes as [M+H]+ ions. We further confirmed that the results [i.e., the retention time, [M+H]+, MS2 and MS3 values, and wavelengths of the maximum absorption (λmax)] obtained from the total ion chromatograms by LC/LIT-MSn and visible spectra by DAD for the extracted dyes were similar to those obtained for the standard dyes.

Analysis of dyes extracted from single fibers
LC/LIT-MSn analyses of the dyes extracted using DMF were successfully performed from single fibers (5 mm in length). All of the fiber dyes were detected by data matching of the precursor ions [M+H]+ and the MS2 and MS3 spectra using search software (ToxID19).

As shown in Fig. 2(a), the existence of Disperse Red 145 in the fiber was confirmed by the DAD-detected peak (retention time = 9.07 min) and extracted ion chromatogram for m/z 381 (retention time = 9.13 min). The ~540 nm maximum of the spectral curve [Fig. 2(b)] indicated that the peaks were derived from the dye. The acquired MS2 and MS3 spectra further confirmed the identity of Disperse Red 145 [Fig. 2(c)].

In contrast, Disperse Blue 60 was difficult to identify by DAD-peak detection and mass chromatography [Figs. 3(a), 3(b)], although a peak retention time of ~13 min and mass spectral peak at m/z 380 [Fig. 3(c)] implied the presence of Disperse Blue 60 in the extracted solution. The information acquired by the MS2 and MS3 spectra clearly showed the presence of Disperse Blue 60 in the fiber [Fig. 3(c)].

Dyes other than Disperse Red 145 and Disperse Blue 60 were also successfully detected in the extracted DMF solutions from the fibers using LC/LIT-MSn. The results are shown in Figs. S2 - S8 (see Supporting Information).

Conclusions
DMF was found to be a more effective solvent for the extraction of disperse dyes from colored polyester threads than acetonitrile/water (4:3, v/v) or methanol/water (1:1, v/v) mixtures. Without relying on comparison clothes, the extracted disperse dyes from single fibers (5 mm in length) were successfully identified using LC/LIT-MSn and a custom-built database for nine dyes, including dyes that were difficult to identify by DAD.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

References