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3D Fluorescence Characterization of Synthetic Organic Dyes—Effect of pH

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ABSTRACT

The fingerprint character and high sensitivity of 3D UV-vis fluorescence spectra offer special advantages for identification of dyes in a museum or forensic setting. However, the extraction process is likely to affect the pH of the medium and, in some cases, may alter the dye itself. We report a study of 65 dyes extracted from wool fibers that are part of the Schweppe Collection of Important Synthetic Dyes. The 3D fluorescence spectra of the dye extracts at pH 1 and pH 14 are compared with the same dyes from the Schweppe solution library, run under the same conditions, as well as with the 3D fluorescence spectra of the dyes taken directly from the solution library without pH control. This analysis leads to guidelines for the use of such spectra in identifying unknown dye samples.

Keywords: Fluorescence; Spectrophotometry; Dye Analysis; Textile Analysis

1. Introduction

We recently reported "fingerprint" patterns obtained from 3-dimensional fluorescence spectrometry [1] for the 65 synthetic organic dyes in the Schweppe dye collection [2]. To test the practical utility of such patterns for identifying dyes, we studied the 3D fluorescence spectra of extracts of these same dyes from woolen fibers, also from the Schweppe collection.

Because of the trifluoroacetic acid extraction procedure, we employed results in a highly acidic solution; we examined the effect of pH on the 3D fluorescence spectra of these dyes and found that many of the spectra are pH dependent. Therefore, we developed a standard protocol in which the fluorescence spectrum of each dye extract is recorded at pH 1 and then again at pH 14. Dye samples from the Schweppe solution library were subjected to the same protocol, and their fluorescence spectra were compared with those from the wool extracts. This comparison led to recommendations on the interpretation of such spectra for problems involving dye identification.

2. Experimental

2.1. Instrumentation

We used a Hitachi F 4500 fluorescence spectrophotometer with a Hitachi 650 - 0116 microcell for running fluorescence spectra; this cell requires 200 μL of solution. The excitation wavelength EX was scanned from 250 - 380 nm and the emission wavelength EM was scanned from 395 - 700 nm. These two ranges intentionally do not overlap in order to avoid exposing the photomultiplier to 1st order scattering from the Xe light source. The scanning rate was 1200 nm/min, and the response time was set at 0.1 sec. With these conditions, a spectrum could be obtained in less than 8 minutes with no apparent loss of detail, compared with slower scan speeds.

Approximate pH measurements with narrow-range pH paper were spot-checked and confirmed using a Fisher Accumet Model AB-15 pH meter with an Accumet glass combination electrode (Fisher 13-620-285).

2.2. Materials

The original solution Schweppe Library of Synthetic Organic Dyes [2] is held at the J. P. Getty Museum in Los Angeles, CA. The samples from this Library in the possession of the Boston Museum of Fine Arts (MFA) are solutions in methanol. The collection includes strands of wool yarn dyed with these same dyes.

For extracting the dyes, we diluted Aldrich spectrophotometric grade trifluoroacetic acid (TFA) (Aldrich 302031) with Sigma-Aldrich Chromasolv-Plus HPLC water (Sigma-Aldrich 34877) to 2 M and also used

Sigma-Aldrich Chromasolv HPLC MeOH (Sigma-Aldrich 34860). For adjusting the pH, we made a 6 M solution of NaOH using Fisher NF/FCC sodium hydroxide (Fisher S 320) and HPLC water. 100% pure medical grade USP modified lanolin (CVS Lanolin Cream, SKU 148868) was used for the lanolin spectrum, as described below.

Since the original Schweppe collection is over 50 years old, some of the library solutions show obvious decomposition, indicated by an absence of color: Acid Black 1 (CI 20470), Basic Green 4 (CI 42000), Acid Blue 93 (CI 42780), Acid Black 2 (CI 50420), Murexide (CI 56085), Mordant Red 3 (CI 58005), and Acid Blue 74 (CI 73015). For these dyes, the fluorescence spectra reported were obtained using fresh dye samples.

The issue of dye concentration has been discussed in [1].

2.3. Extraction of the Dyes

The extraction of both synthetic and natural dyes from textile samples for analysis has been a subject of prior investigation [see for example 3 and 4]. Souto [3] compared a variety of extraction methods for sampling synthetic organic dyes from textiles. That work suggests that dilute TFA is the most effective extracting agent for most of the dyes studied. HCl/methanol is also commonly used [4,5]. While Souto removed the TFA after extraction by heating under vacuum, we found that the resulting material was still acidic. Since dye spectra may be pH dependent and, indeed, a number of the Schweppe dyes are actually pH indicators, we opted to adjust the pH to standard levels rather than attempting to remove the TFA, thus providing well-defined protonated and deprotonated dye species for fluorometric analysis.

To extract a sample, we clipped a fragment of wool (about 2 mg) from a Schweppe library wool sample and placed it at the bottom of a 0.5 mL polypropylene microcentrifuge tube. We added 20 μL 2 M TFA, agitated with a glass rod, and let stand at room temperature for 2 min. We then added 30 μL MeOH and let stand for another 4 minutes at room temperature. The sensitivity of fluorescence spectrometry is such that sufficient dye was extracted in most cases even under these gentle conditions. Finally, the extract was diluted with 250 μL H_2O. The dyes insufficiently extracted under these conditions were Acid Violet 7 (CI 18055), Acid Black 1 (CI 20470), Basic Yellow 2 (CI 41000), Acid Green 6 (CI 42075), and Mauveine (CI 50245).

2.4. pH Adjustment

The dye solution as extracted from the wool fibers with TFA is highly acidic, even after dilution, with a pH of 1 indicated by narrow range pH paper and confirmed using a glass electrode pH meter. After the 3D fluorescence spectrum was run at pH 1, 10 μL of 6 M NaOH solution was added to the microcuvette and thoroughly mixed. If necessary, additional NaOH solution was added to bring the pH to 14. The pH 14 spectrum was then run.

3. Results and Discussion

The Supplementary Information—**Table 1** [http://simmons.academia.edu/LenSoltzberg] contains, for each of the 46 Schweppe dyes giving a usable match between the wool extract and library spectra, the 3D fluorescence spectra of the wool extracts and library solutions at pH 1 and pH 14, along with the unbuffered library spectrum and corresponding molecular structure. We refer to those spectra using the Schweppe number; e.g., "S10". Spectra illustrating particular findings are given in the figures below.

3.1. Spectral Artifacts

Certain distinct artifacts characteristic of fluorescence spectra need to be taken into account when examining the spectra reported here. These artifacts include Rayleigh scattering and Raman scattering from the solvent. These artifacts have been discussed in detail in [1] but are shown again here in **Figure 1** to facilitate reading the dye spectra shown in the present work. 3D fluorescence spectra of pH 1 and pH 14 blank solutions showed no features other than these instrumental artifacts.

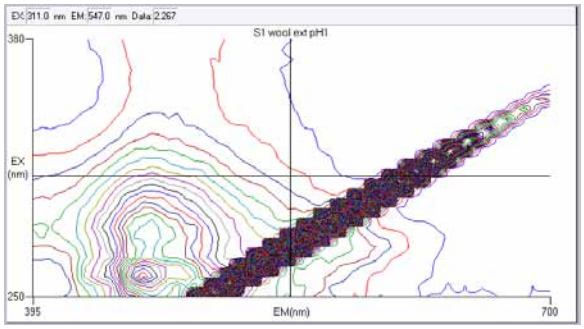
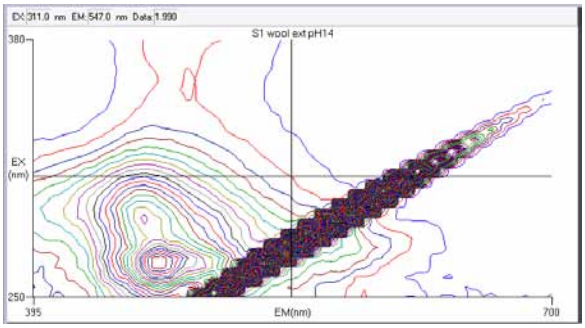
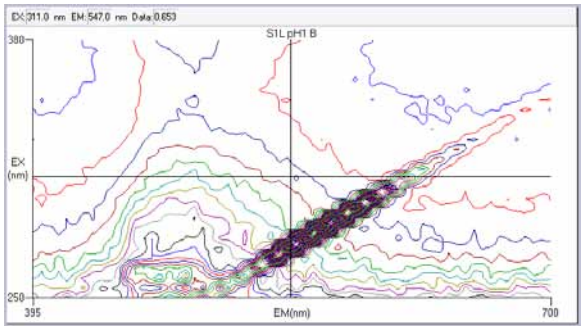
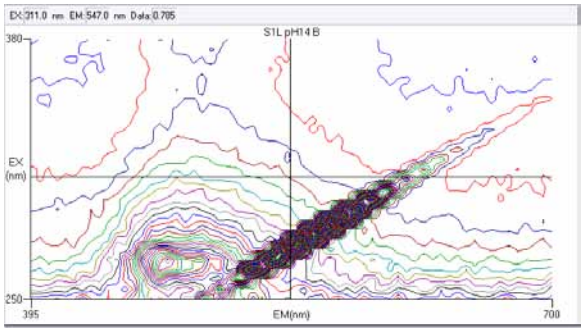
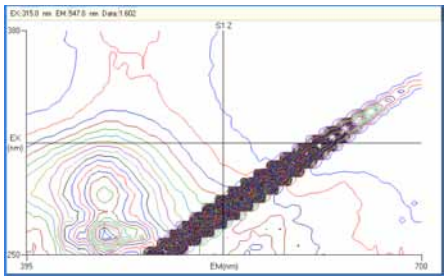
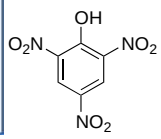
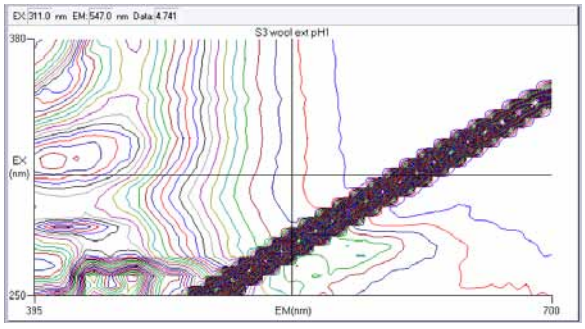
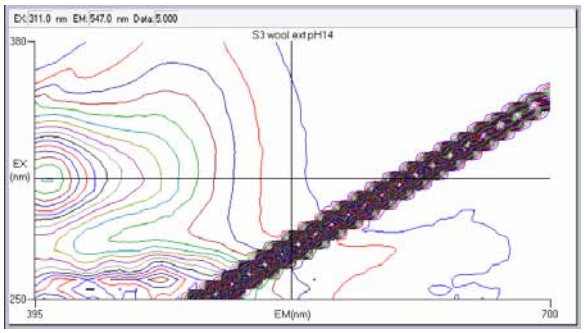
Care needs to be taken to avoid confusion due to a peak centered near λ_{EX} = 300 nm λ_{EM} = 400 nm, often seen in the pH 14 wool extract spectra (**Figure 2(a)**) and apparently due to lanolin (**Figure 2(b)**). A weak fluorescence spectrum has been previously reported for lanolin [7]. For weakly fluorescent or poorly extracted dyes, the lanolin peak may overlap with or obscure dye features. Examination of the pH 14 dye library spectrum shows whether the dye itself has a peak at that location.

3.2. Effect of pH on Dye Spectra

While a detailed correlation of fluorescence spectra with molecular structure is a complex matter [8], it is no surprise that these spectra can be strongly influenced by pH [9]. For example, while the 3D fluorescence spectra of S 51 at the pH 1/pH 14 extremes are virtually identical (**Figure 3**), the spectrum of S16 changes markedly with pH (**Figure 4**). Protonation/deprotonation of -OH, -COOH, $-SO_3H$, or $-NH_2$ groups commonly found in synthetic dyes, plus the consequent change in conjugation, may be expected to alter both the excitation and the emission profiles, leading to changes in the 3D fluorescence pattern.

Of the 65 dyes in the Schweppe collection, the 3D

Table 1. Dye can be identified from 3D fluorescence spectra.

	Acidic	Basic
S x	**[CI (Colour Index) Number CI Name]**	
wool	**dye extracted from wool fibers—pH 1**	**dye extracted from wool fibers—pH 14**
vial	**dye from solution library—pH 1**	**dye from solution library—pH 14**
Sdb	**dye from solution library—no pH adjustment**	**dye structure**
S 1	**[CI 10305 picric acid]**	
wool		
vial		
Sdb		
S 3	**[CI 10316 Acid Yellow 1]**	
wool		

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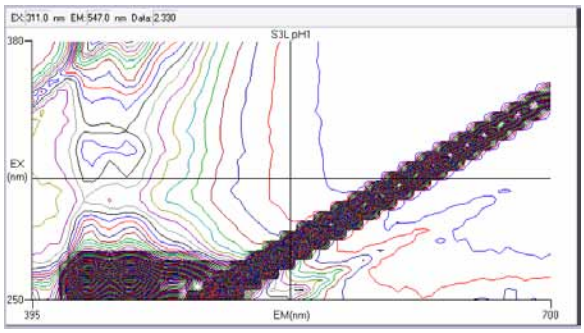
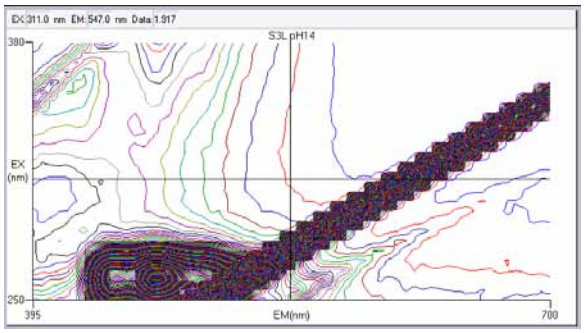
vial





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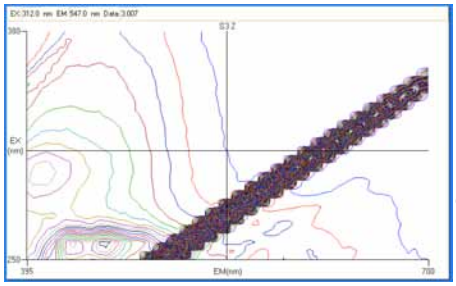
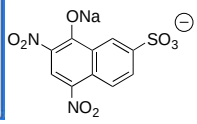
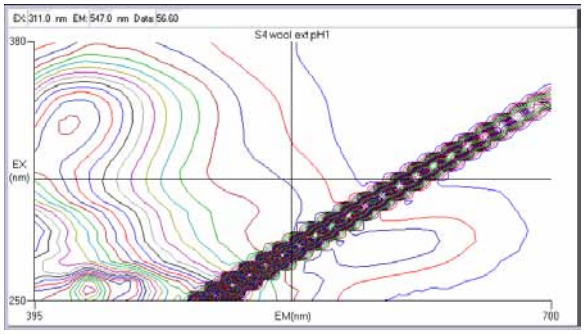
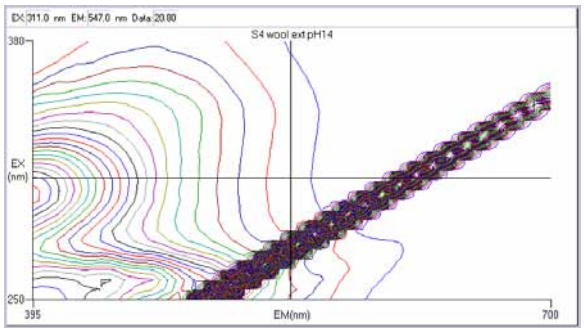




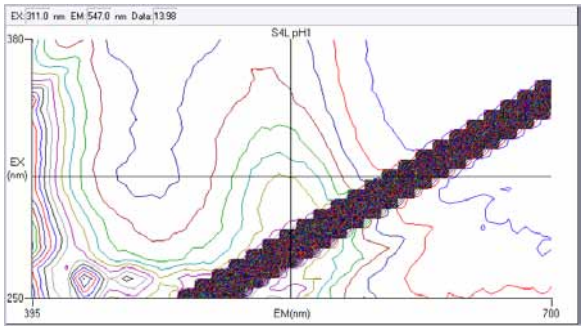
S 4 **[CI 11270 Basic Orange 2]**

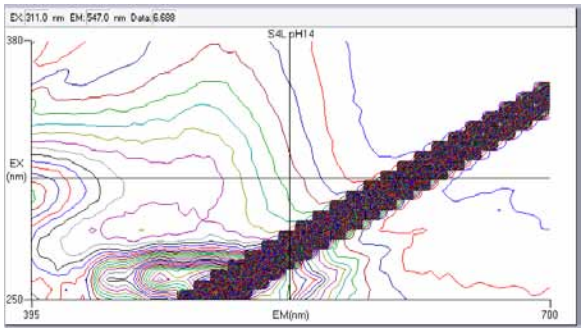
wool





vial





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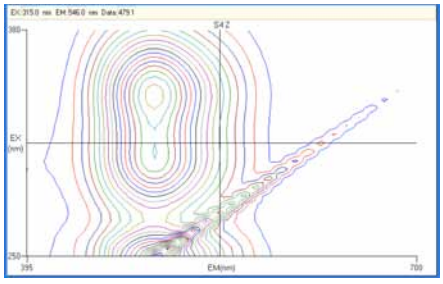

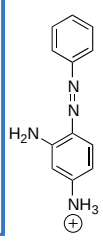

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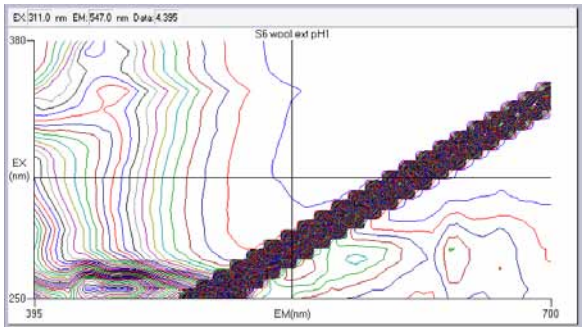
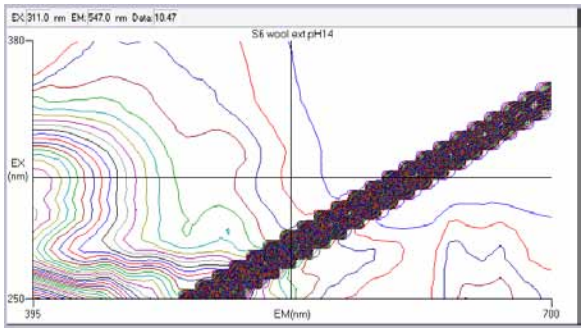
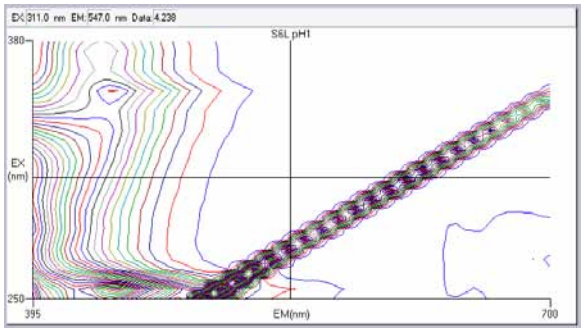
S 6	**[CI 13080 Acid Orange 5]**

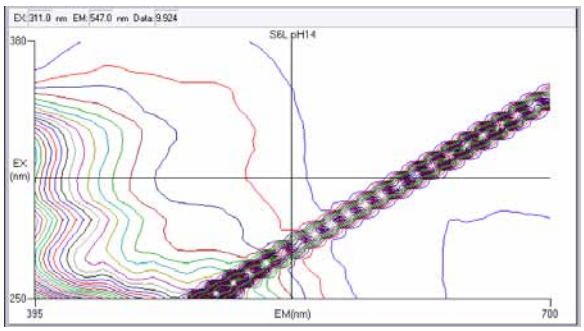
wool





vial





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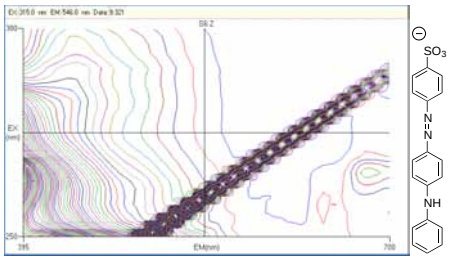
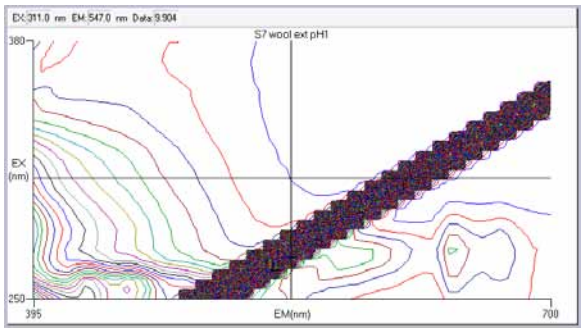
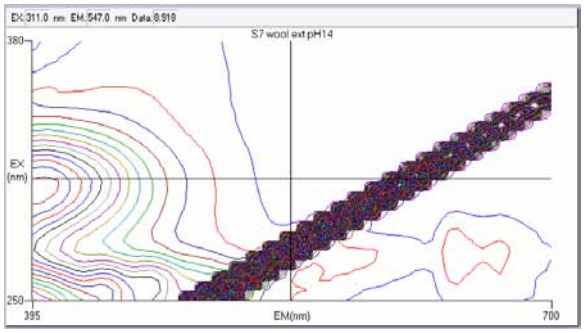


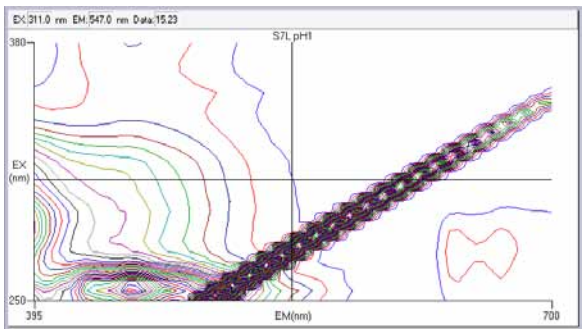
S 7	**[CI 13090 Acid Orange 1]**

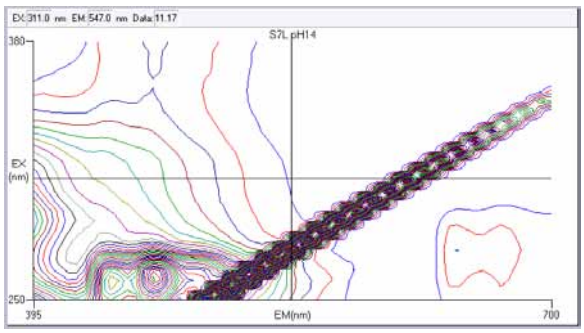
wool





vial





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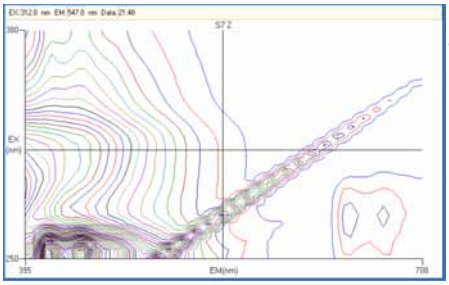
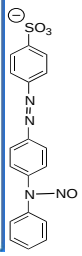
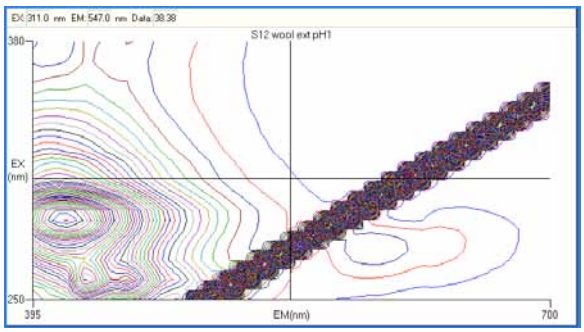
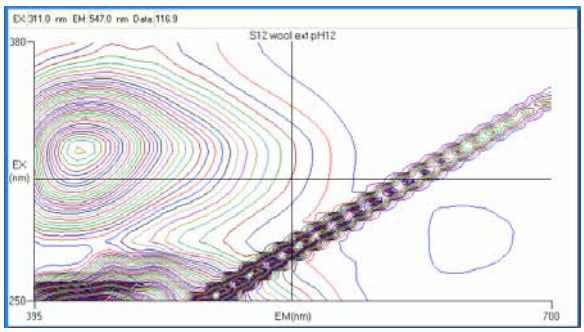




S 12 **[CI 14625 Acid Brown 6]**

wool





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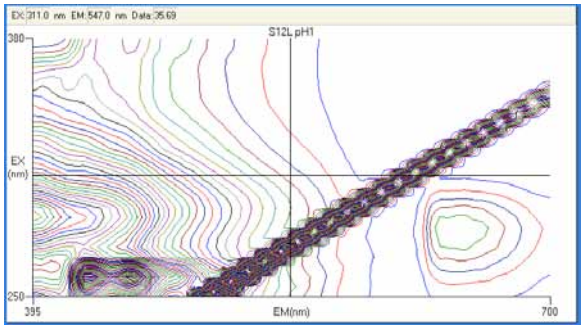

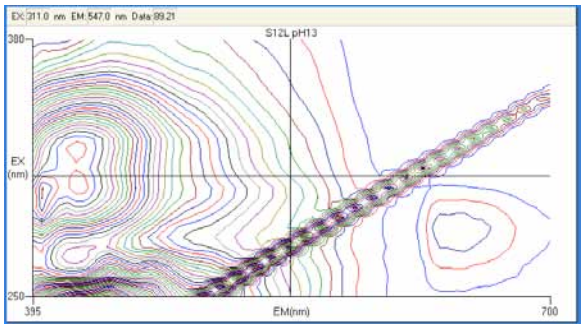

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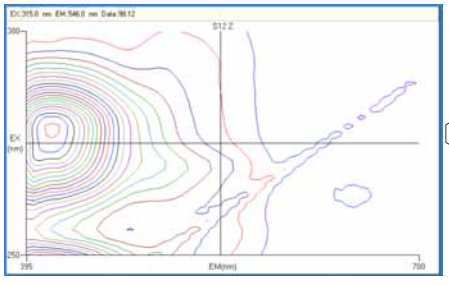
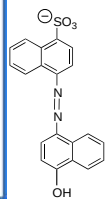
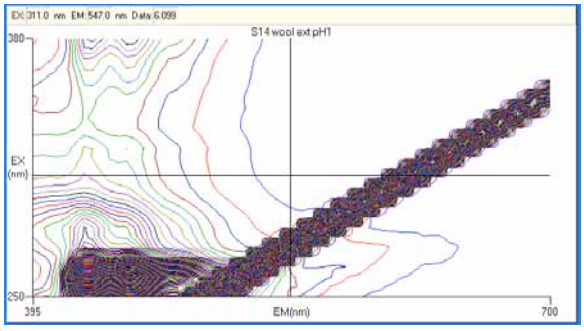
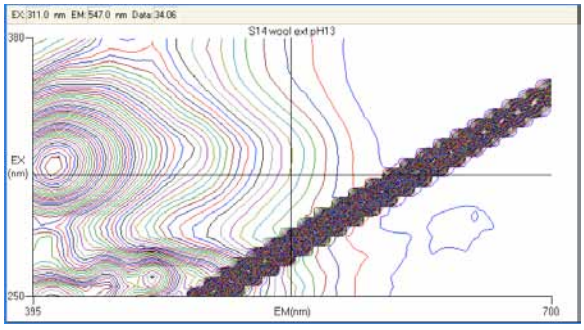




S 14 **[CI 15620 Acid Red 88]**

wool





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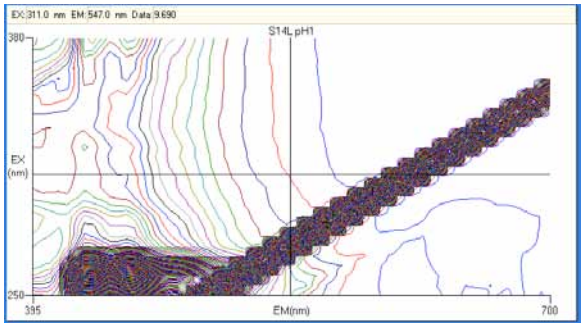
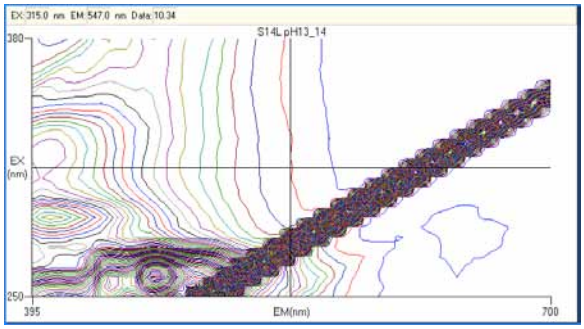
vial





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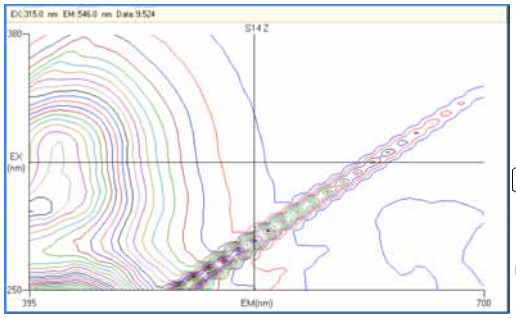
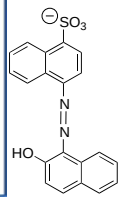
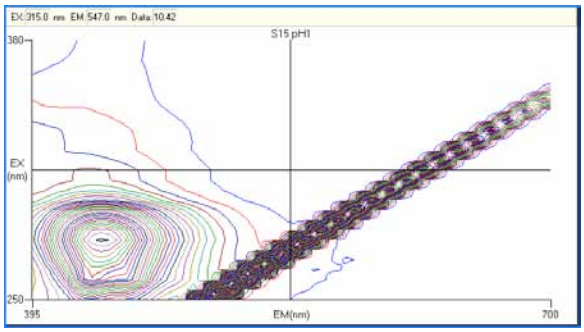
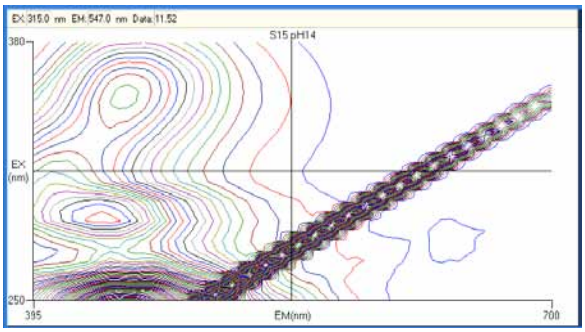




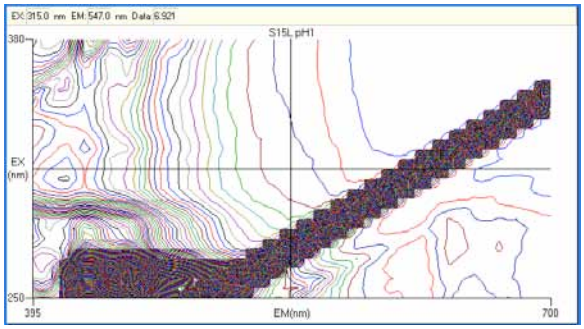
S 15 **[CI 15635 Acid Red 9]**

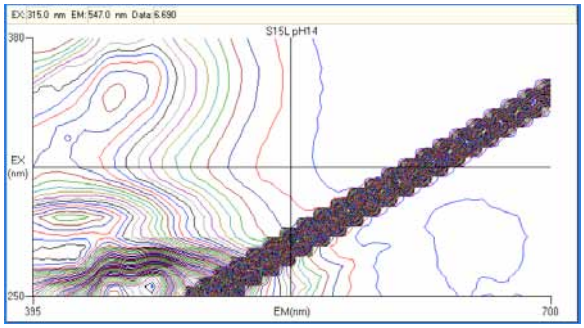
wool



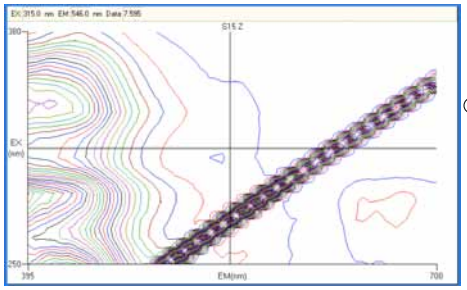


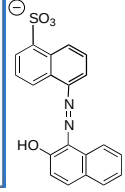
vial





Sdb





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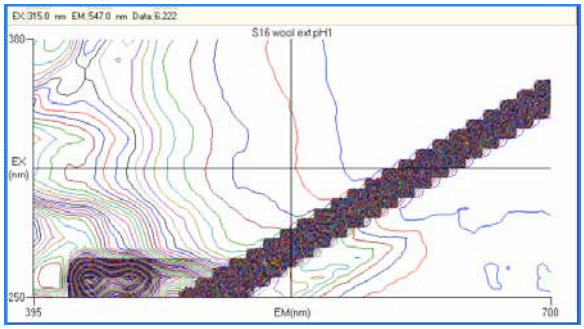
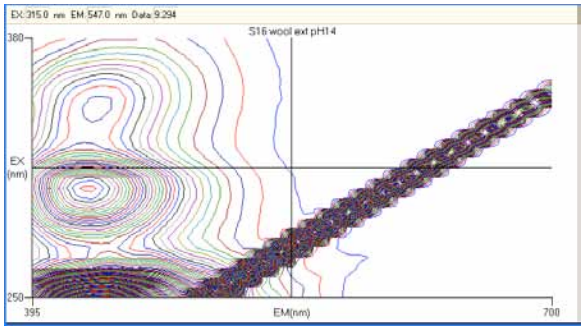
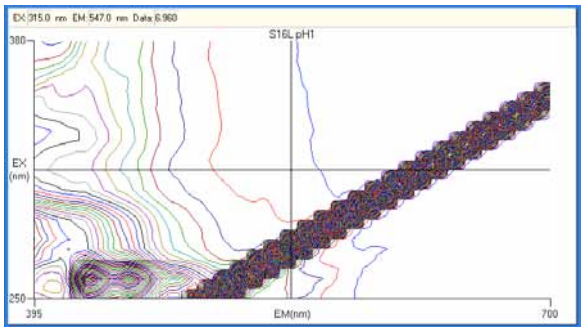
S 16	**[CI 15970 Acid Orange 12]**

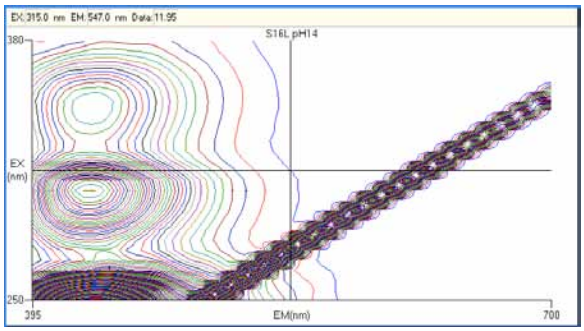
wool





vial





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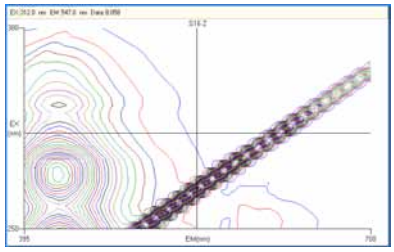
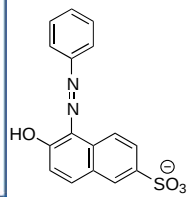
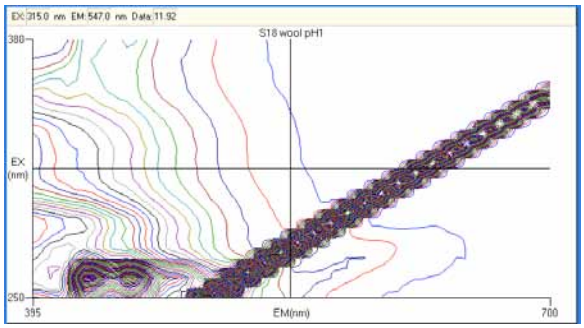
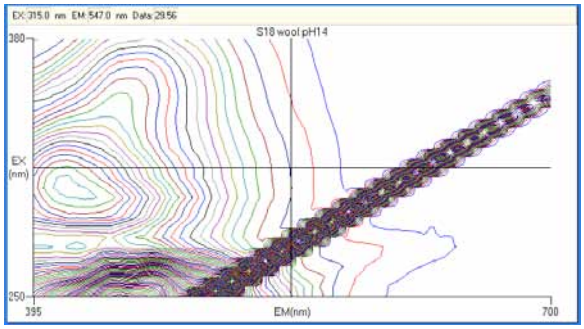




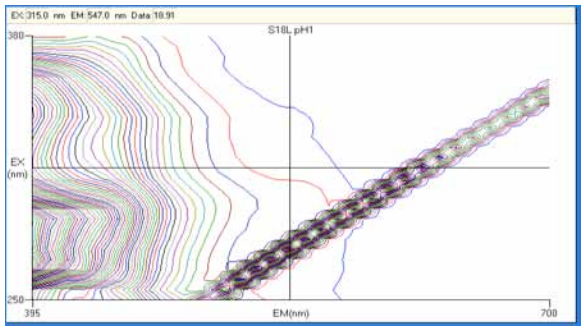
S 18	**[CI 16050 Acid Red 25]**

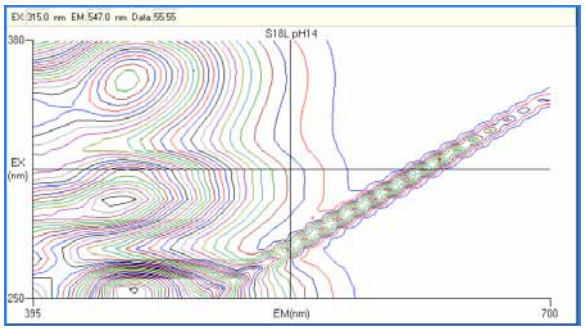
wool





vial





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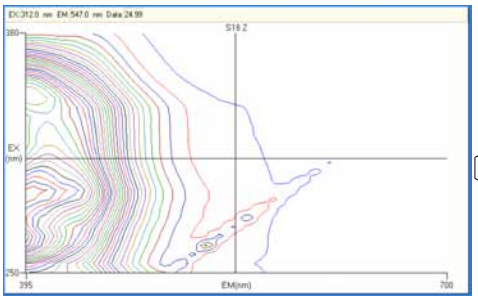
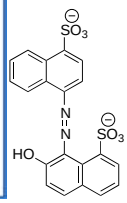
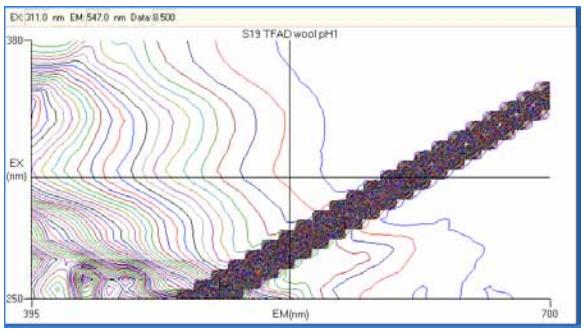
Sdb

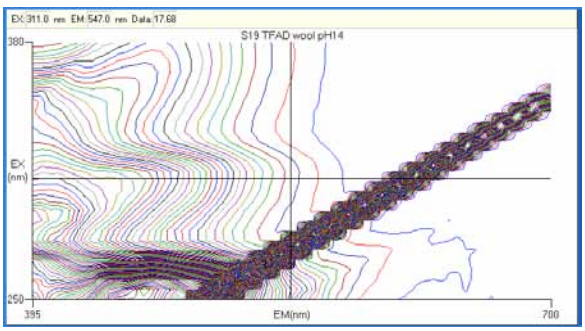




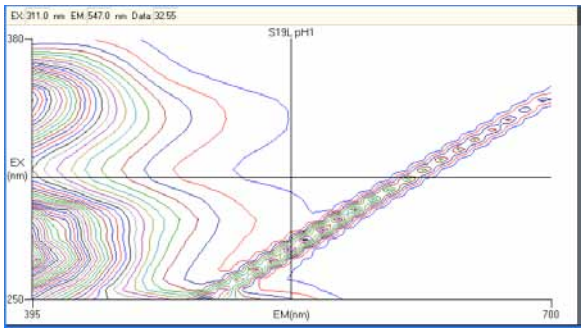
S 19 **[CI 16100 Acid Orange 14]**

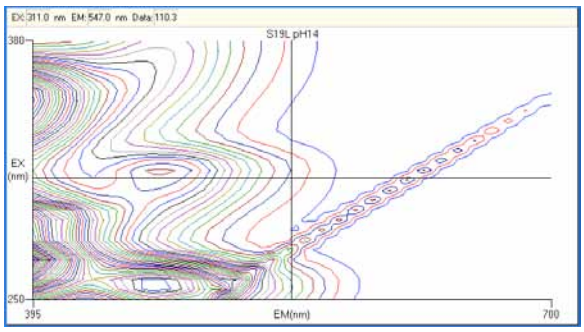
wool





vial





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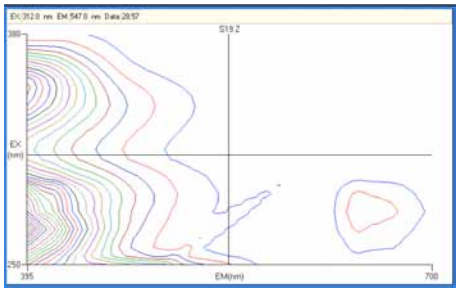
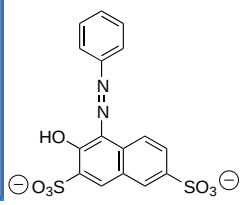
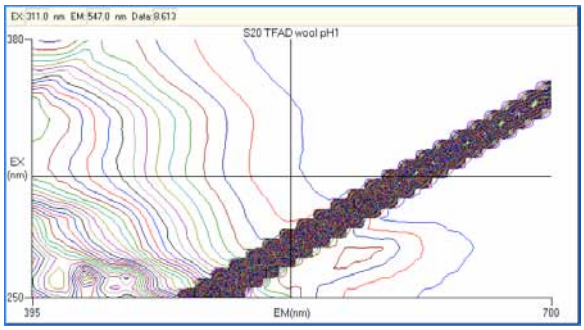
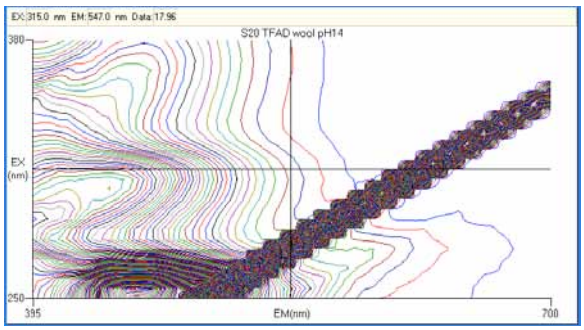




S 20 **[CI 16150 Acid Red 26]**

wool





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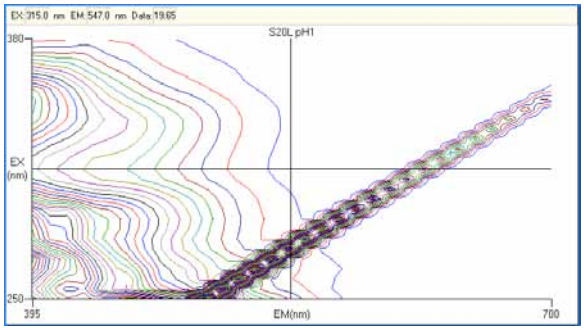
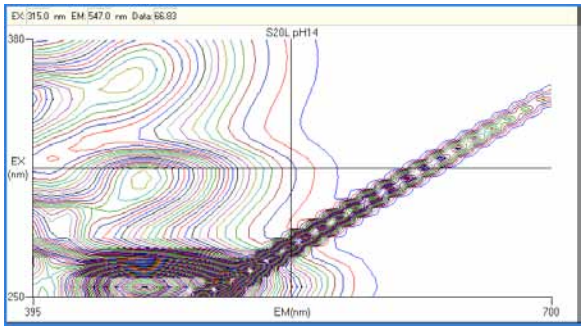
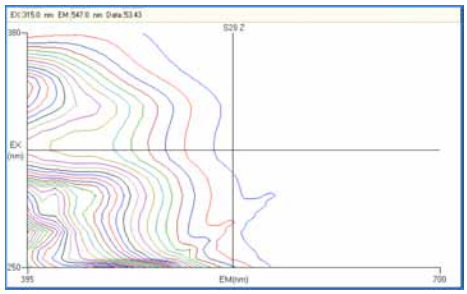
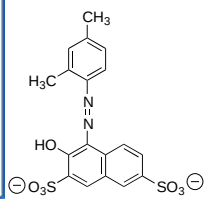
vial





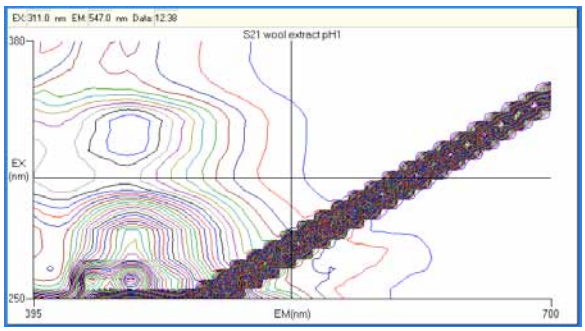
Sdb

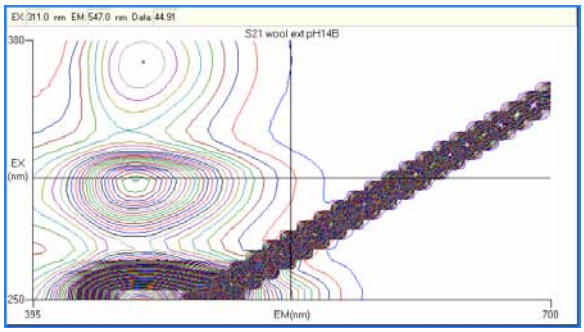




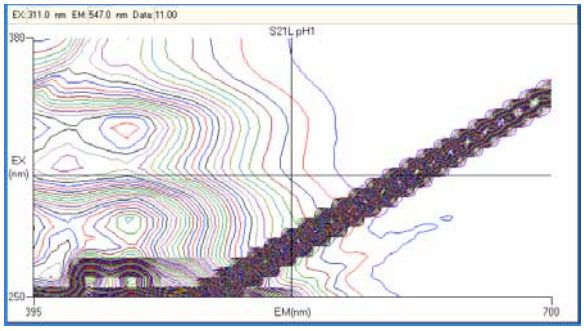
S 21 **[CI 16180 Acid Red 17]**

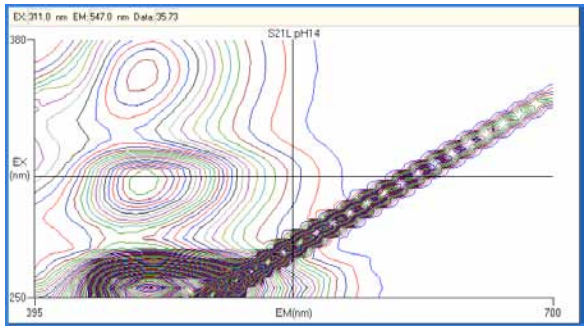
wool



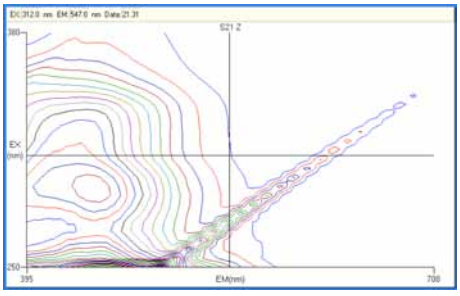


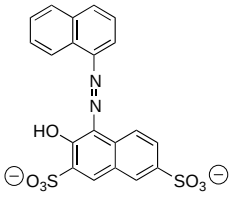
vial





Sdb





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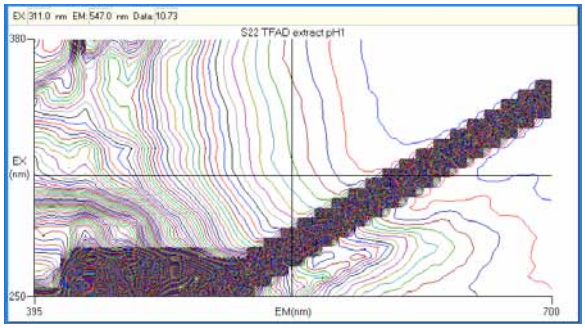
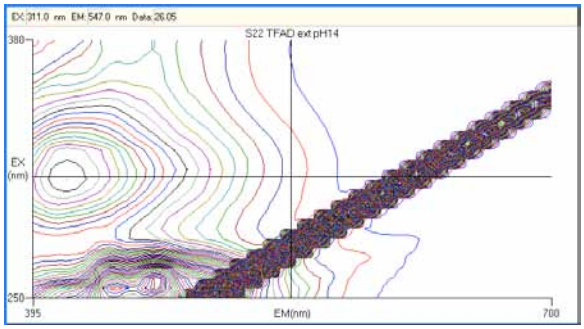
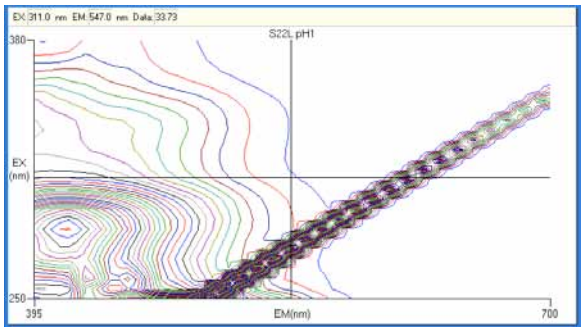
S 22	**[CI 16185 Acid Red 27]**

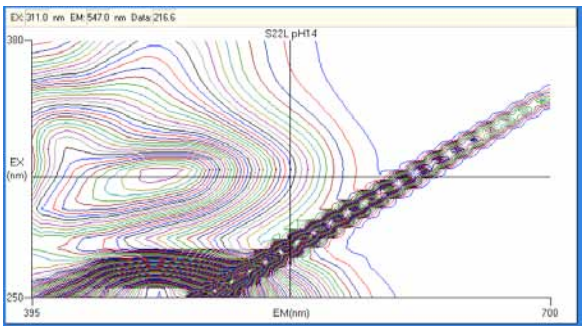
wool



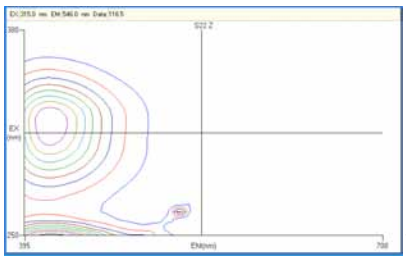


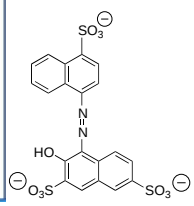
vial





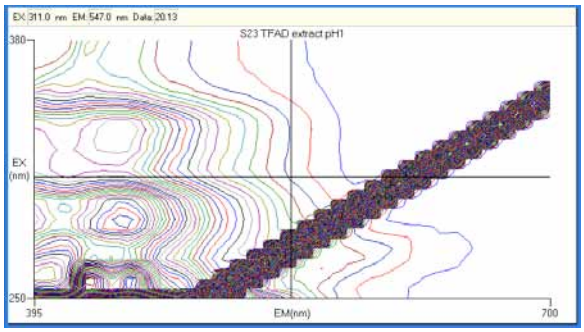
Sdb

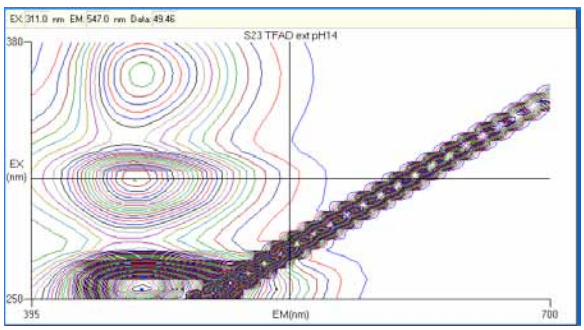




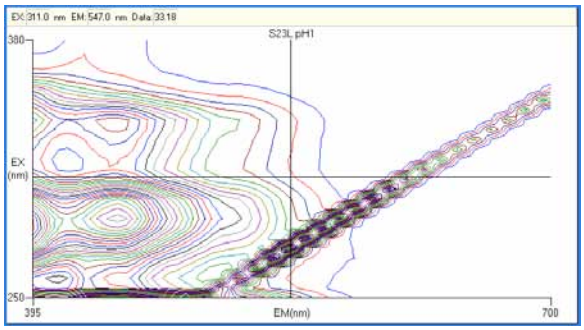
S 23	**[CI 16230 Acid Orange 10]**

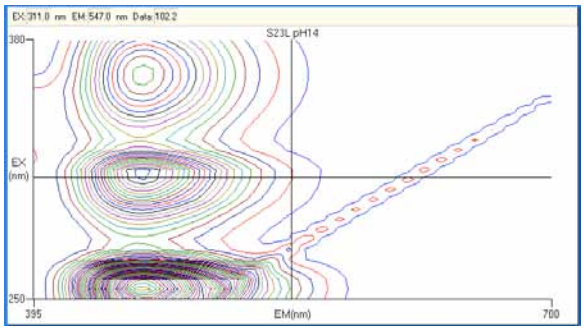
wool





vial





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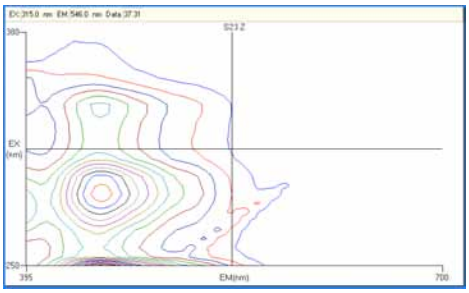
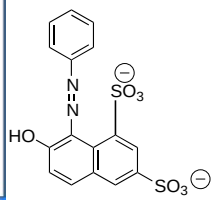
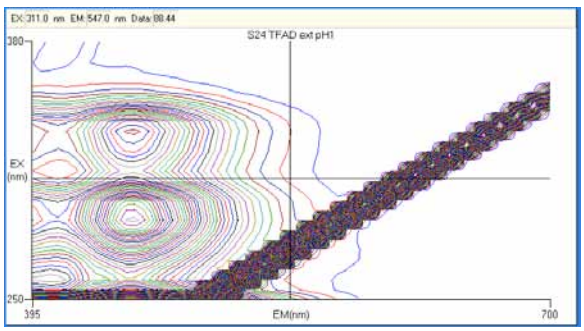
Sdb

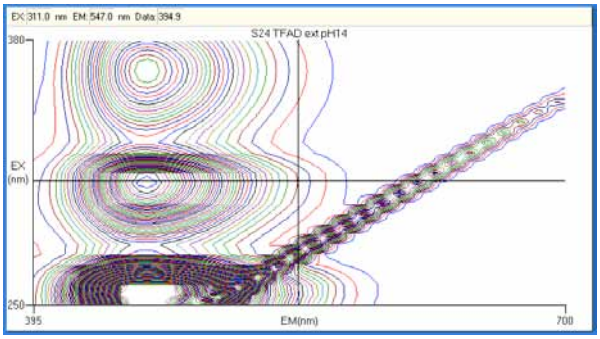




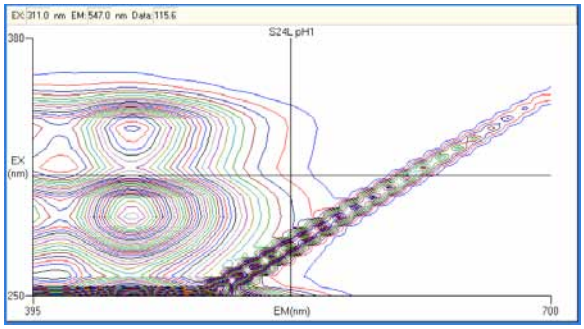
S 24 **[CI 16250 Acid Red 44]**

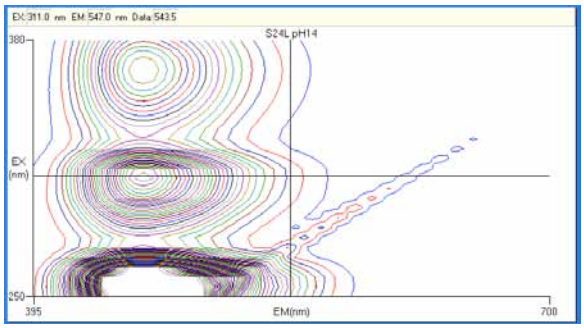
wool



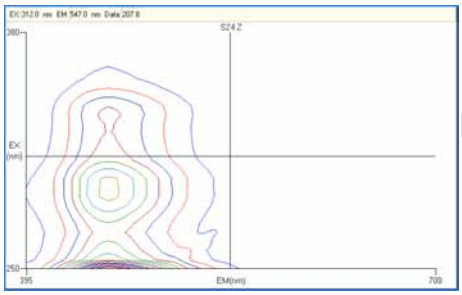


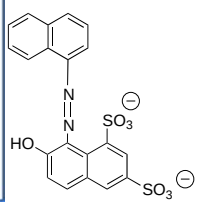
vial





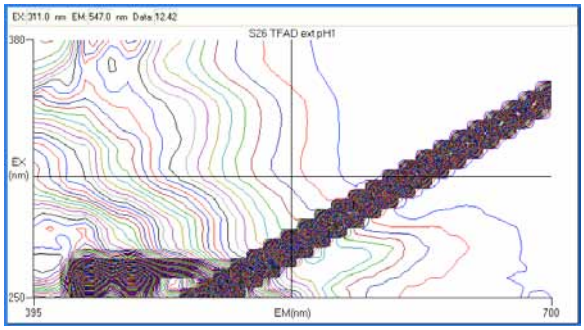
Sdb

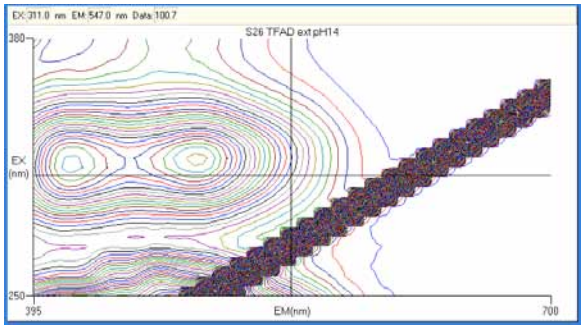




S 26 **[CI 16290 Acid Red 41]**

wool





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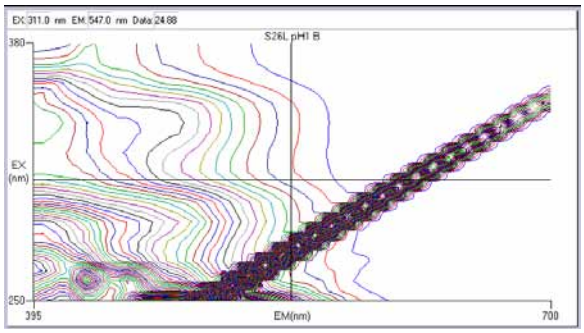
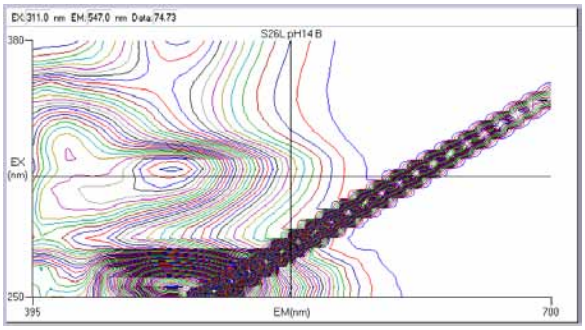
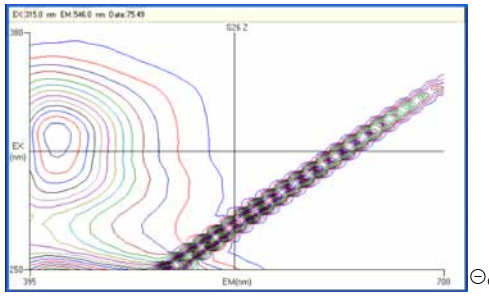
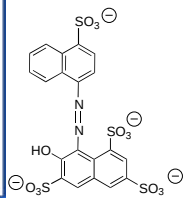
vial





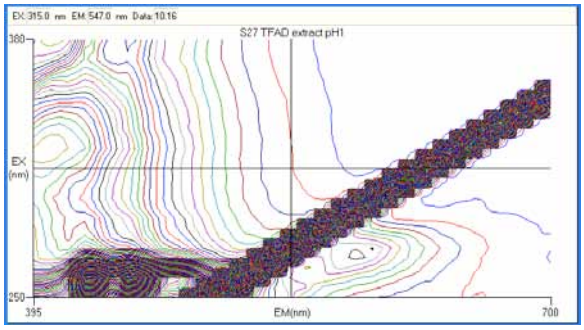
Sdb

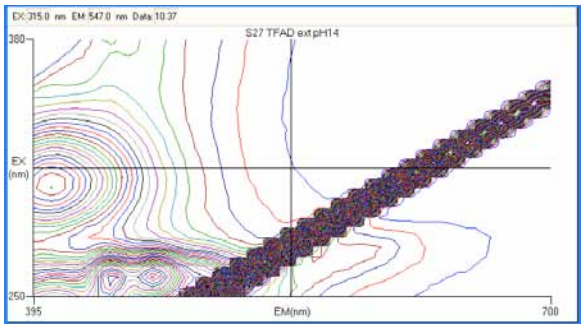




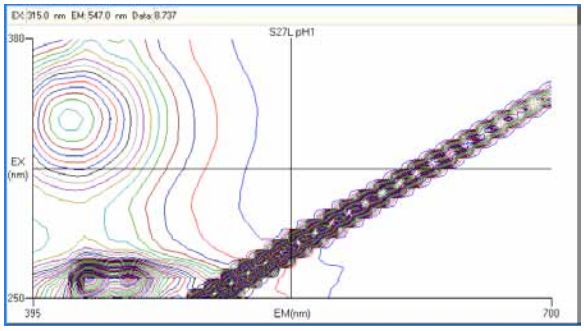
S 27 **[CI 17200 Acid Red 33]**

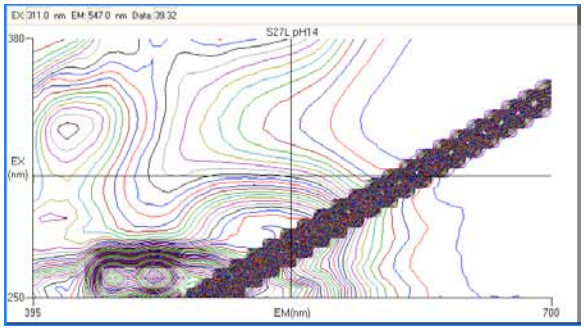
wool



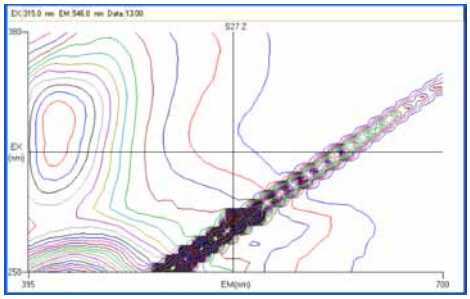


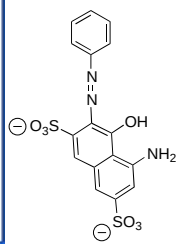
Vial





Sdb





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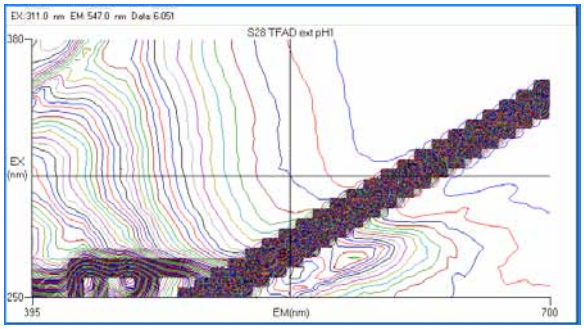
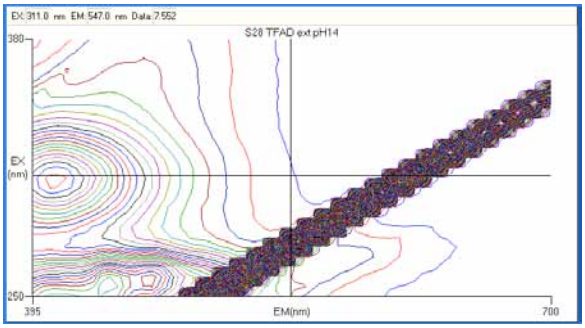
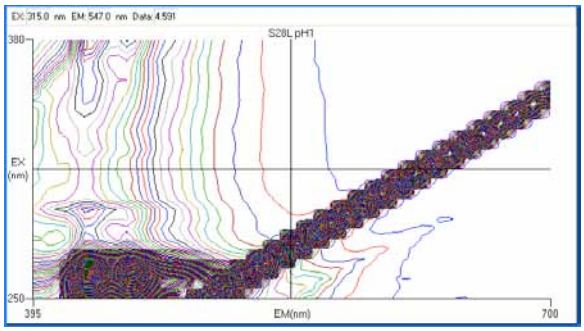
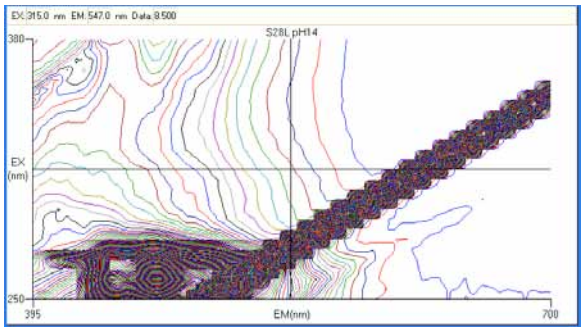
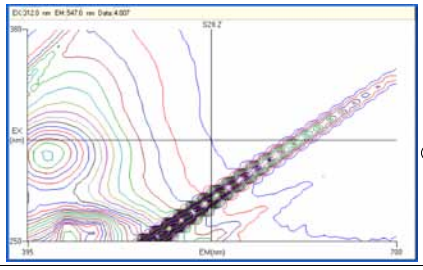
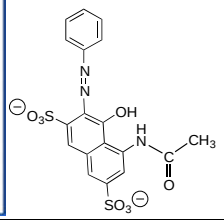
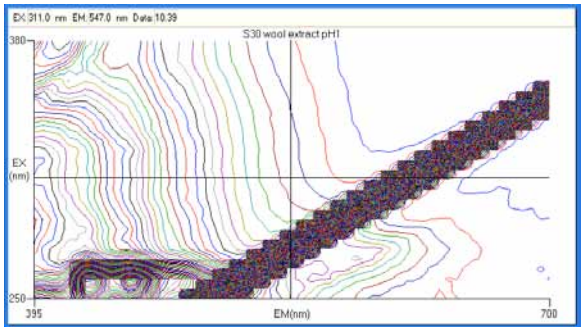
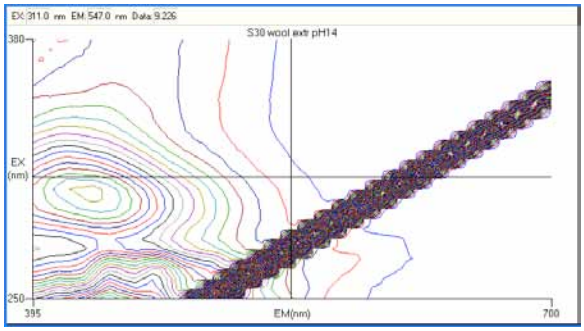
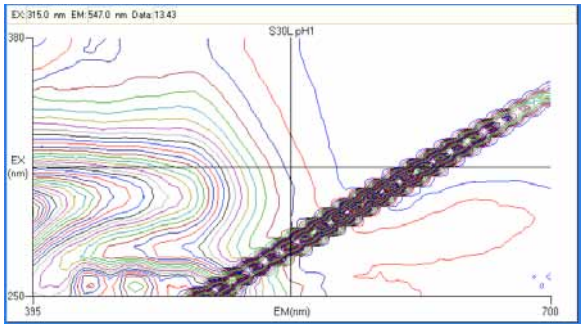
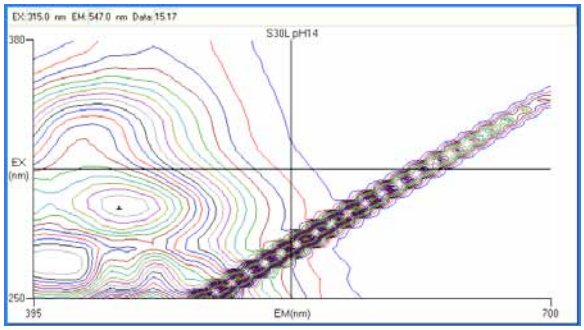
S 28	**[CI 18050 Acid Red 1]**
wool	 
vial	 
Sdb	 

S 30	**[CI 18820 Acid Yellow 11]**
wool	 
vial	 

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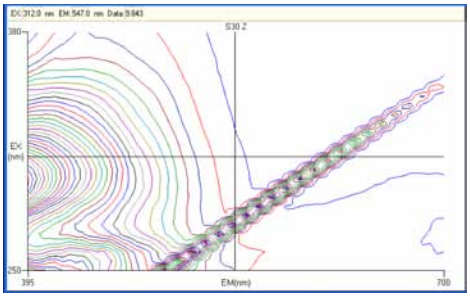
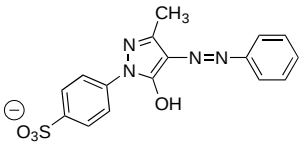
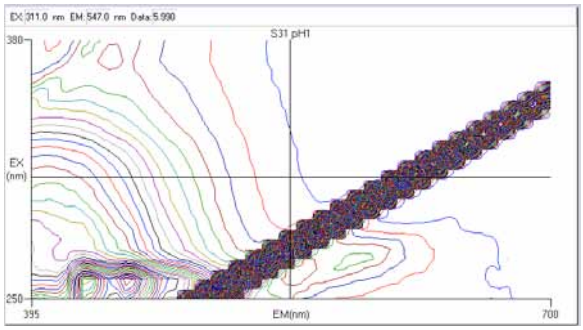
Sdb

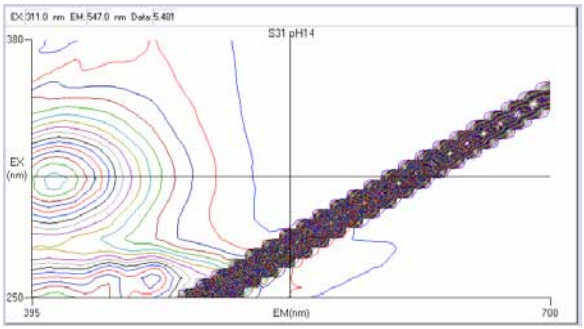




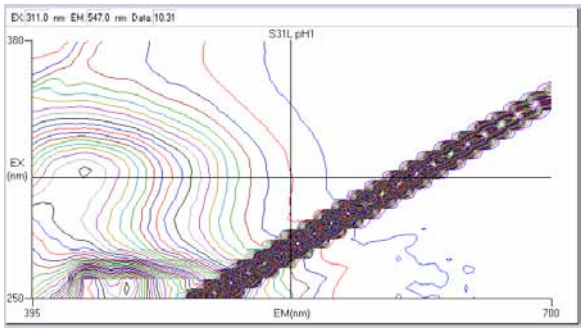
S 31 **[CI 19140 Acid Yellow 23]**

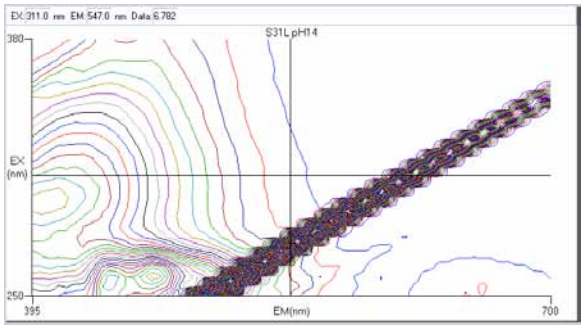
wool



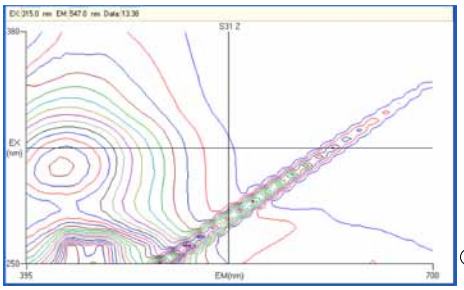


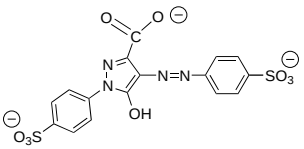
vial





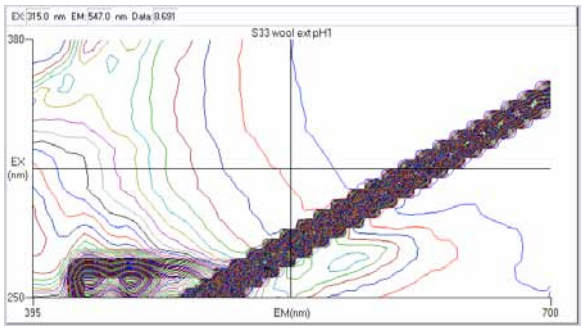
Sdb

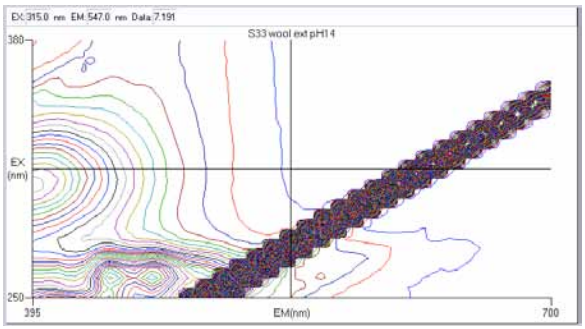




S 33 **[CI 21000 Basic Brown 1]**

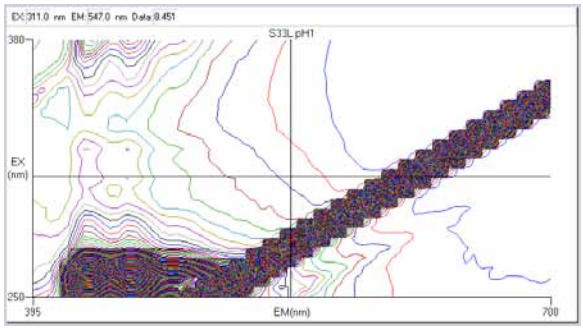
wool

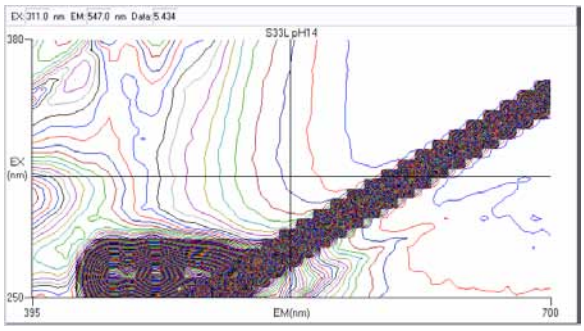




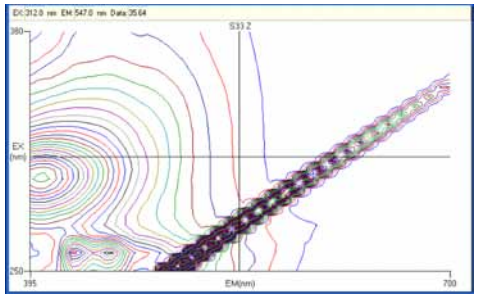
Continued

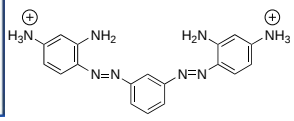
vial





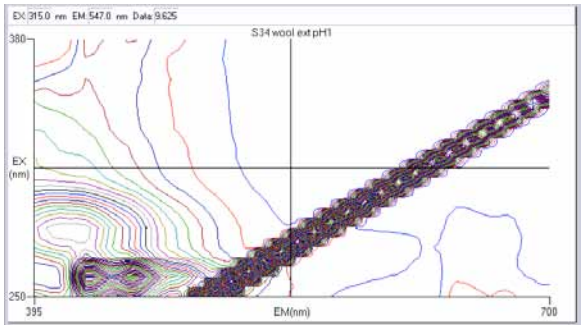
Sdb

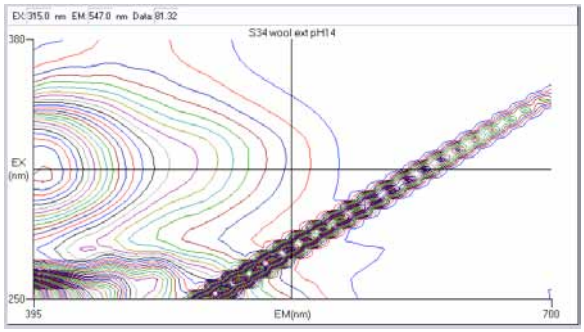




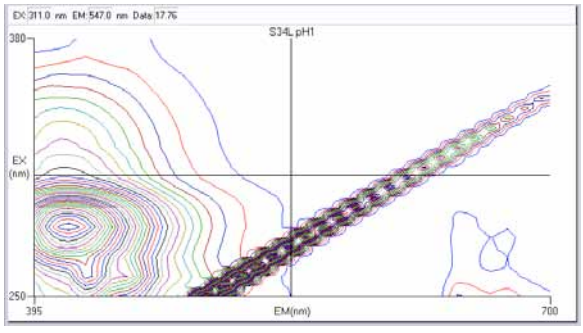
S 34 **[CI 22120 Direct Red 28]**

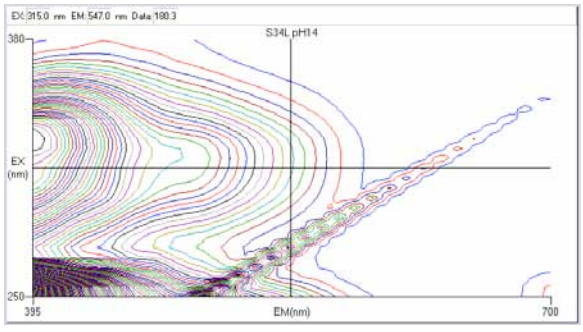
wool



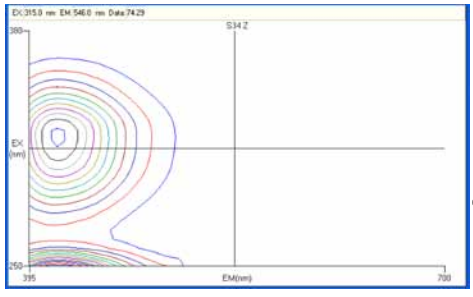


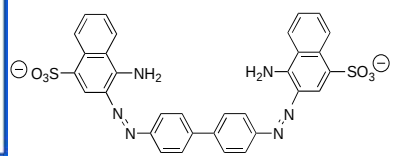
vial





Sdb

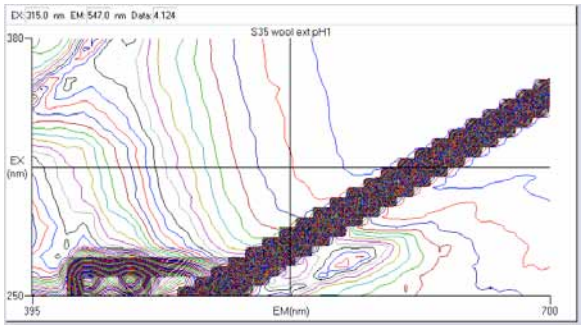


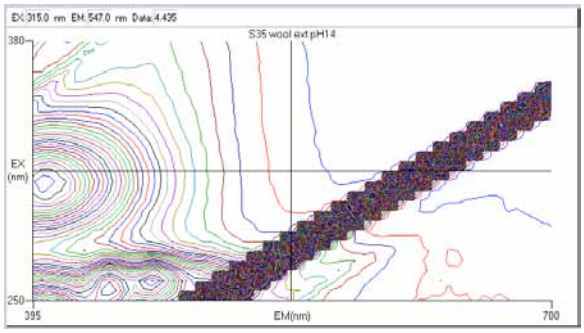


Continued

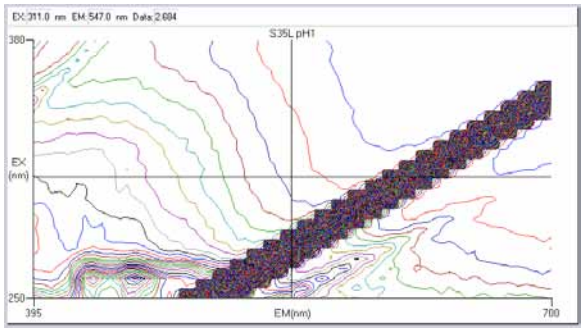
S 35 **[CI 24890 Direct Yellow 4]**

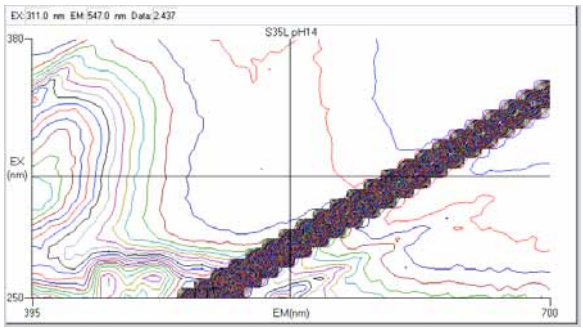
wool



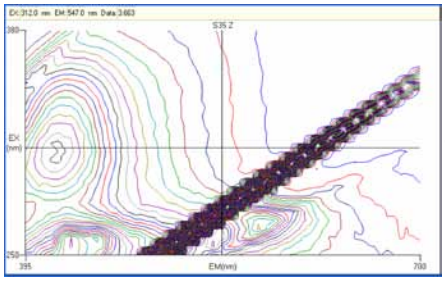


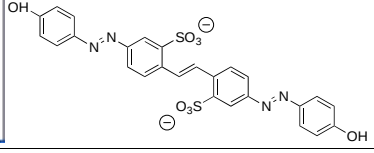
vial





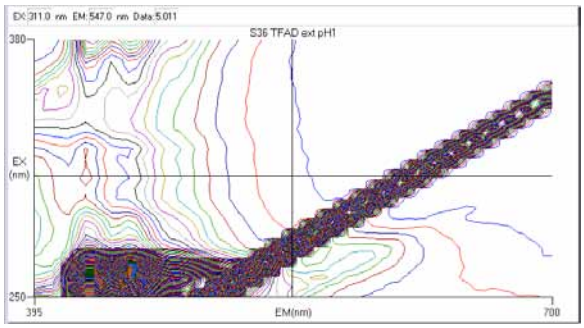
Sdb

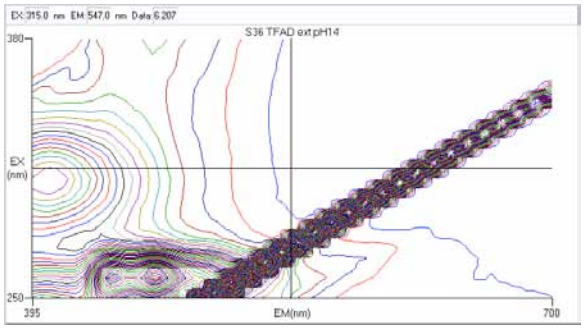




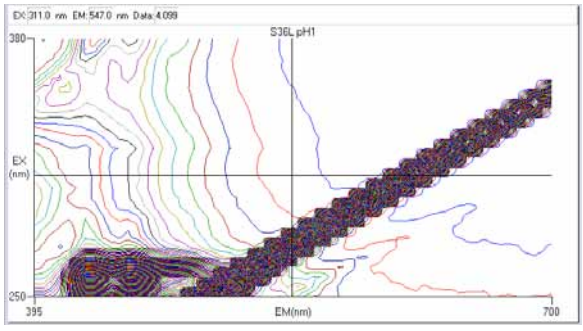
S 36 **[CI 26900 Acid Red 151]**

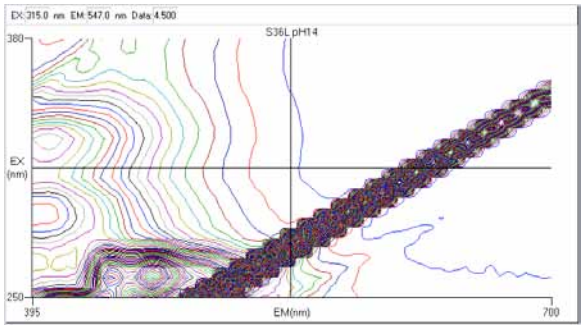
wool





vial





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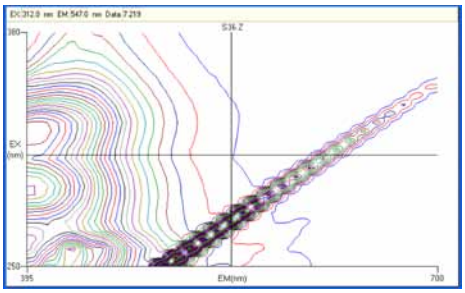
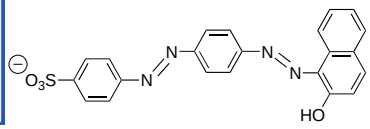
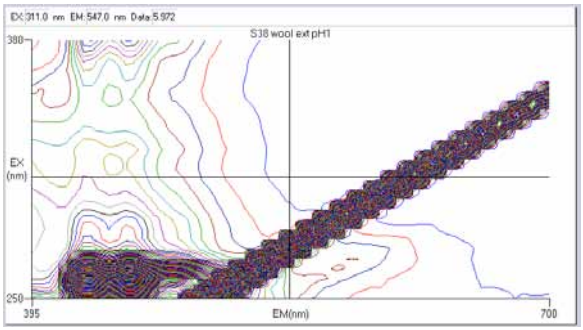
Sdb

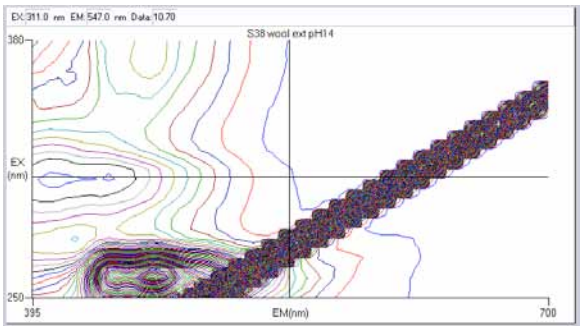




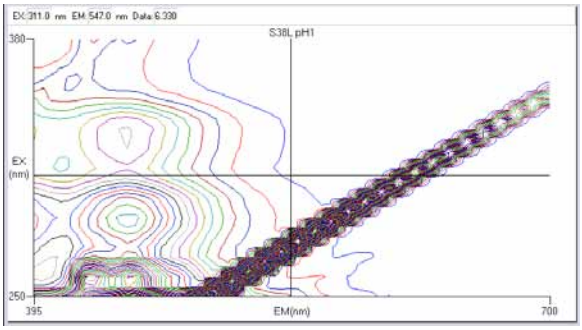
S 38	**[CI 27290 Acid Red 73]**

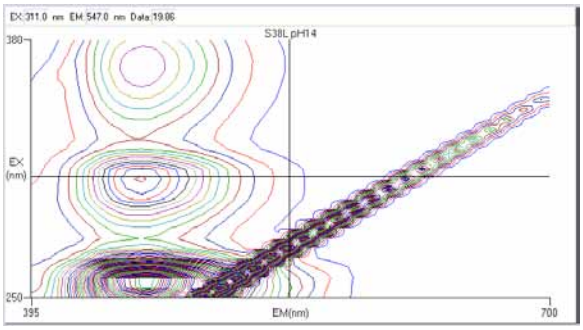
wool



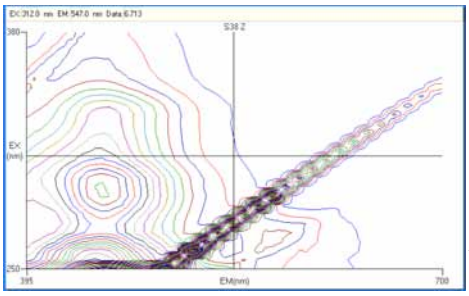


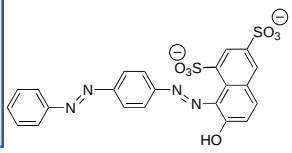
vial





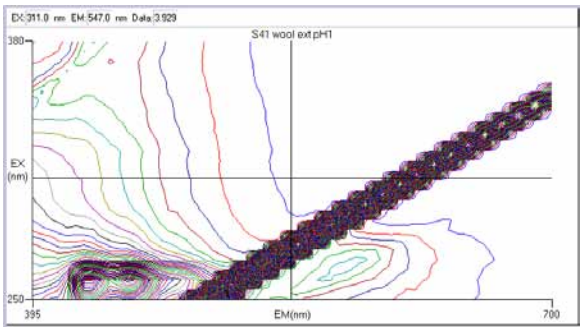
Sdb

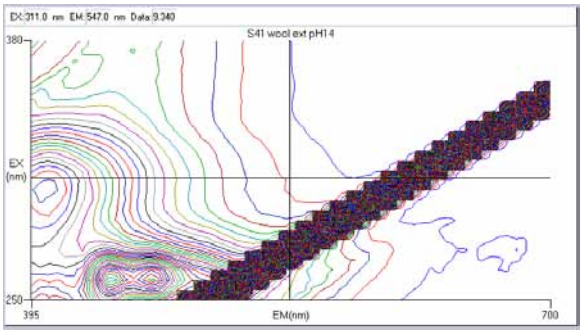




S 41	**[CI 42040 Basic Green 1]**

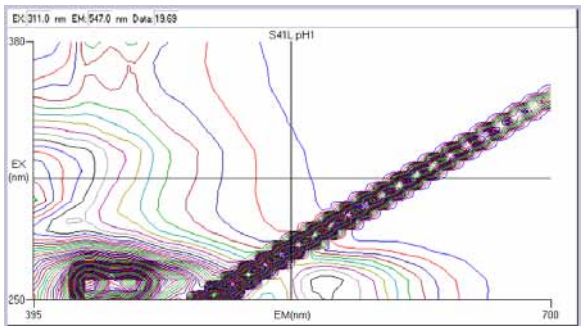
wool

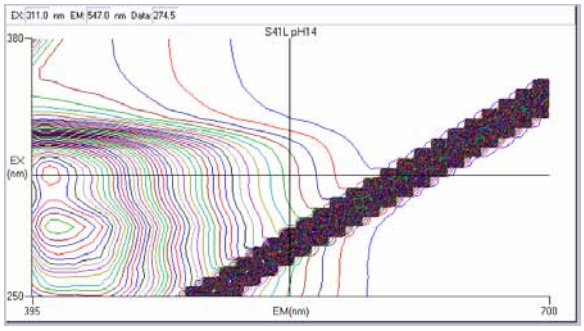




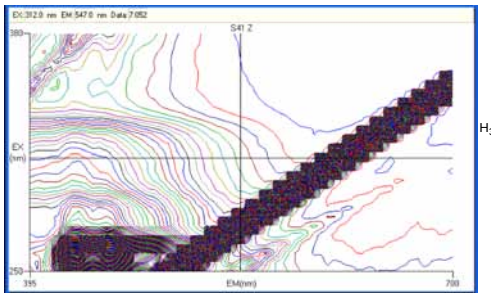
Continued

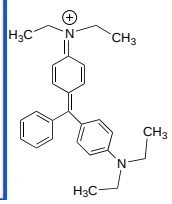
vial





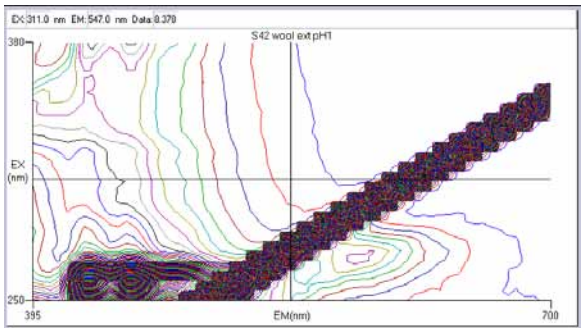
Sdb

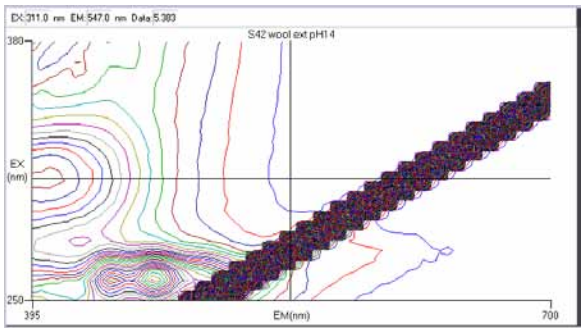




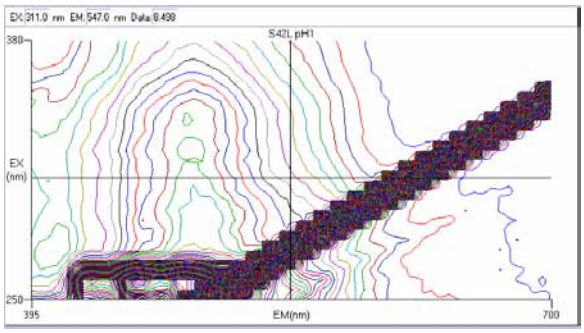
S 42 **[CI 42051 Acid Blue 3]**

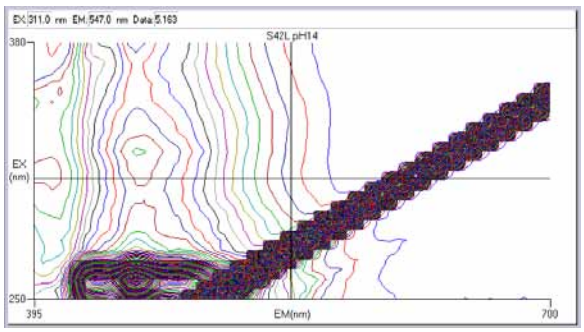
wool



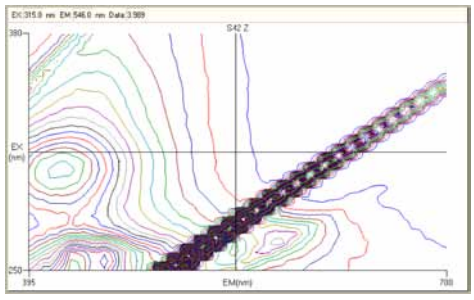


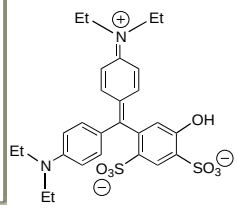
vial





Sdb

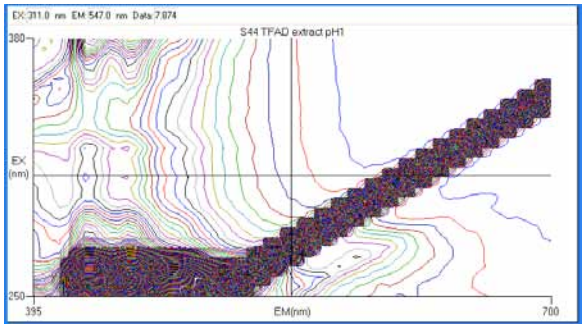


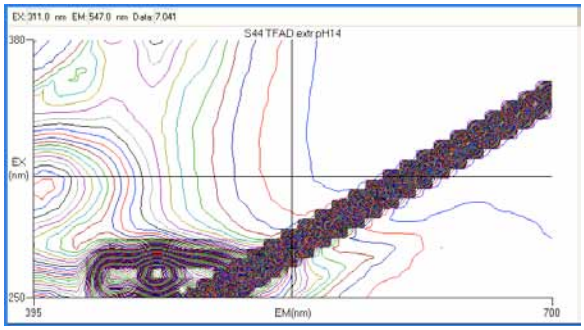


Continued

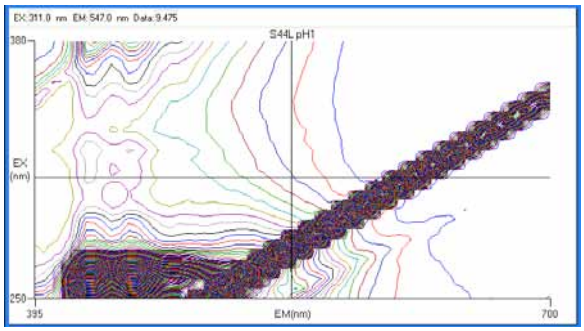
S 44	**[CI 42510 Basic Violet 14]**

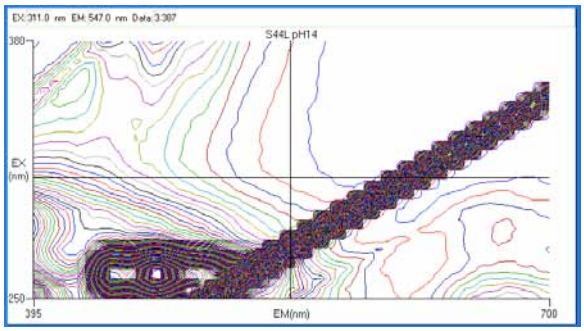
wool





vial





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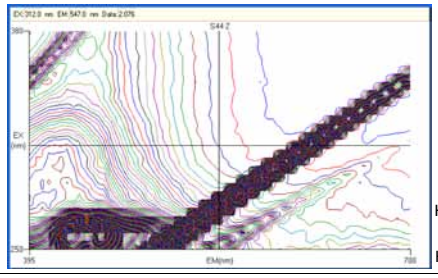
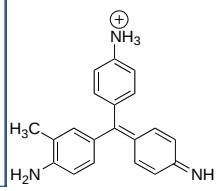
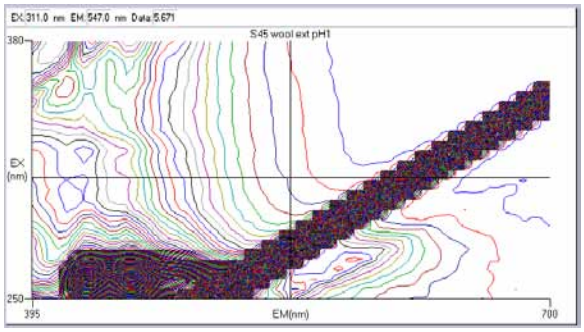
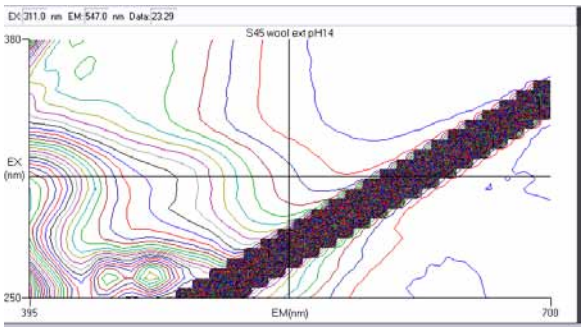




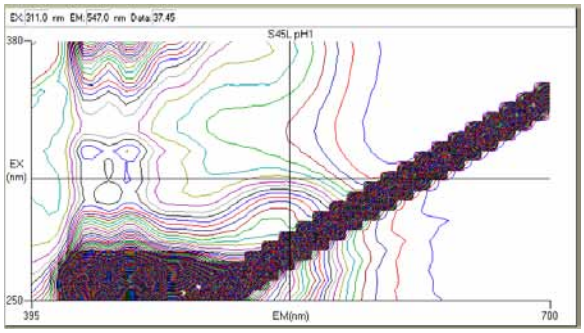
S 45	**[CI 42535 Basic Violet 1]**

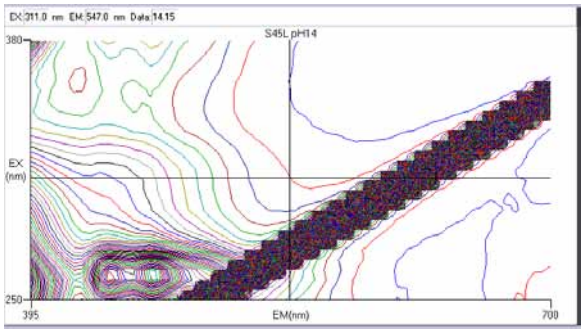
wool





vial





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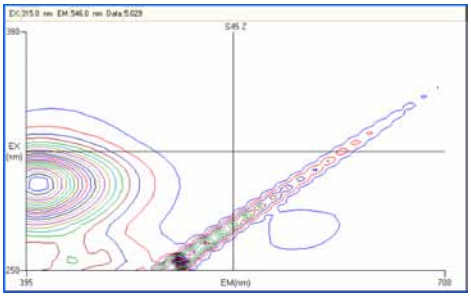
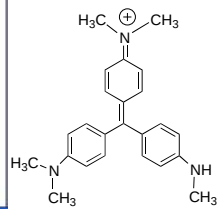
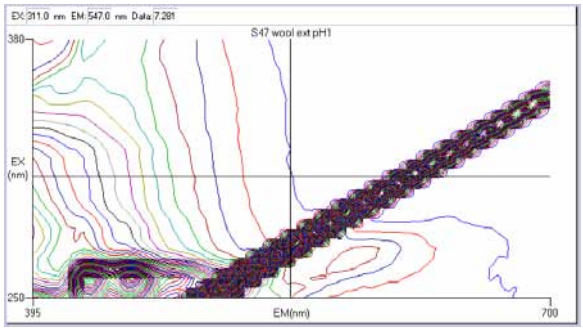
Sdb

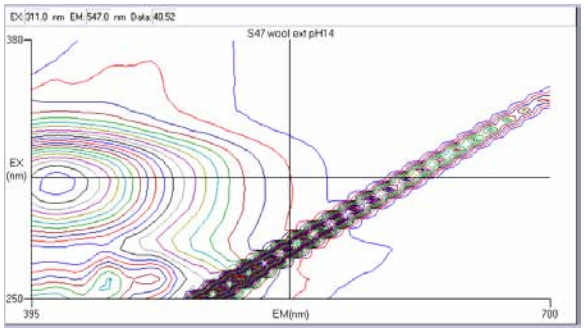




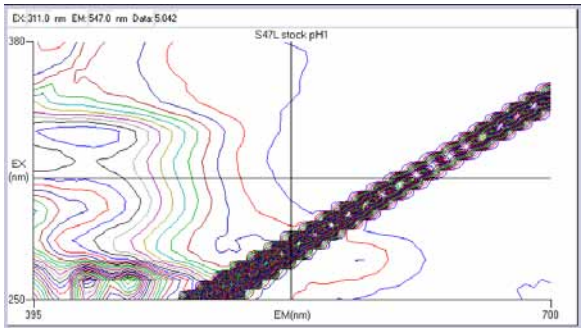
S 47 **[CI 42780 Acid Blue 93]**

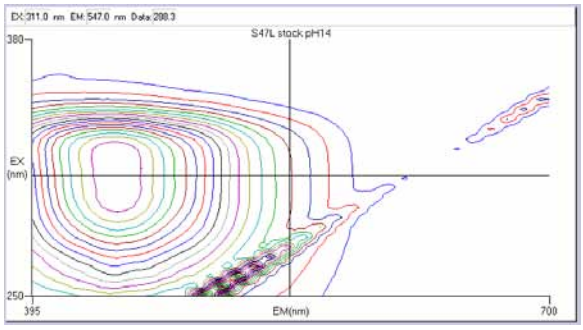
wool



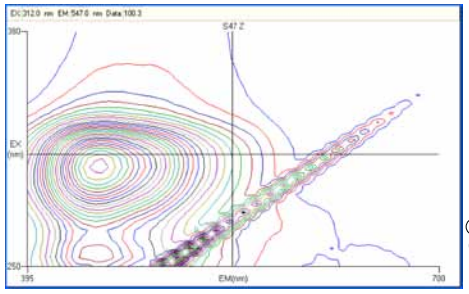


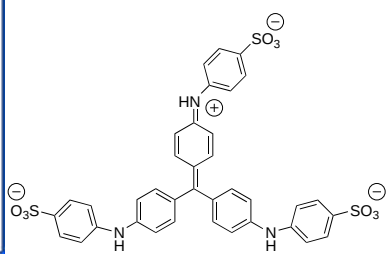
vial





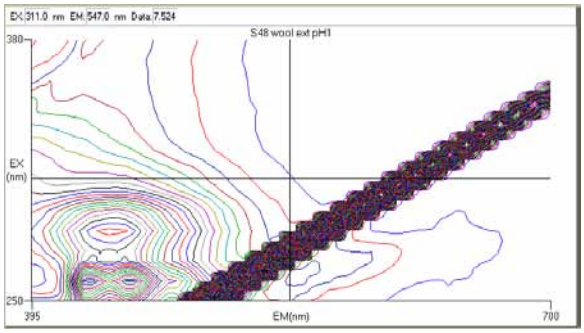
Sdb

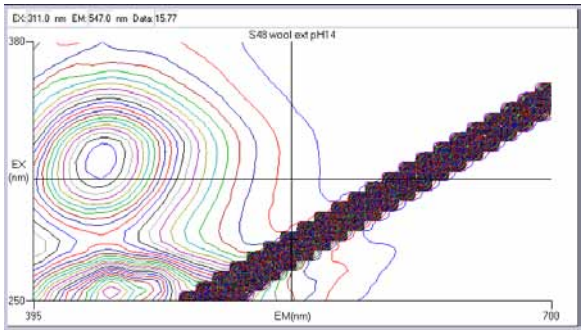




S 48 **[CI 44040 Basic Blue 11]**

wool





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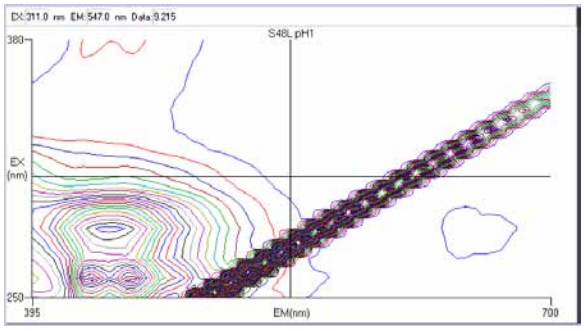
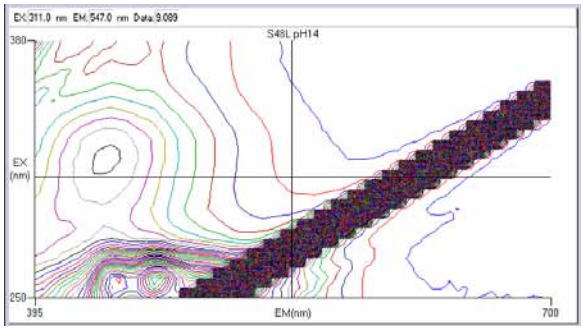
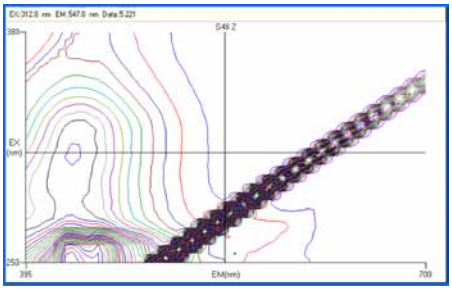
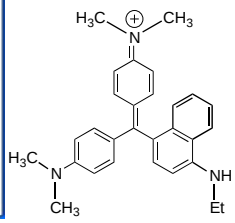
vial





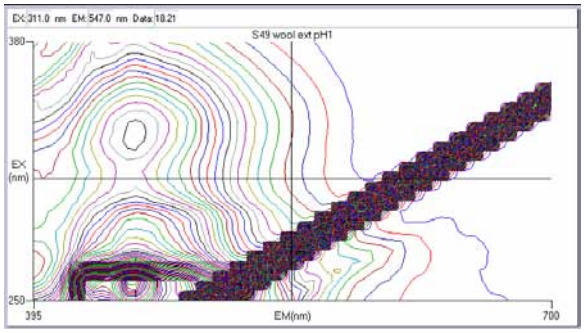
Sdb

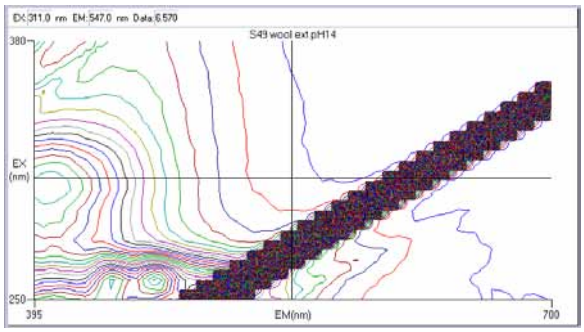




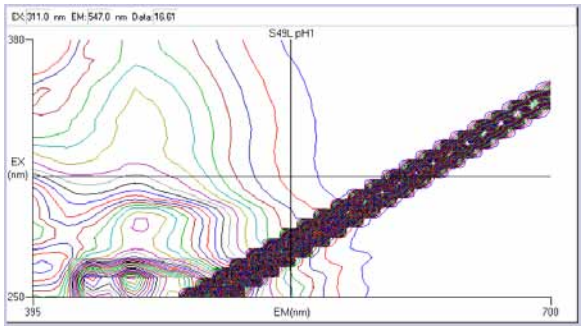
S 49 **[CI 44045 Basic Blue 26]**

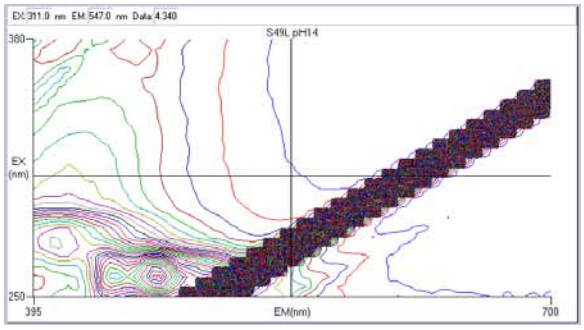
wool



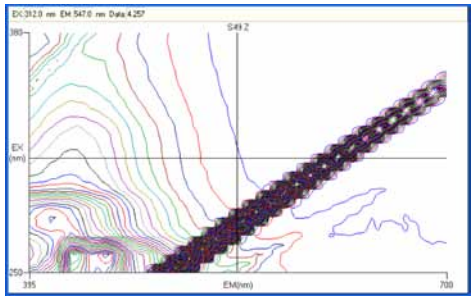


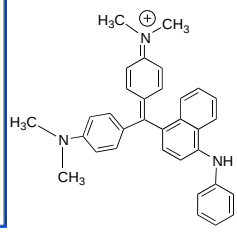
vial





Sdb

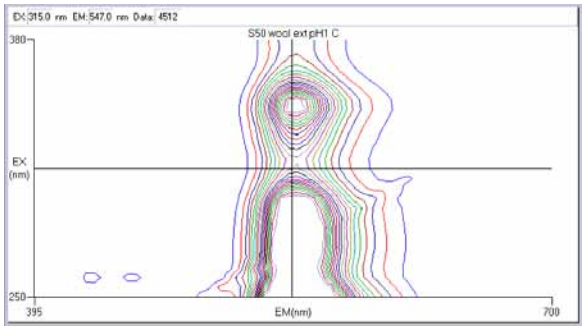


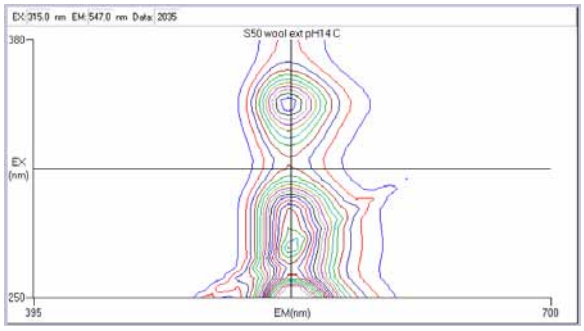


Continued

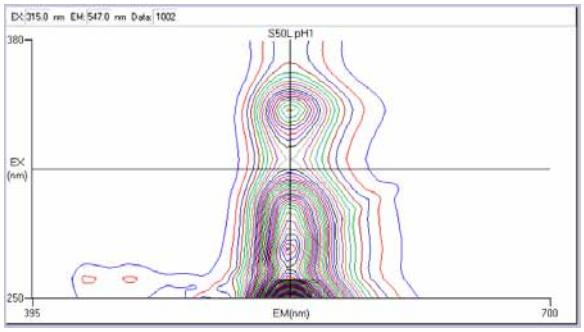
S 50 **[CI 45160 Basic Red 1]**

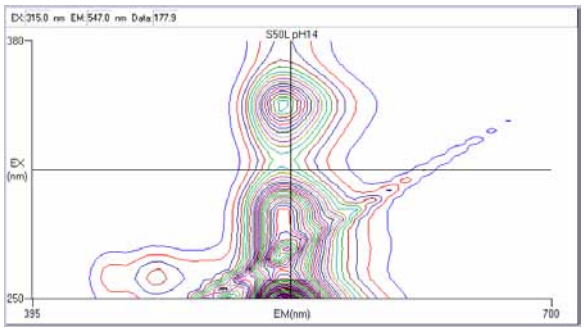
wool





vial





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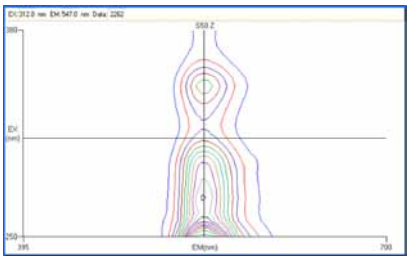
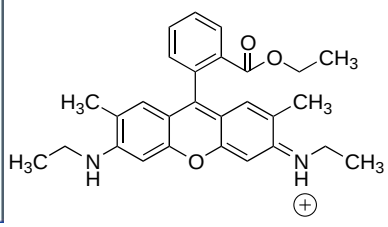
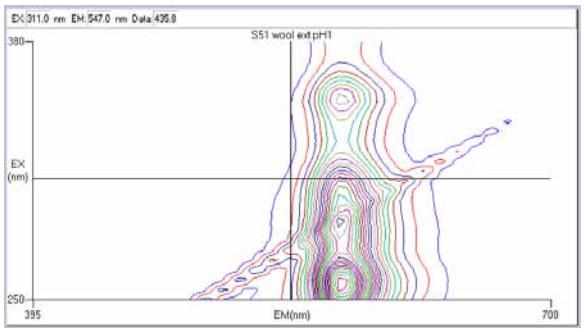
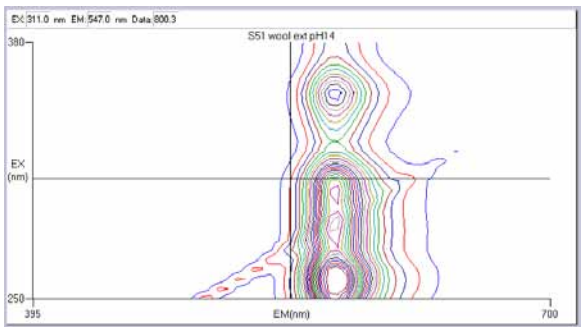




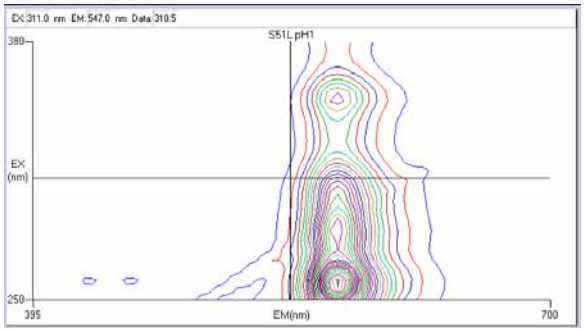
S 51 **[CI 45170 Solvent Red 49]**

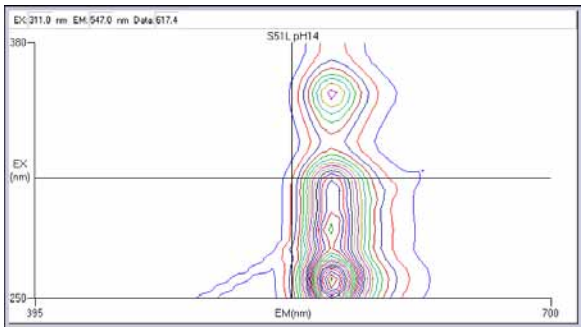
wool





vial





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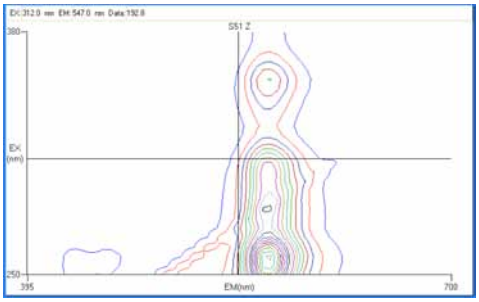
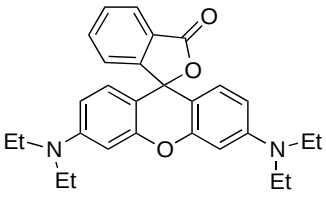
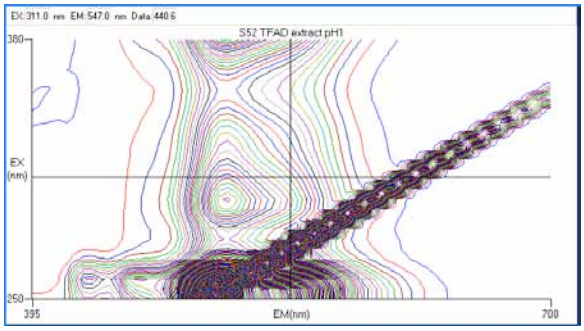
Sdb

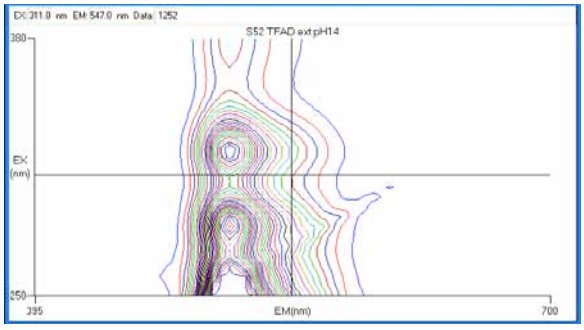




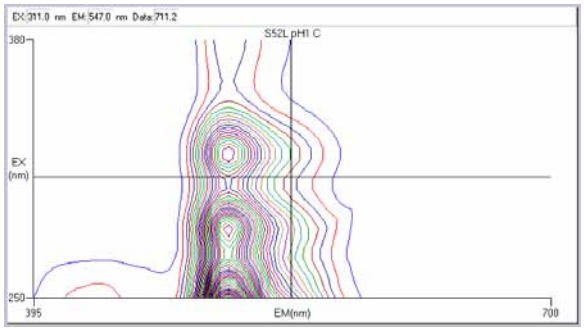
S 52 **[CI 45350 Acid Yellow 73]**

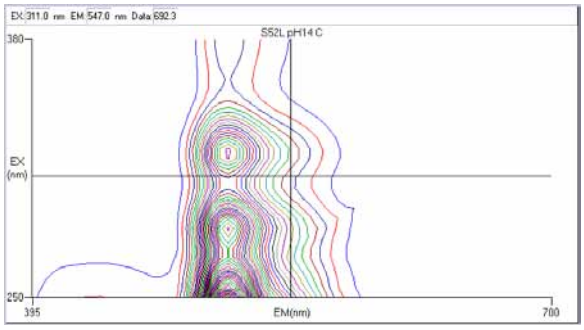
wool



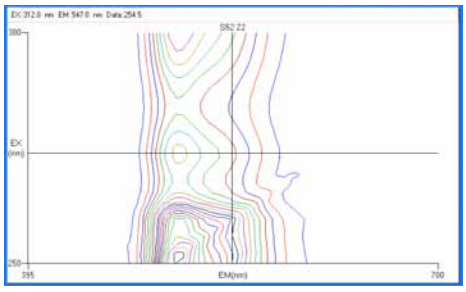


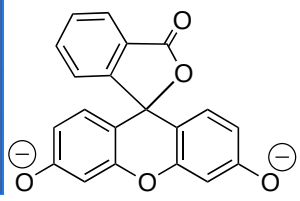
vial





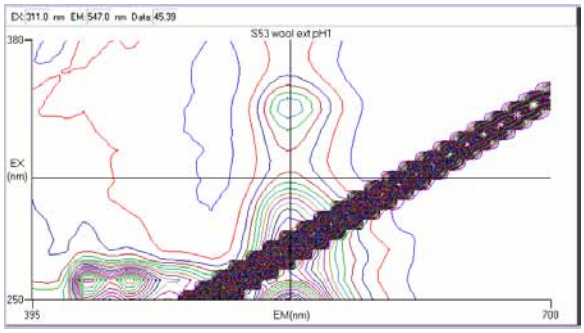
Sdb

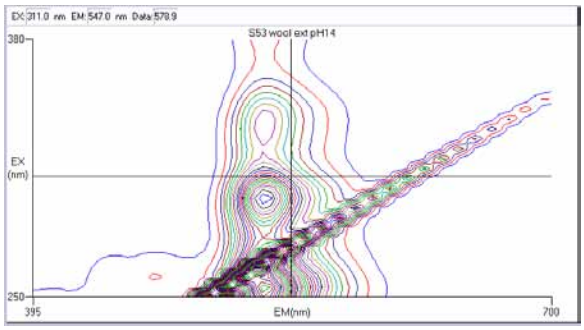




S 53 **[CI 45380 Acid Red 87]**

wool





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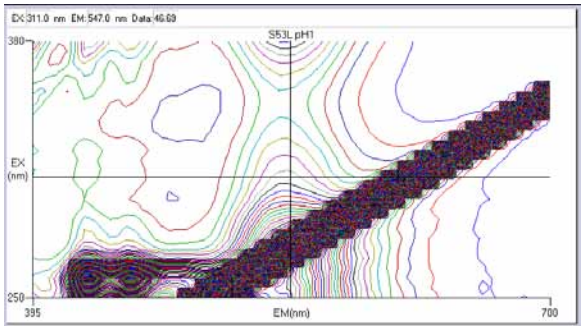
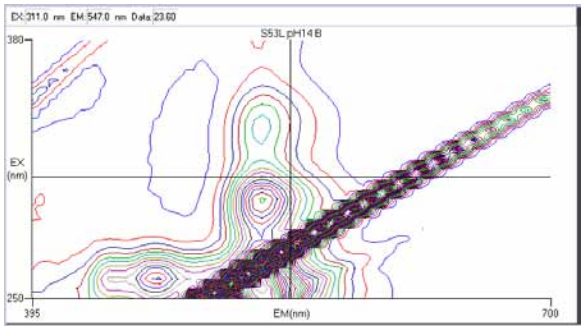
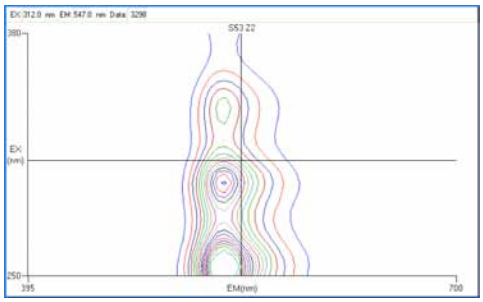
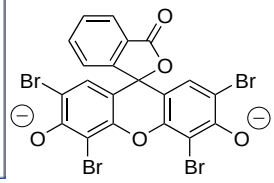
vial





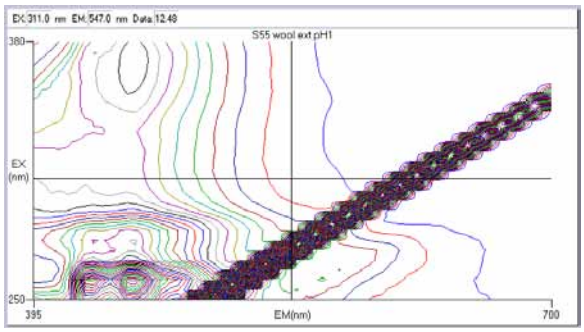
Sdb

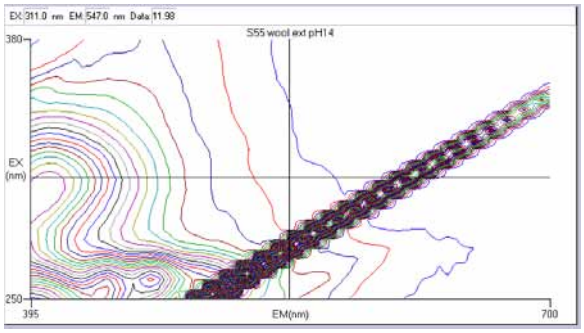




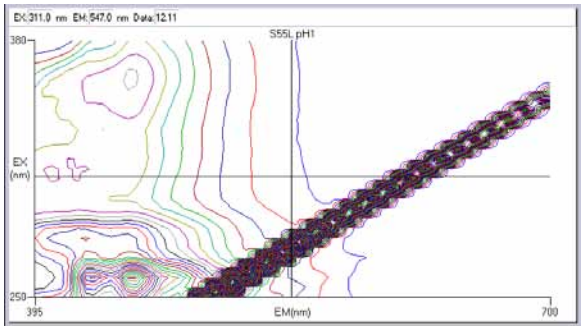
S 55 **[CI 47005 Acid Yellow 3]**

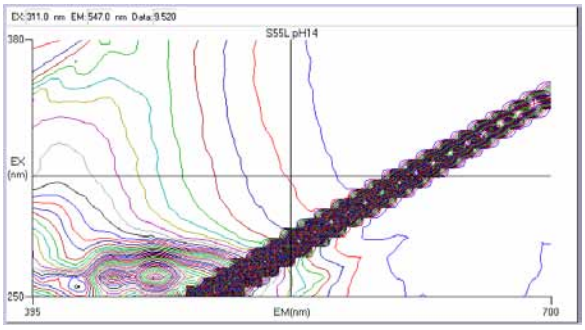
wool





vial





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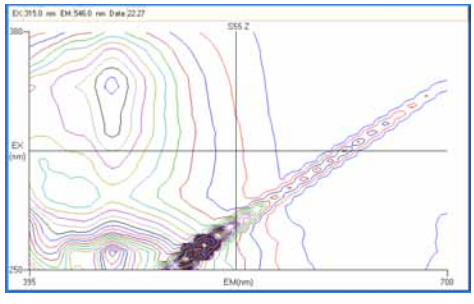

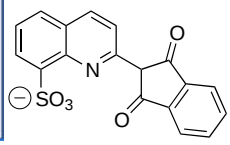

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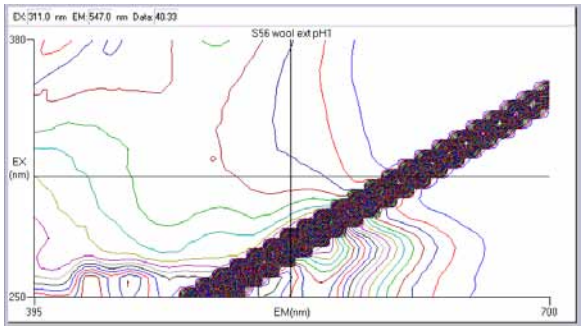
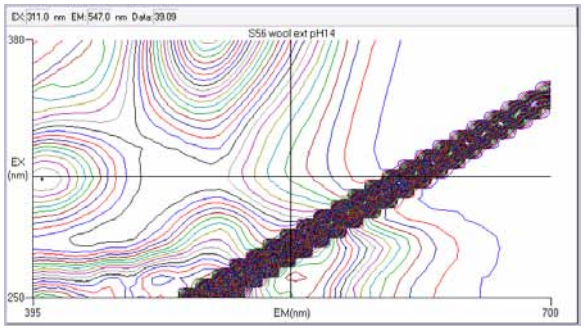
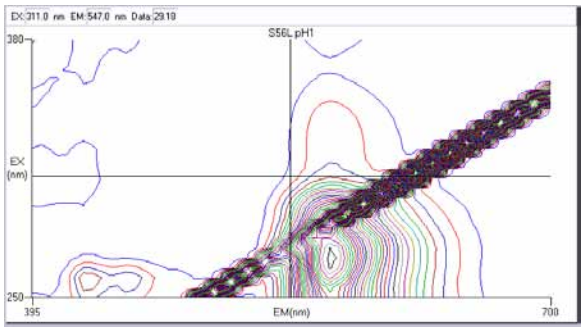
S 56	**[CI 50240 Basic Red 2]**

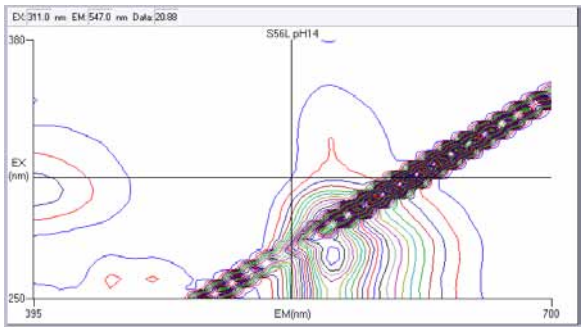
wool





vial





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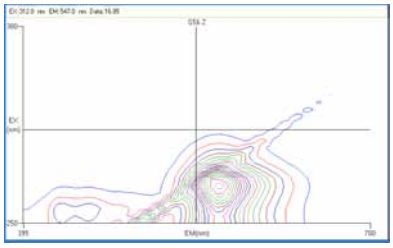
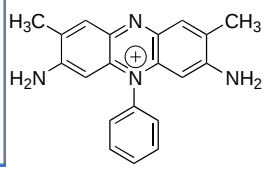
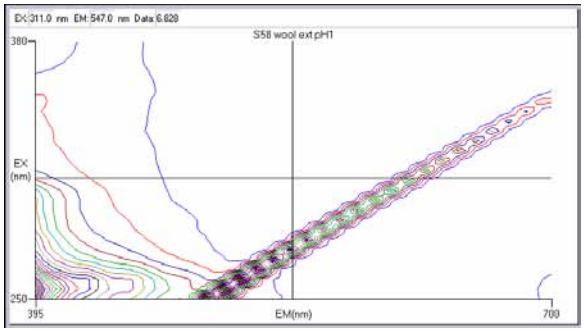
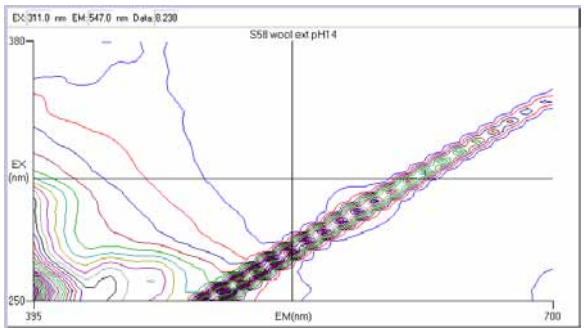




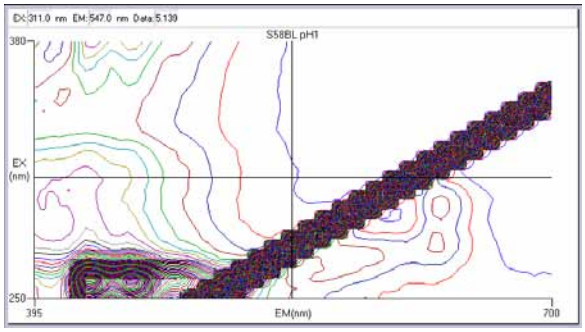
S 58	**[CI 50420 Acid Black 2]**

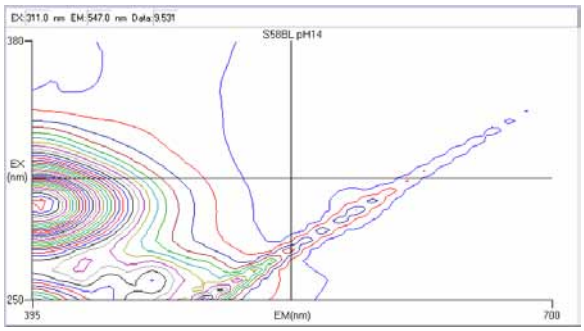
wool





vial





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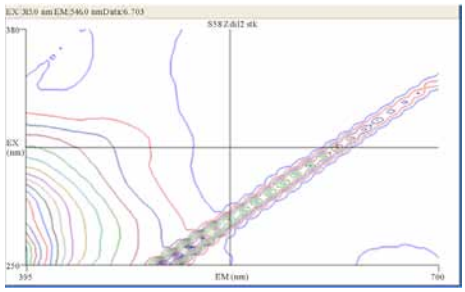
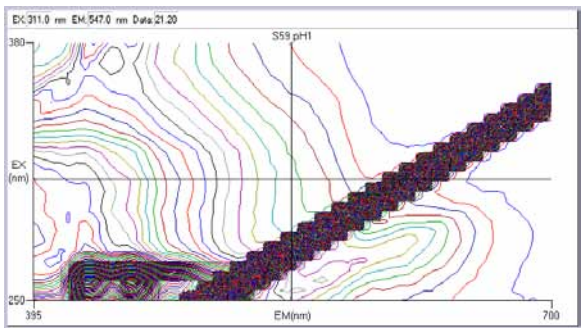
Sdb

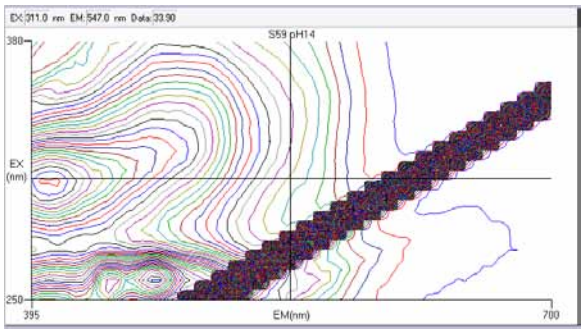


uncertain structure

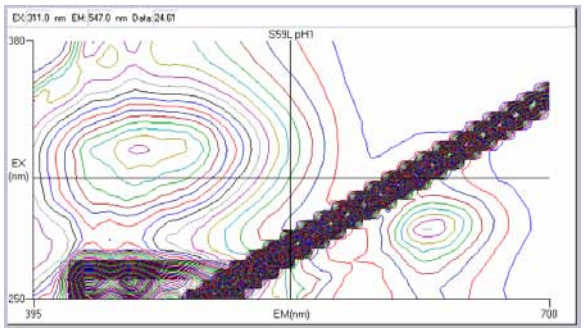
S 59 **[CI 52015 Basic Blue 9]**

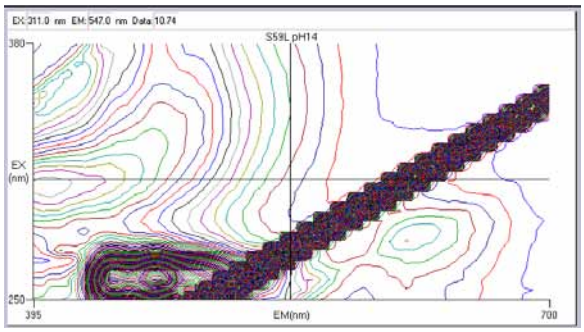
wool



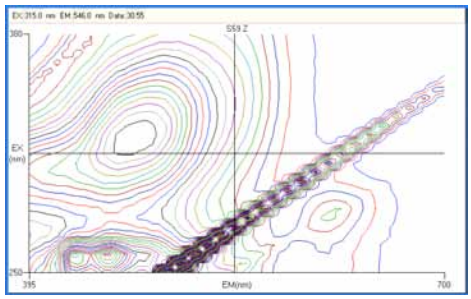


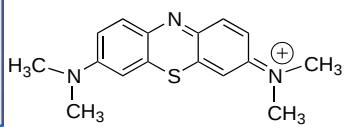
vial





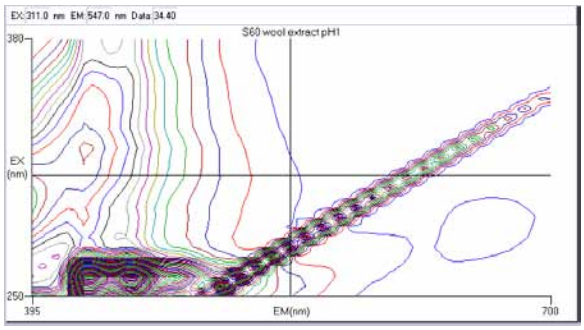
Sdb

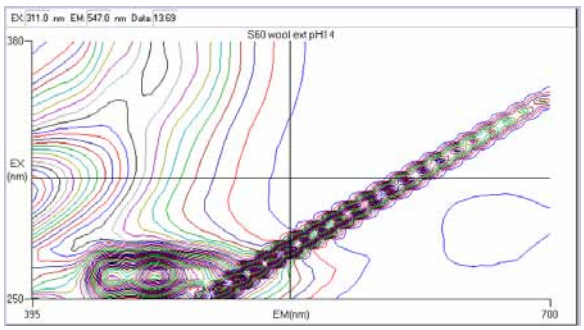




S 60 **[CI 56085 Murexide]**

wool





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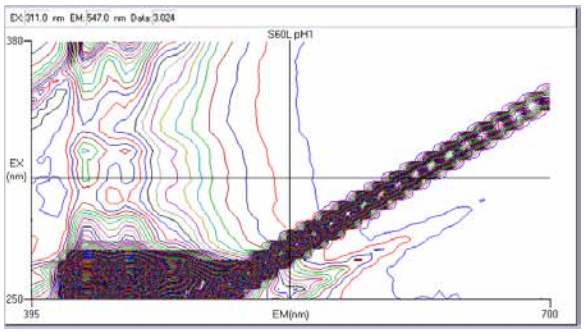
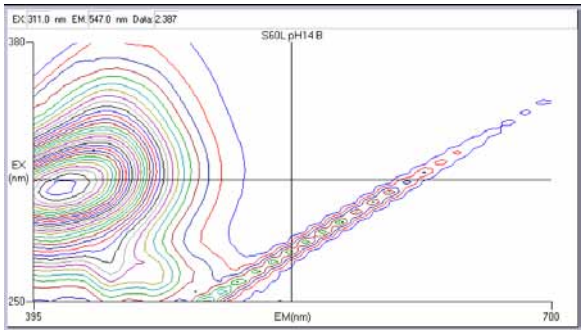
vial





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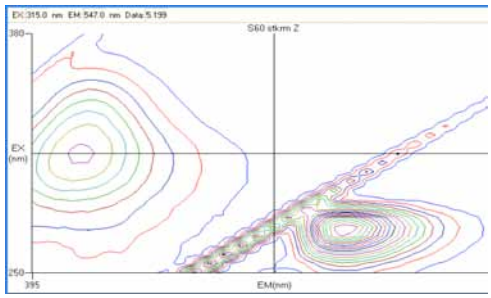
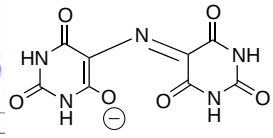
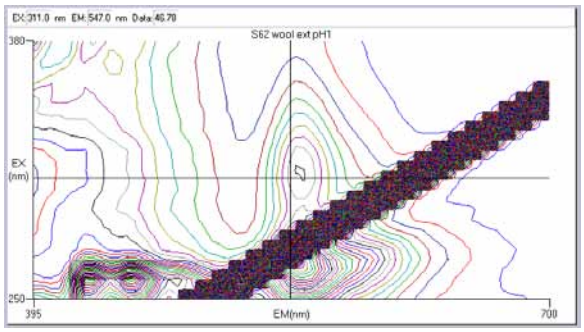
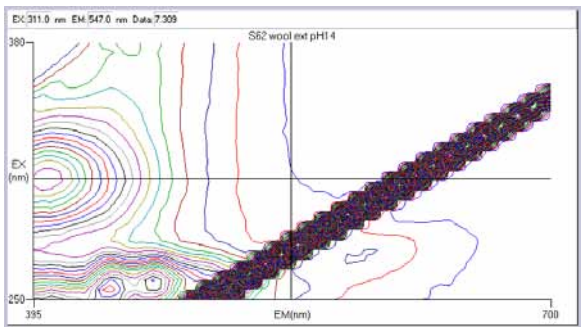




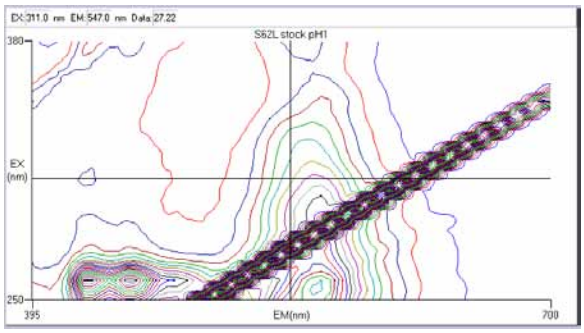
S 62 **[CI 58005 Mordant Red 3]**

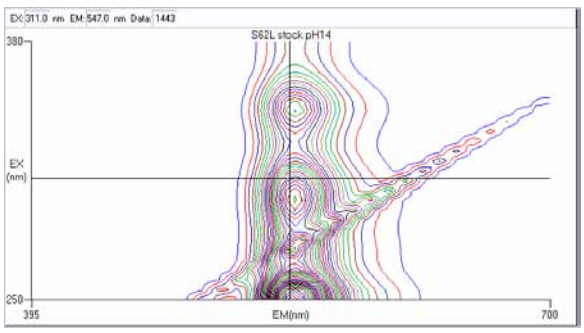
wool





vial





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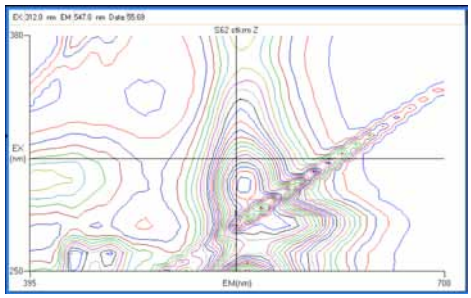

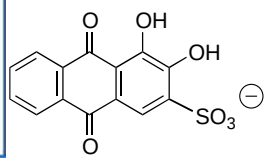

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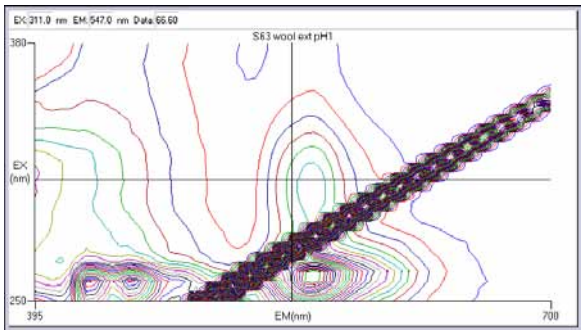
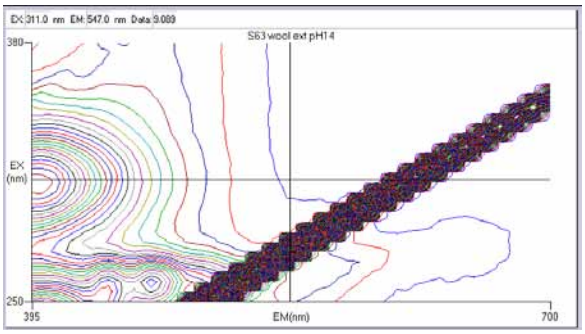
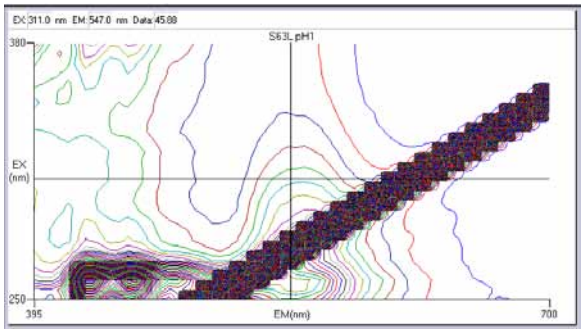
S 63 **[CI 58219 Alizarin Red PS]**

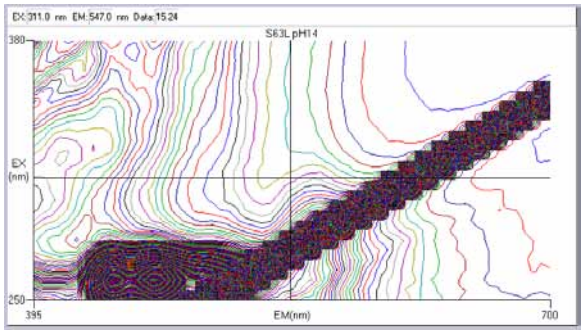
wool





vial





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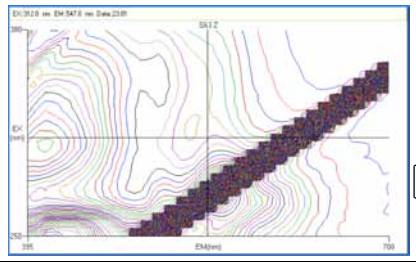
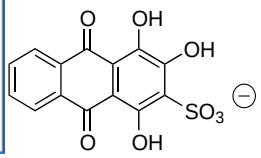
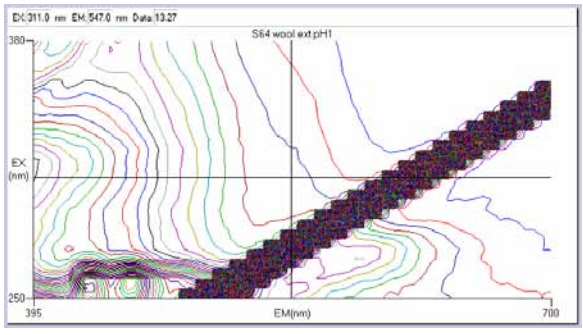
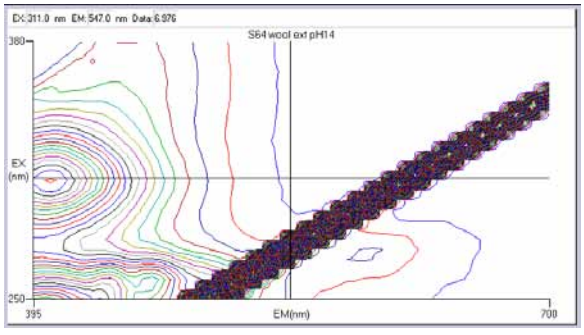




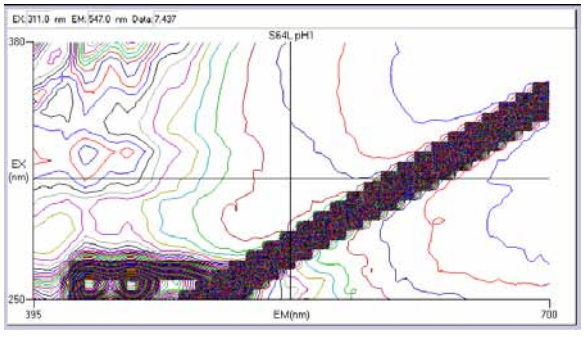
S 64 **[CI 58260 Mordant Red 2]**

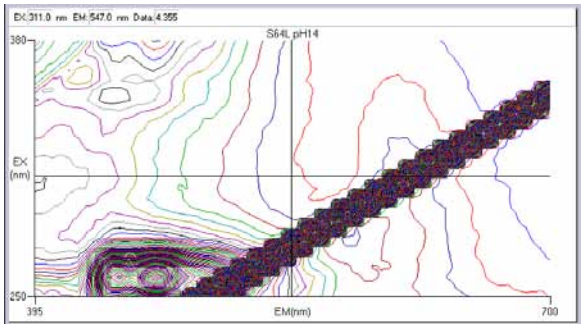
wool





vial





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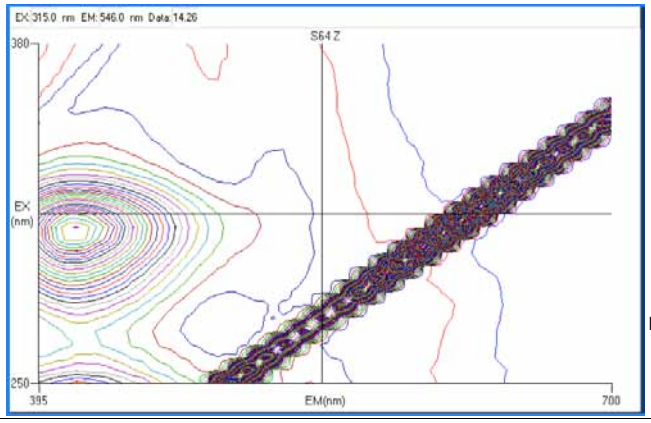
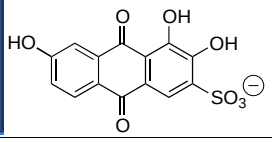
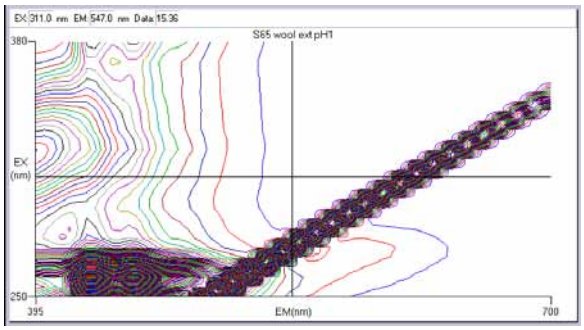
Sdb

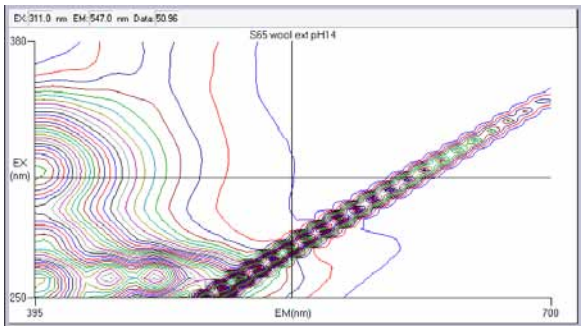




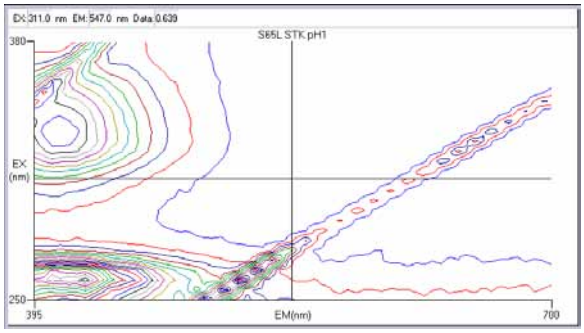
S 65 **[CI 73015 Acid Blue 74]**

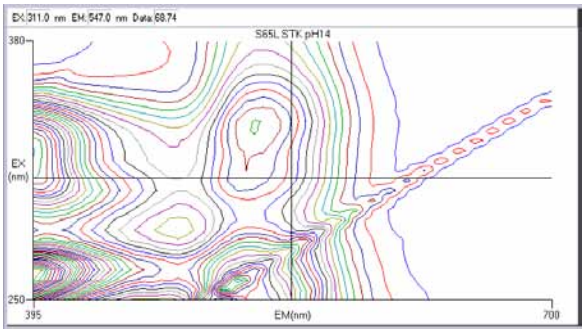
wool



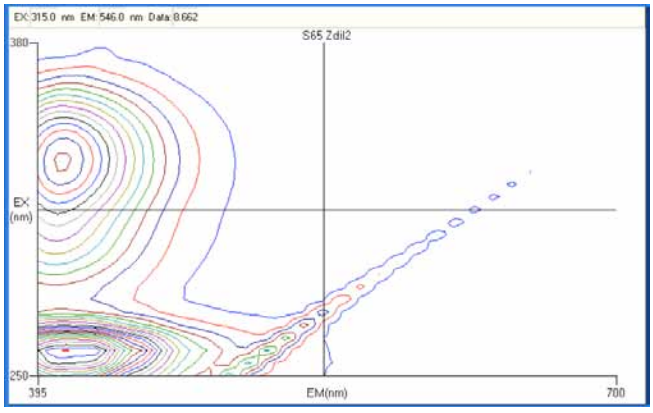


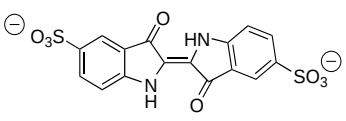
vial





Sdb





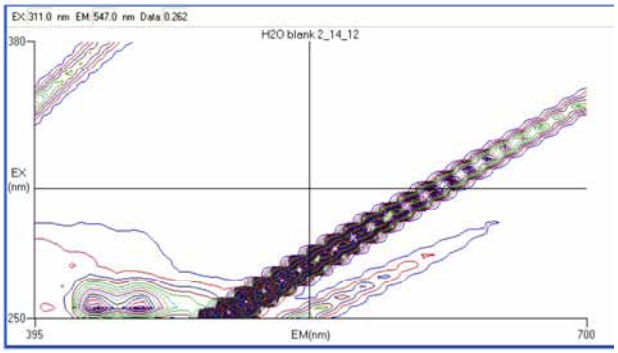
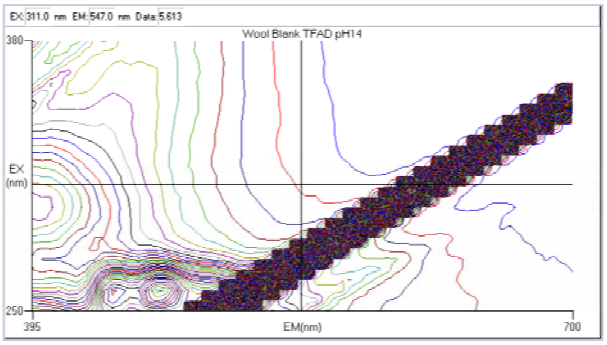
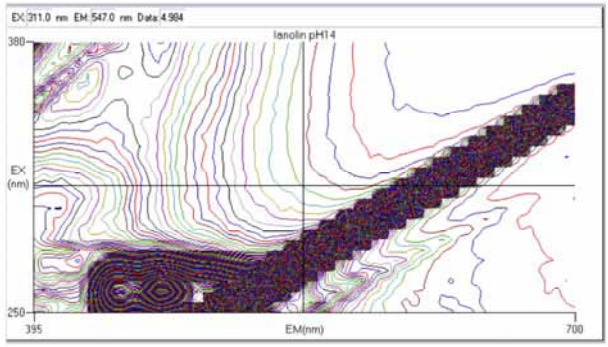


Figure 1. 3D fluorescence spectrum of HPLC grade water showing diagonal features resulting from (left to right) Raman scattering of 1st order Xe lamp photons by water vibration mode ν 3, Rayleigh scattering of 2nd order Xe lamp photons, and Raman scattering of 2nd order Xe lamp photons by water vibration mode ν 2. The mask-like artifact in the lower left is a transient feature [6].



(a)



(b)

Figure 2. 3D fluorescence spectrum of (a) TFA extract from undyed wool, adjusted to pH 14. Peak near EX = 300 nm and EM = 400 nm has been attributed to lanolin. [7]; (b) TFA/MeOH/lanolin supernatant, adjusted to pH 14.

fluorescence spectra for only 7 of the dyes were unaffected by the change from pH 1 to pH 14. Twenty-four of the Schweppe dyes are actually acid/base indicators [10]. The unbuffered library spectrum in such cases is often recognizably a combination of the pH 1 and pH 14 spectra. To eliminate the ambiguity of dealing with such mixed spectra, we collected fluorescence spectra at the extremes of pH 1 and pH 14 to ensure that the dye molecules were entirely in one molecular form (pH 1) or the other (pH 14).

3.3. Categories of Spectral Library Matching

S 51 (Solvent Red 49, **Figure 3**) typifies a dye for which both the pH 1 and pH 14 extracts match the corresponding library spectra and also are virtually identical with each other. For S 16 (Acid Orange 12, **Figure 4**), although the acidic and basic spectra are quite different from each other, the wool extract and library spectra nonetheless give excellent matches for both pH 1 and pH 14.

In contrast, S 41 (Basic Green 1, **Figure 5**) gives a reasonably good match at pH 1 but not at pH 14; it is possible that the lanolin peak (see **Figure 2(a)**) is distorting the wool extract spectrum at pH 14. However, there are also dyes, such as S 15 (Acid Red 9, **Figure 6**), for which the pH 14 spectra match well while the pH 1 spectra do not.

3.4. Decomposition of Dyes at Extreme pH

It is known that S 60 (Murexide) irreversibly decomposes at pH values lower than 4.5 and higher than 9 [11]. However, the rate constant for the decomposition is such that a useable fluorescence spectrum can be obtained if the extraction and spectrum are completed within about 20 min. We did not encounter pH-related decomposition problems with other Schweppe dyes.

3.5. Guidelines for Dye Identification from 3D Fluorescence Spectra

To employ the methods described here for identifying an unknown dye, one should extract the dye, adjust the pH, and run the spectra as described in Section 2. Then:

1) Compare the pH 14 and pH 1 spectra with the 46 library ("vial") spectra in the Supplementary Information—**Table 1** [http://simmons.academia.edu/LenSoltzberg]. First look for a match among the pH 14 spectra. If such a match is found, check the corresonding pH 1 spectra for a match as well. These steps may result in a positive identification.

2) If no match is found in step (1), repeat the comparison against the 19 spectra in the Supplementary Information—**Table 2** [http://simmons.academia.edu/LenSoltzberg]. Compare the spectra of the unknown sample against only the library spectra, since in **Table 2**, our extract spectra do not match the library spectra, in several cases probably

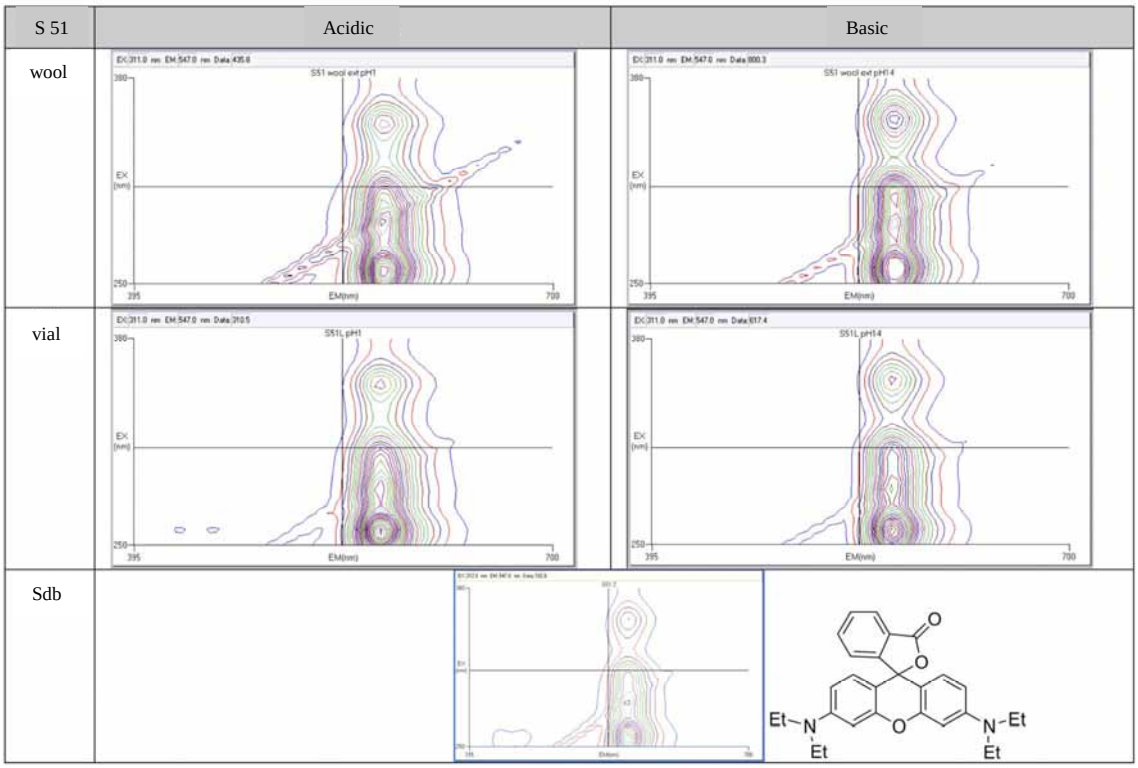


Figure 3. Dye extract showing no pH dependence of the 3D fluorescence spectrum, pH 1 (left) versus pH 14 (right). Excellent match between wool extract ("wool", upper) and library spectra ("vial", lower). Unbuffered Schweppe database spectrum (Sdb) is at bottom next to the structure. S 51 is CI 45170 (Solvent Red 49).

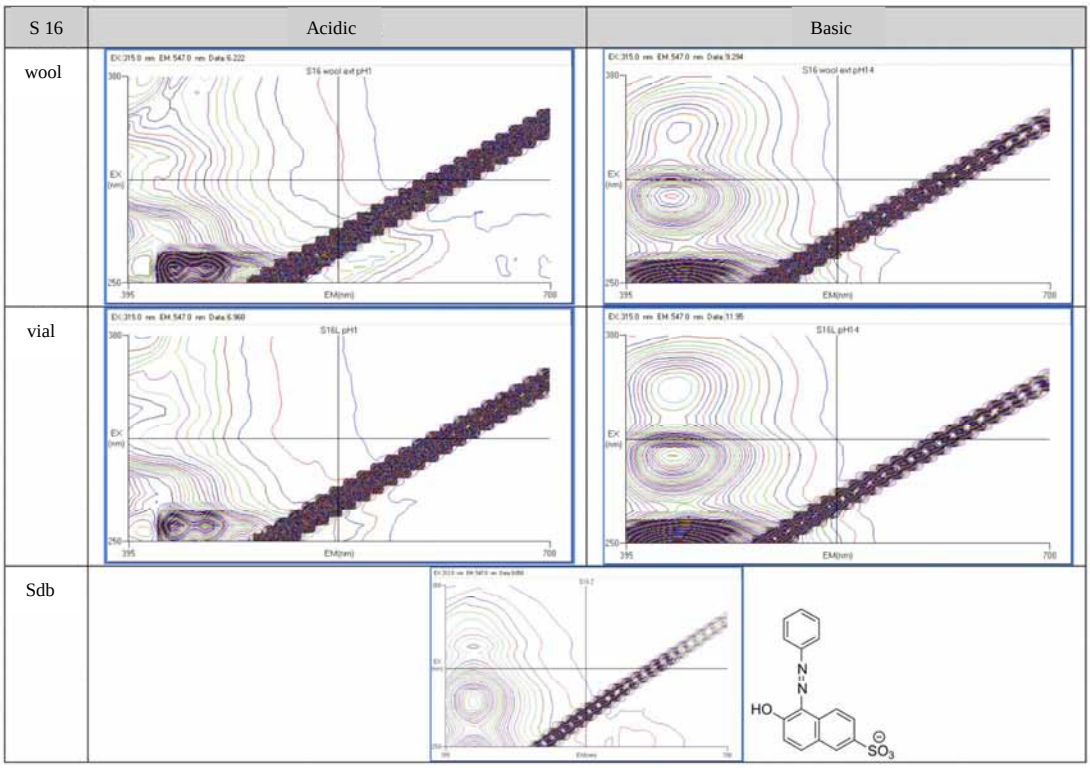


Figure 4. Dye extract showing pH dependence of the 3D fluorescence spectrum. Excellent match between wool extract and library spectra. S 16 is CI 15970 (Acid Orange 12).

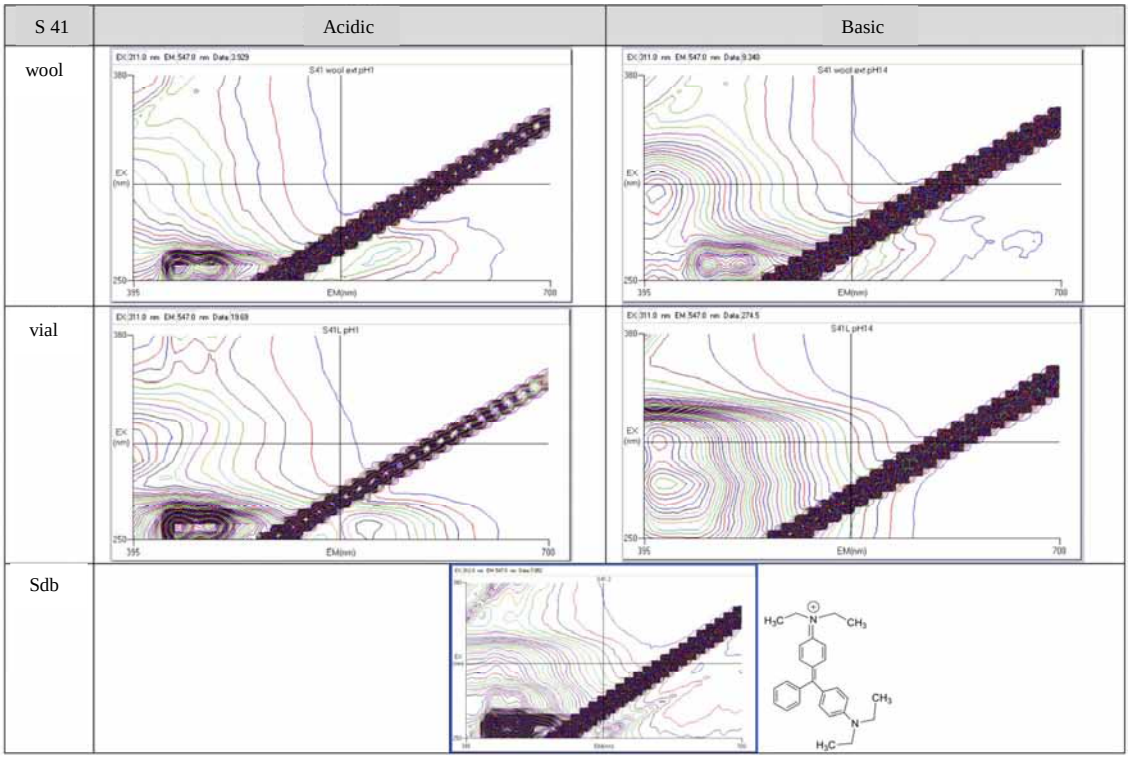


Figure 5. Dye extract showing good match between pH 1 wool extract and pH 1 library spectrum. S 41 is CI 42040 (Basic Green 1).

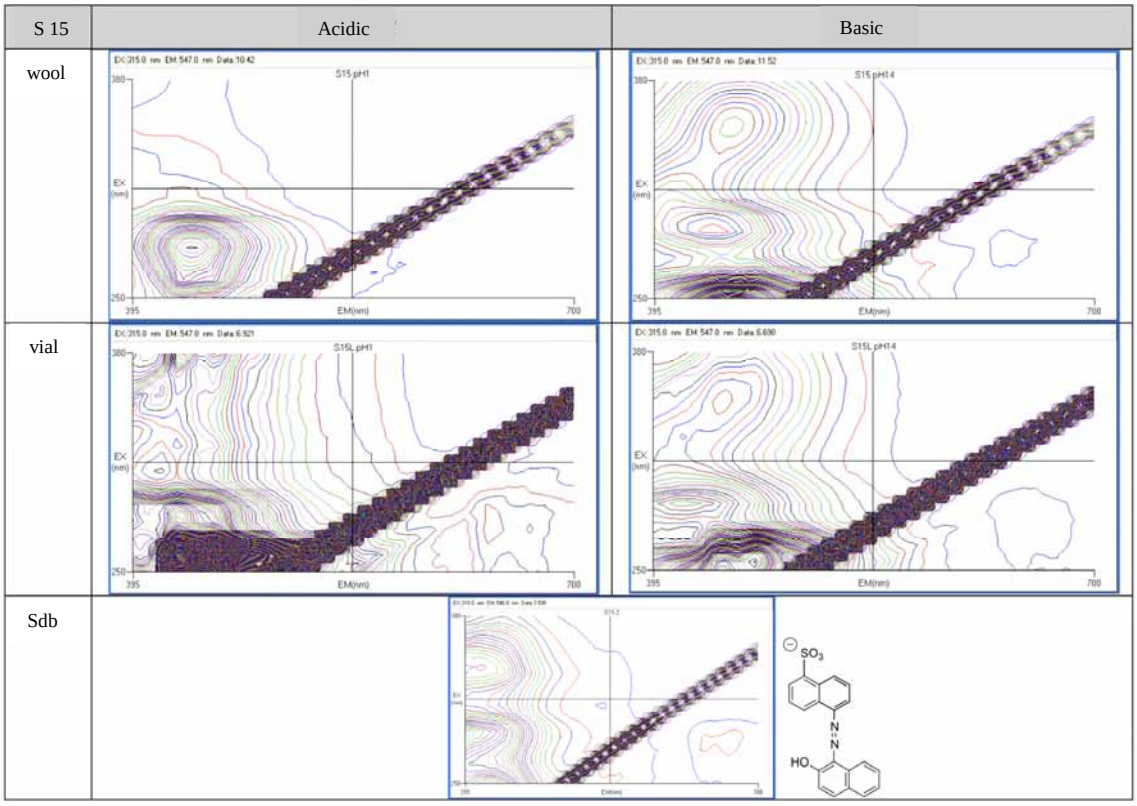
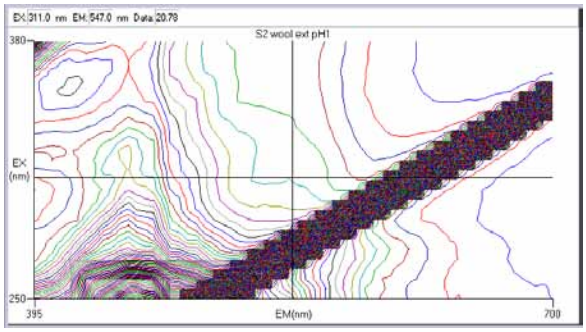
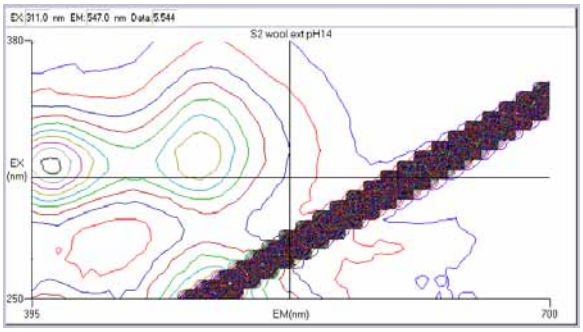
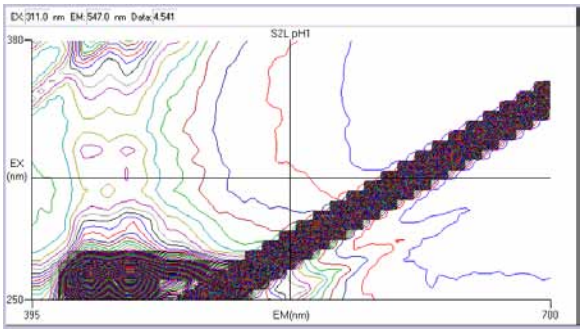
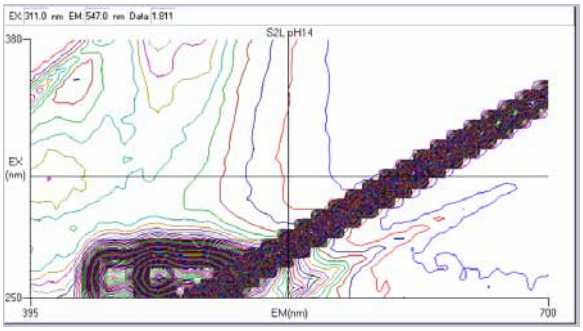
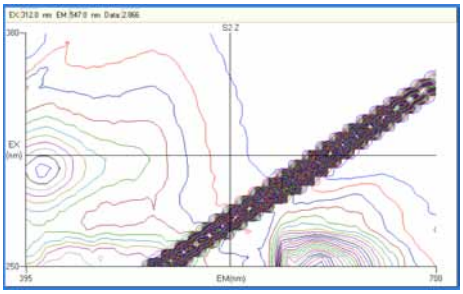
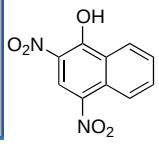
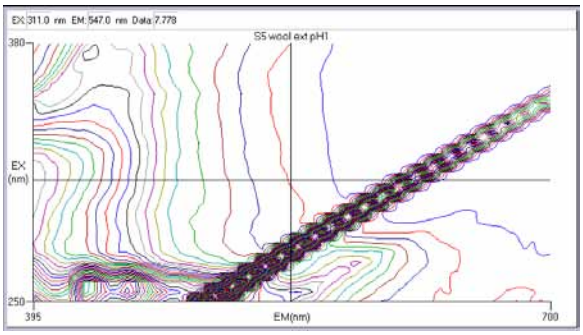
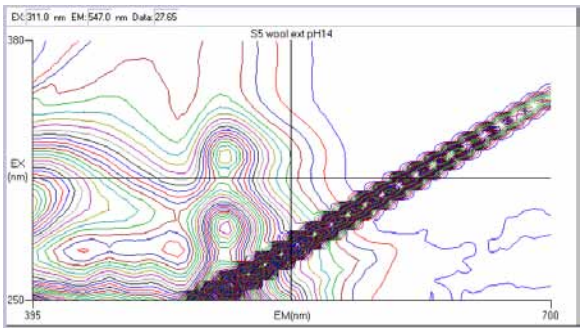


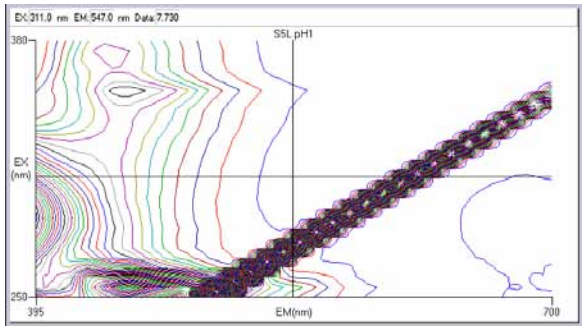
Figure 6. Dye extract showing good match between pH 14 wool extract and pH 14 library spectrum. S 15 is CI 15635 (Acid Red 9).

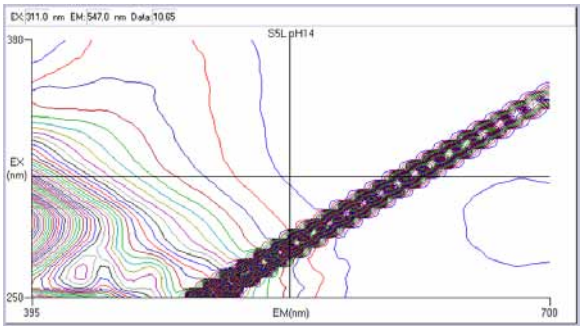
Table 2. Dye cannot be identified from 3D fluorescence spectra.

	Acidic	Basic
S x	**[CI (Colour Index) Number CI Name]**	
wool	**dye extracted from wool fibers—pH 1**	**dye extracted from wool fibers—pH 14**
vial	**dye from solution library—pH 1**	**dye from solution library—pH 14**
Sdb	**dye from solution library—no pH adjustment**	**dye structure**
S 2	**[CI 10315 Acid Yellow 24]**	
wool		
vial		
Sdb	 	
S 5	**[CI 13065 Acid Yellow 36]**	
wool		

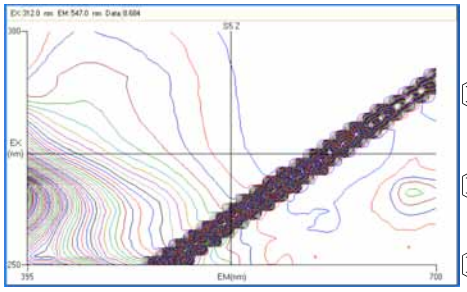
Continued

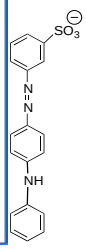
vial





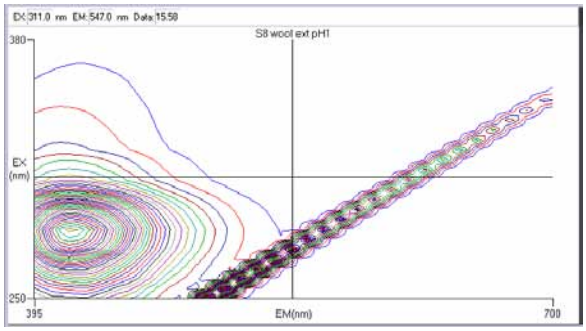
Sdb

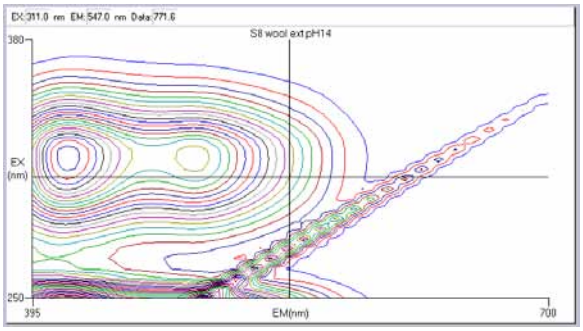




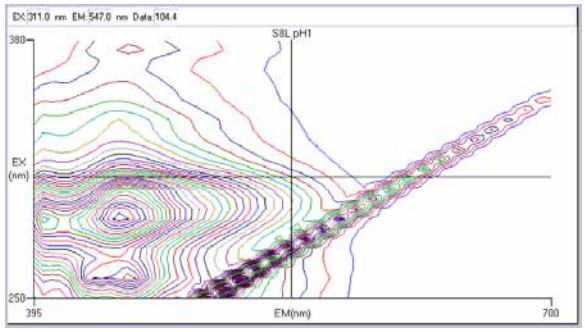
S 8 **[CI 13355 Acid Red 74]**

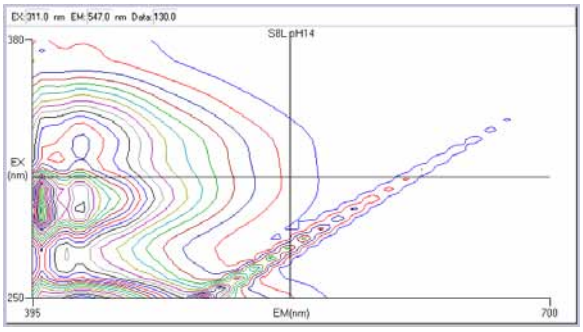
wool



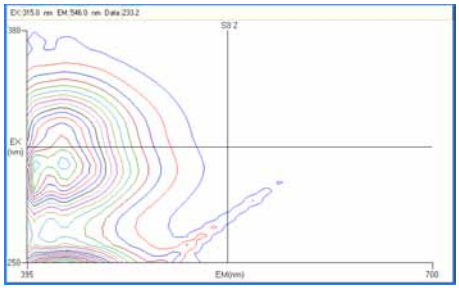


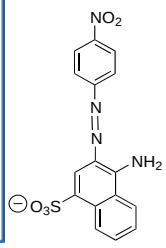
vial





Sdb

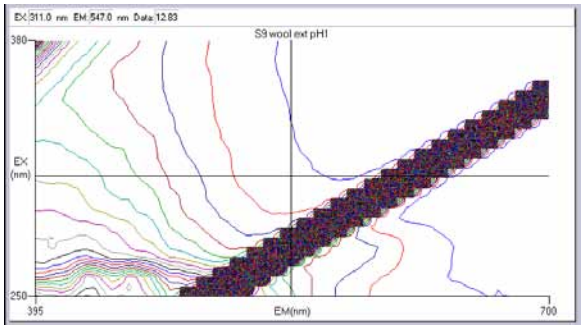


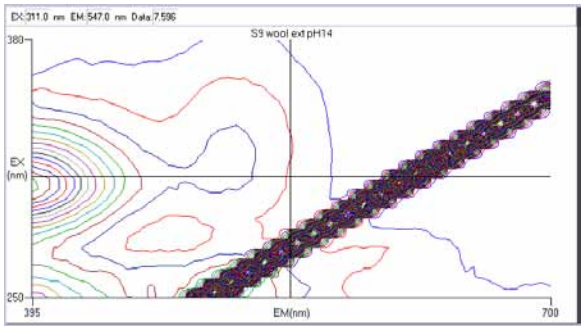


Continued

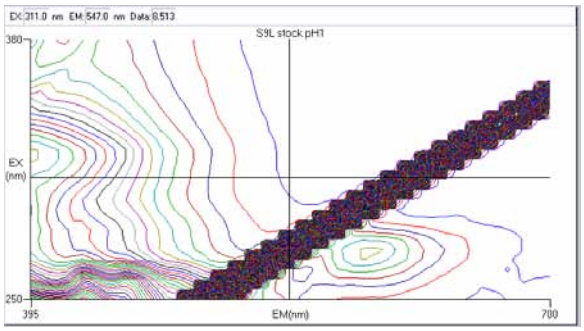
S 9 **[CI 14025 Mordant Yellow 1]**

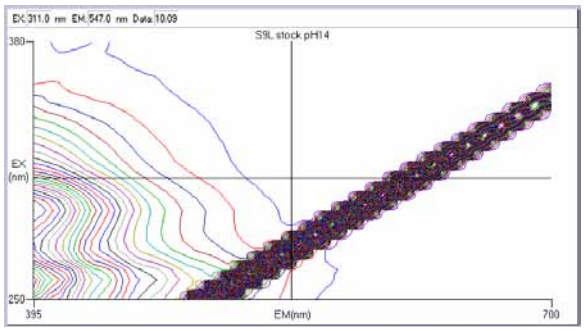
wool





vial





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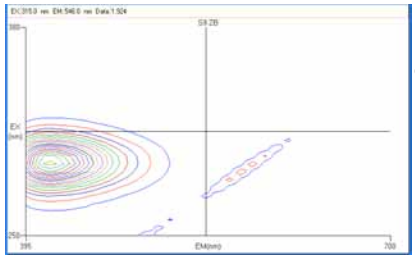
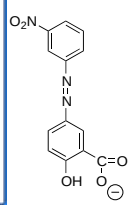
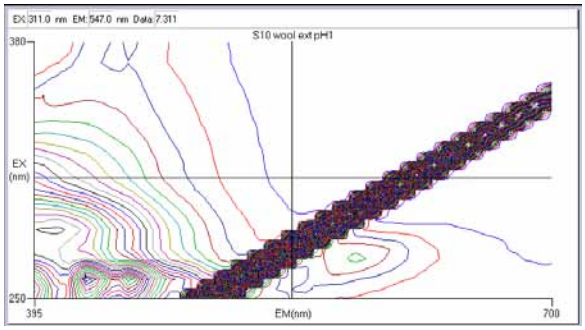
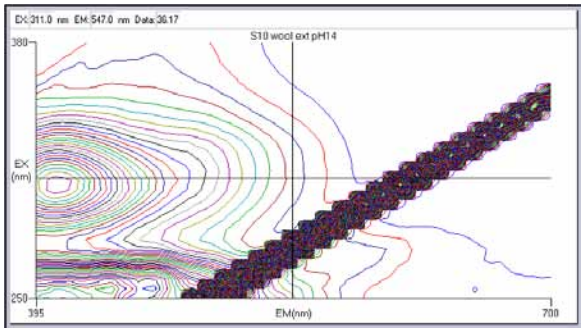




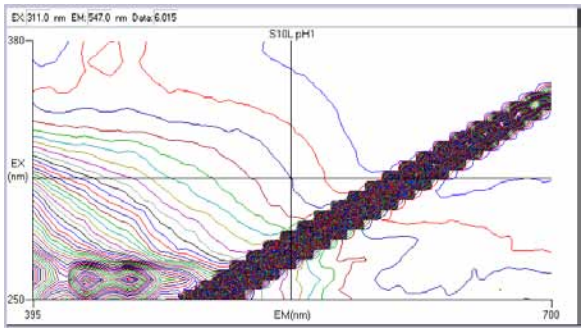
S 10 **[CI 14270 Acid Orange 6]**

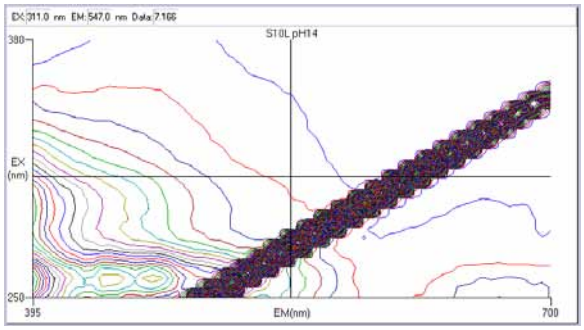
wool





vial





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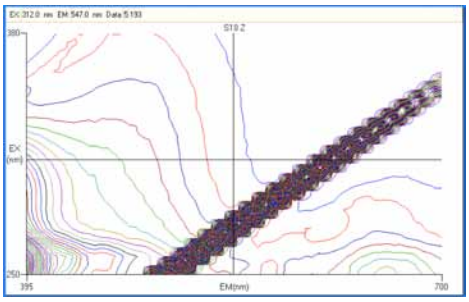
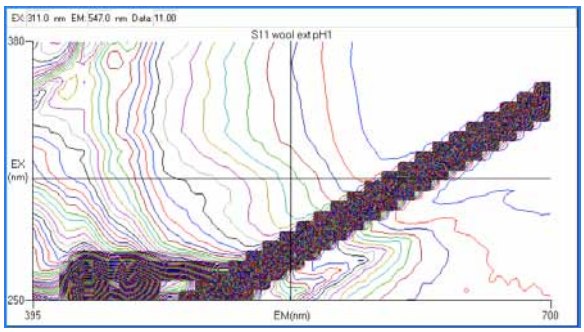
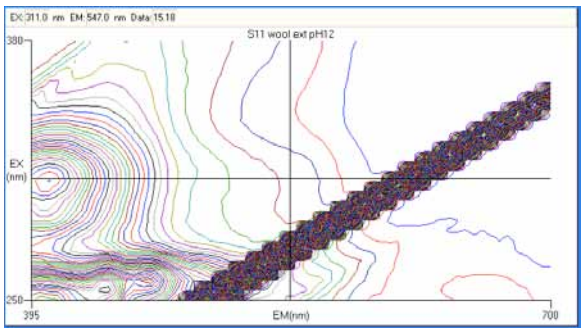
Sdb



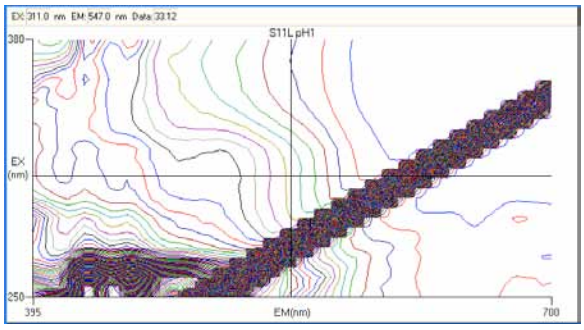
S 11 **[CI 14600 Acid Orange 20]**

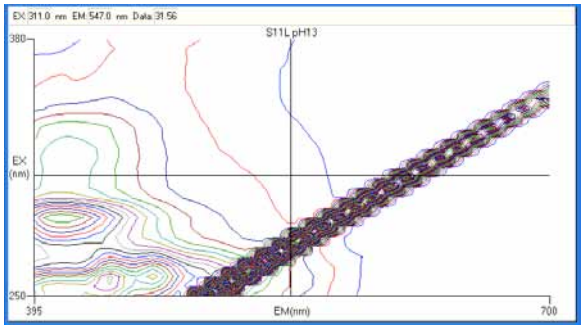
wool



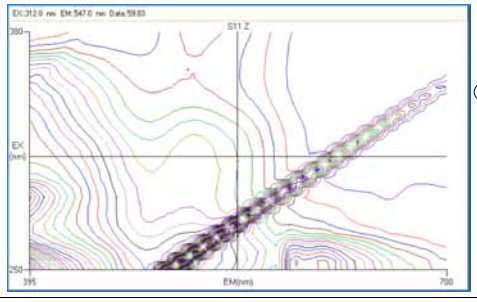


vial



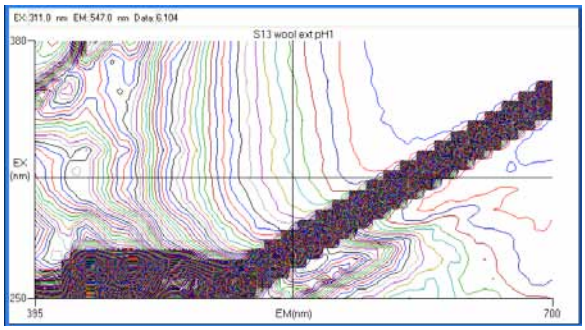


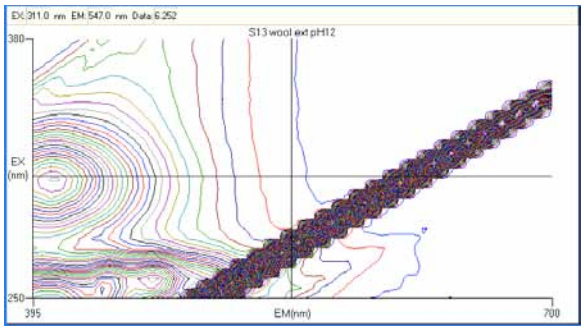
Sdb



S 13 **[CI 15510 Acid Orange 7]**

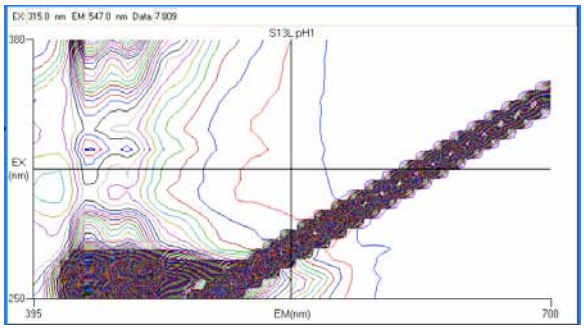
wool

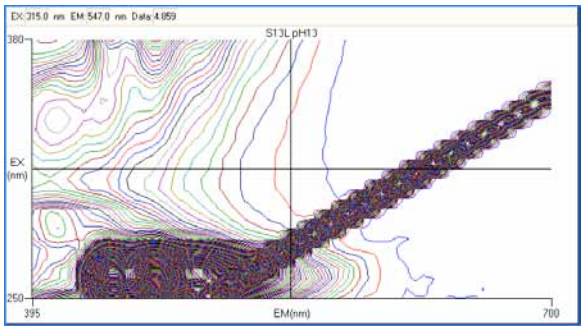




Continued

vial





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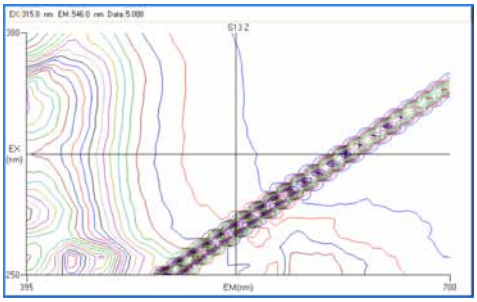
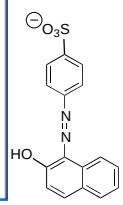
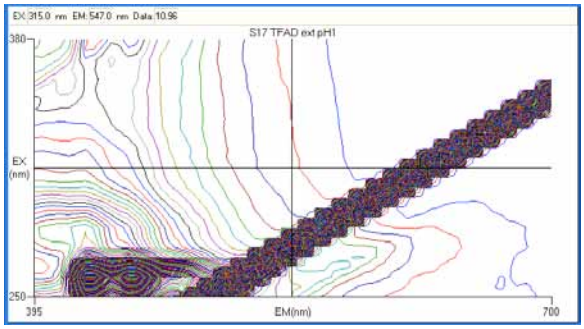
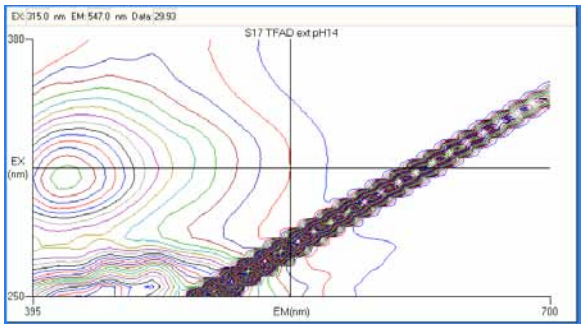




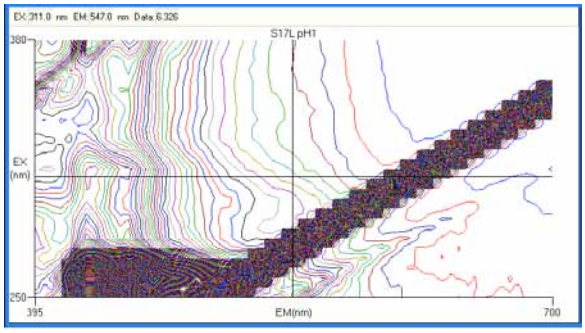
S 17 **[CI 16045 Acid Red 13]**

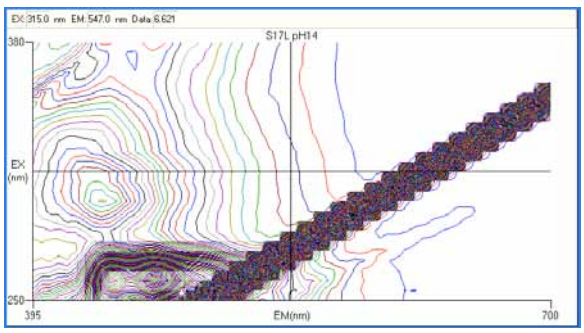
wool



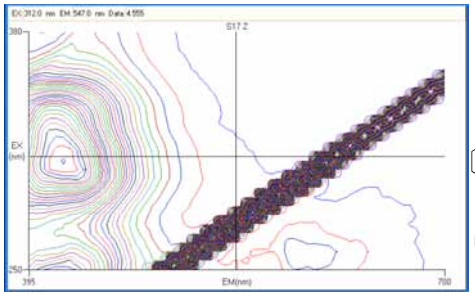


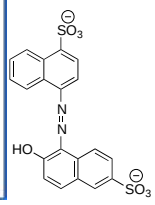
vial



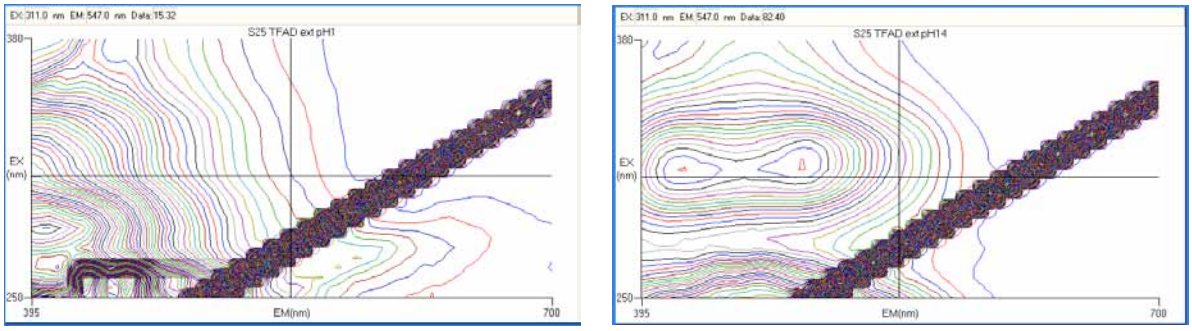
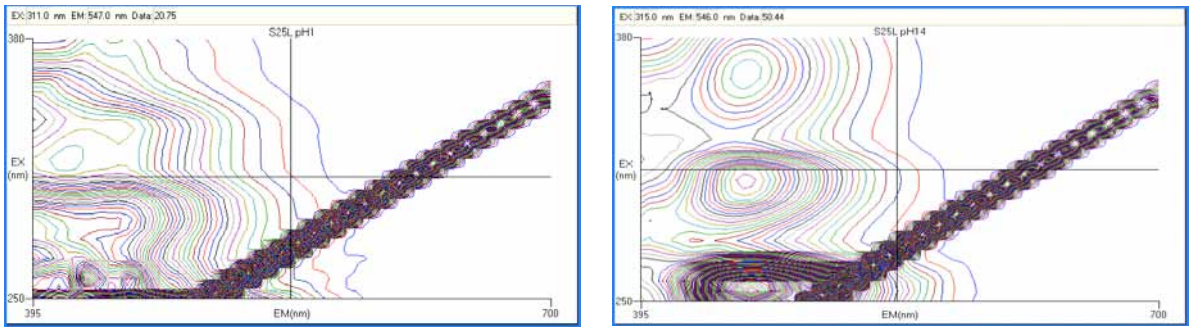
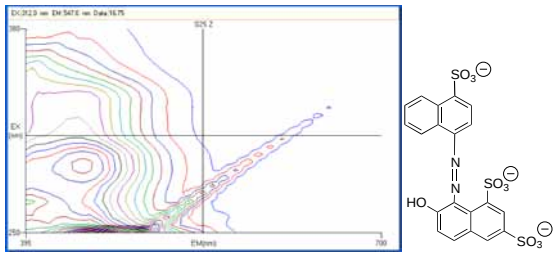


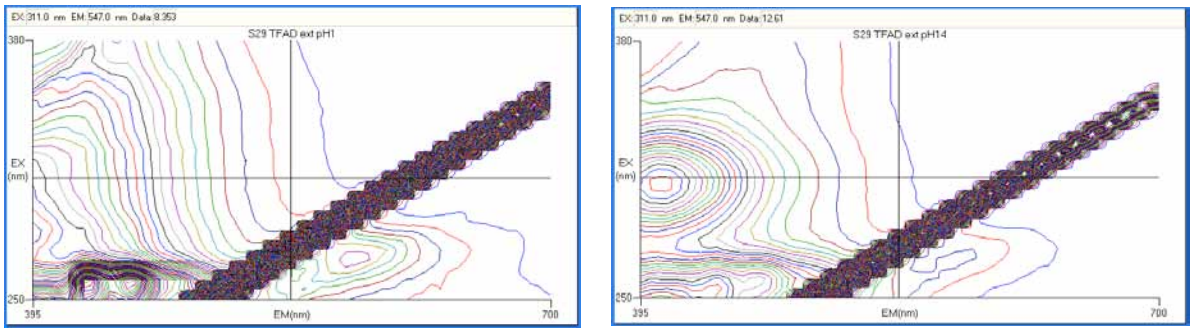
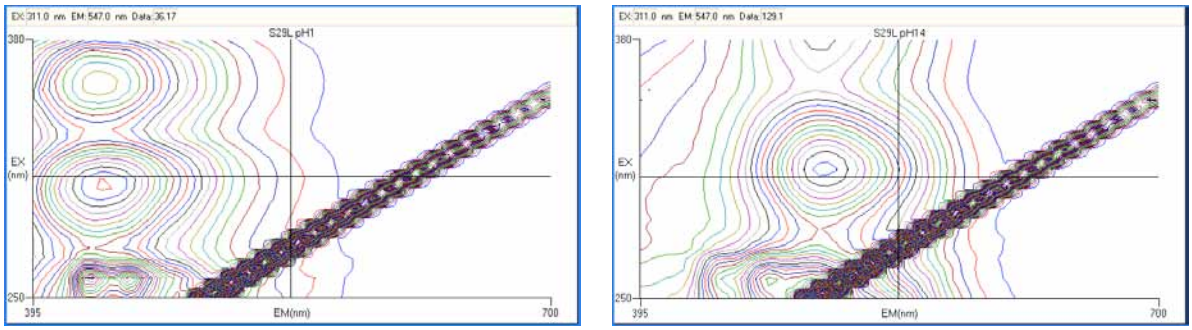
Sdb





Continued

S 25	**[CI 16255 Acid Red 18]**
wool	
vial	
Sdb	

S 29	**[CI 18055 Acid Violet 7]**
wool	
vial	

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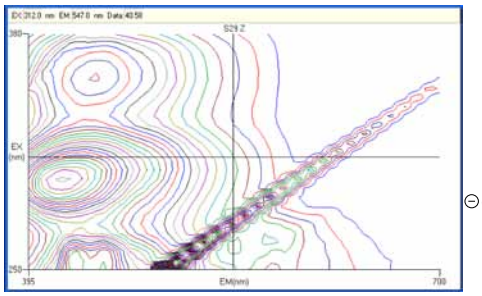
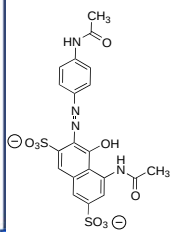
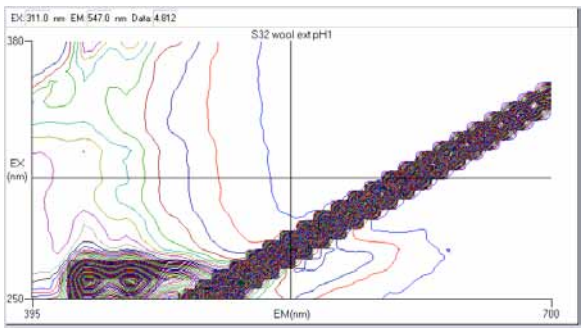
Sdb

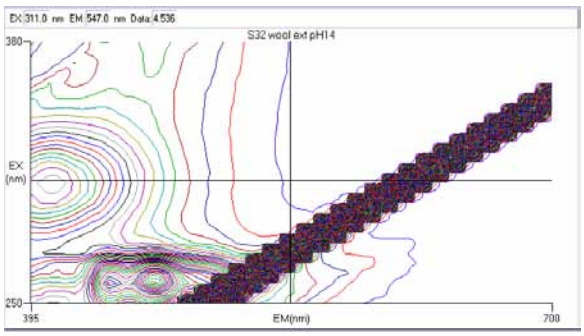




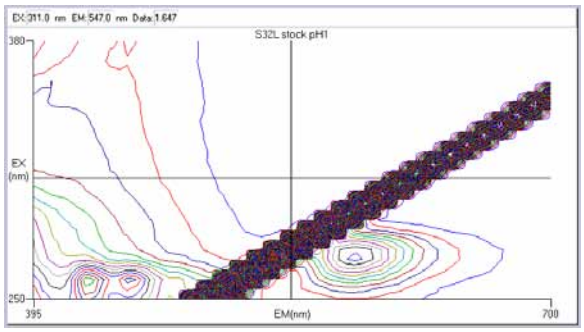
S 32 **[CI 20470 Acid Black 1]**

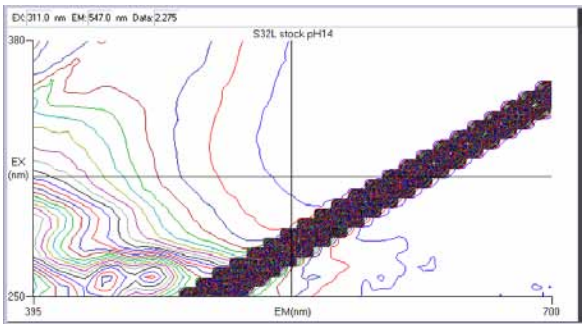
wool



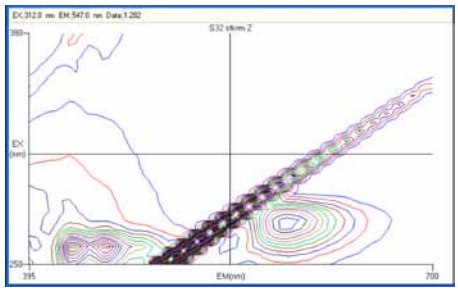


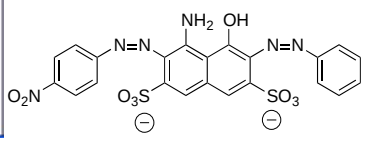
vial





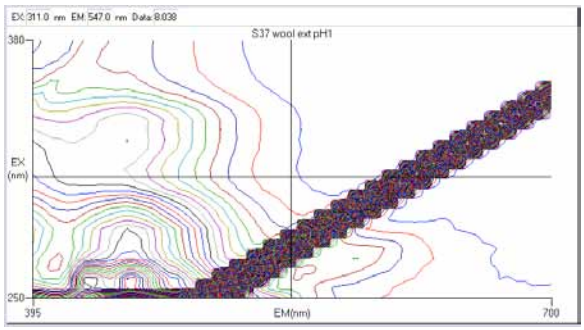
Sdb

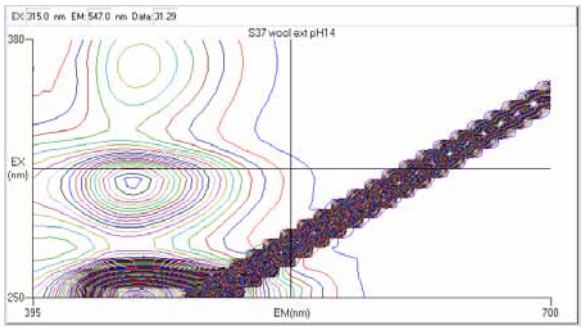




S 37 **[CI 27200 Acid Red 115]**

wool





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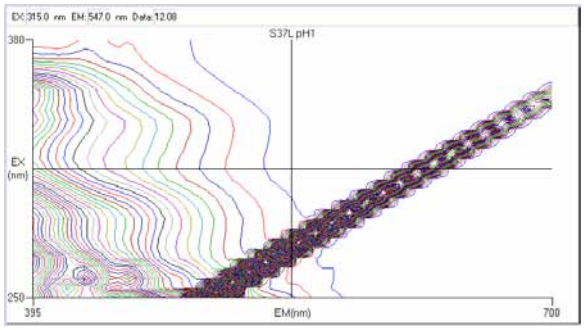
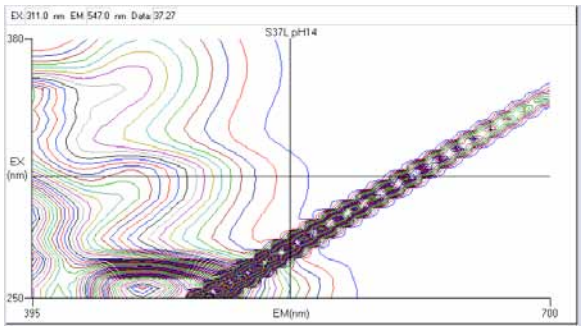
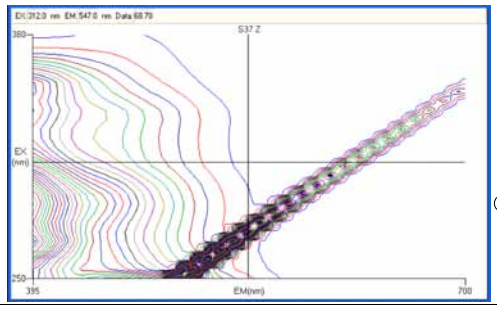
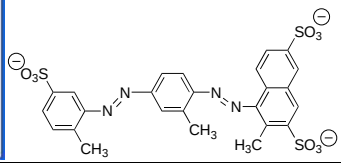
vial





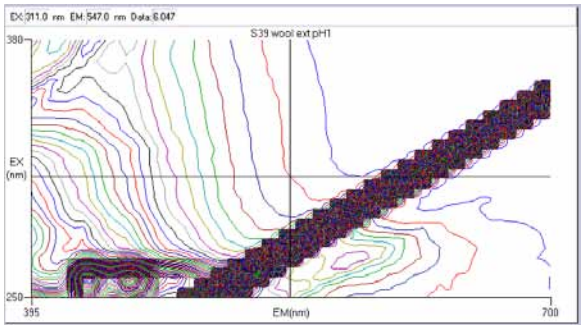
Sdb

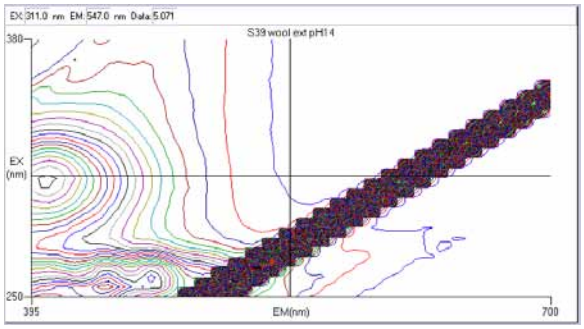




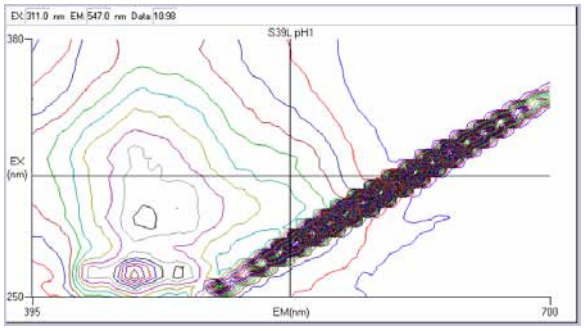
S 39 **[CI 41000 Basic Yellow 2]**

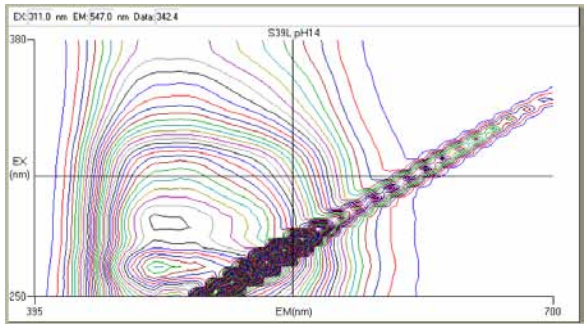
wool



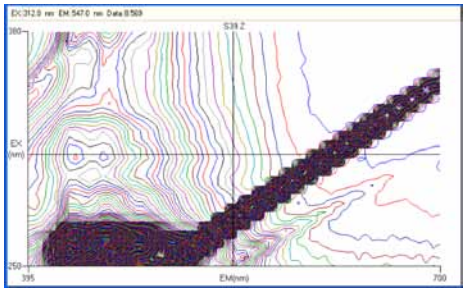


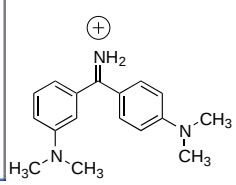
vial





Sdb





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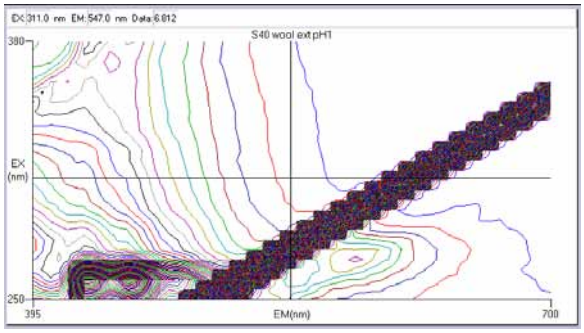
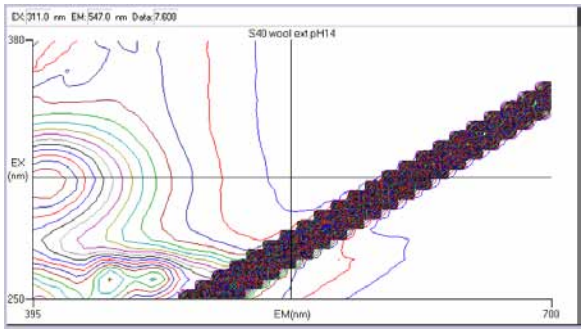
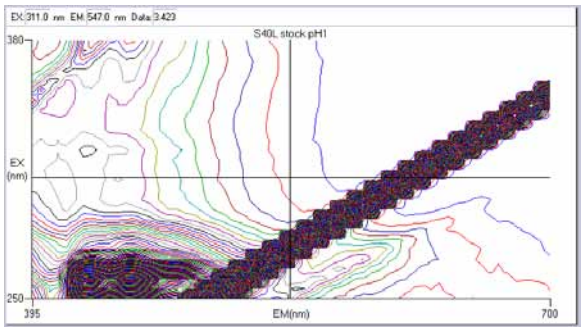
S 40	**[CI 42000 Basic Green 4]**

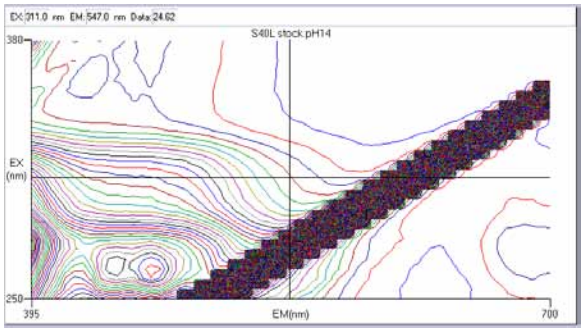
wool



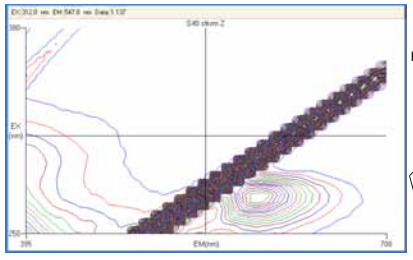


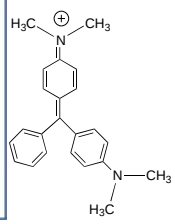
vial





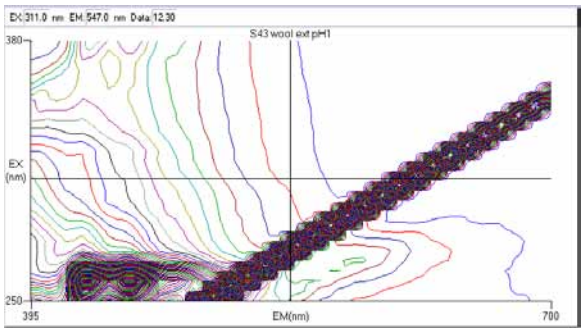
Sdb

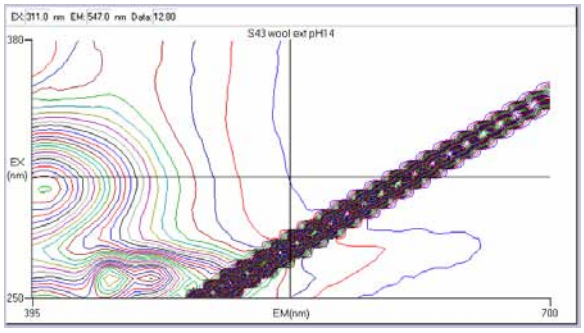




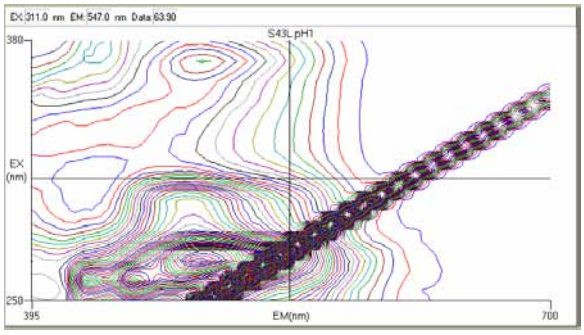
S 43	**[CI 42075 Acid Green 6]**

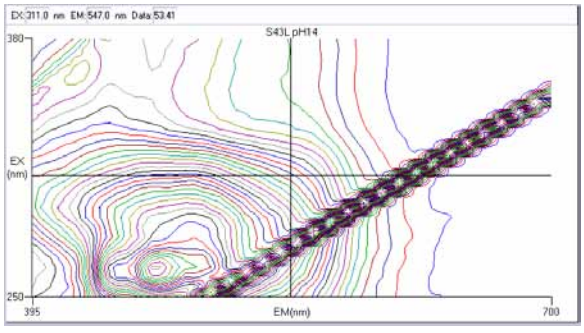
wool





vial





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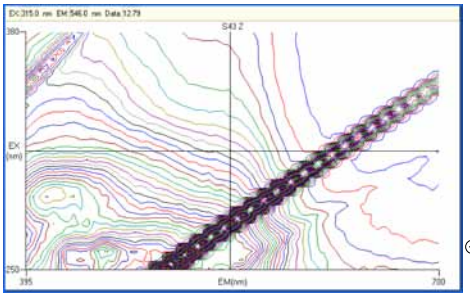
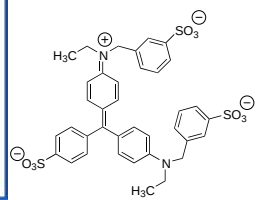
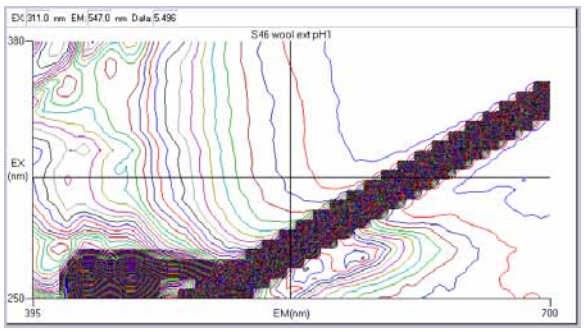
Sdb

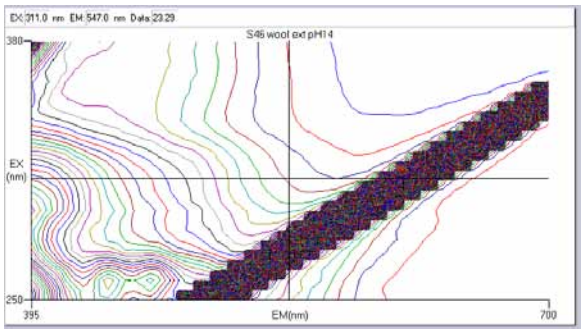




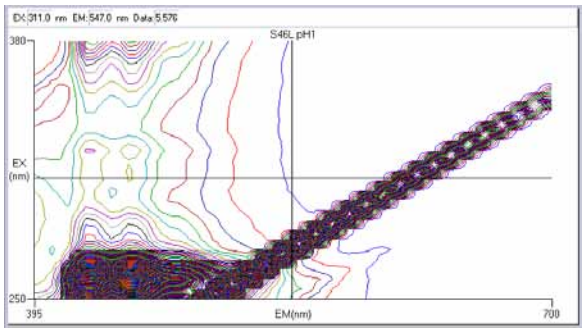
S 46 **[CI 42555 Basic Violet 3]**

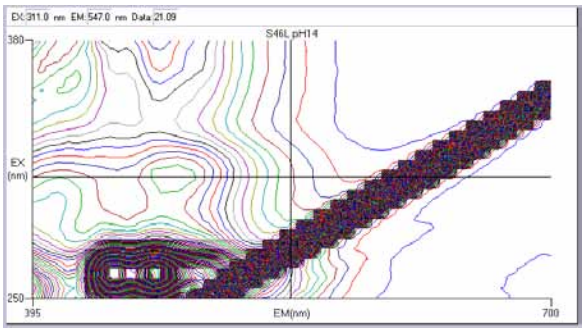
wool



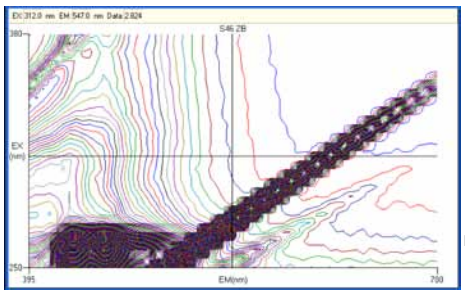


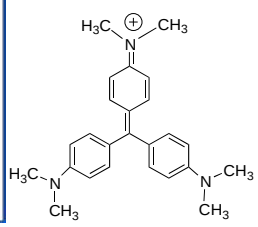
vial





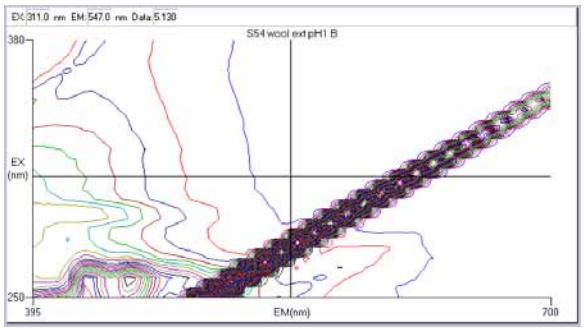
Sdb

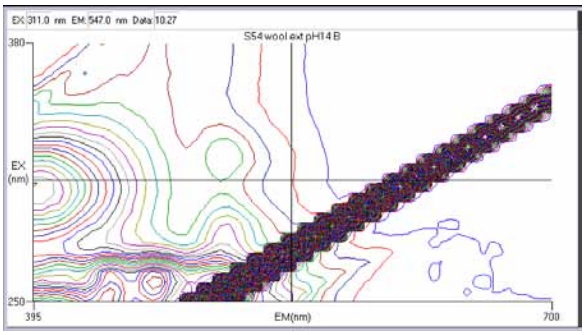




S 54 **[CI 45430 Acid Red 51]**

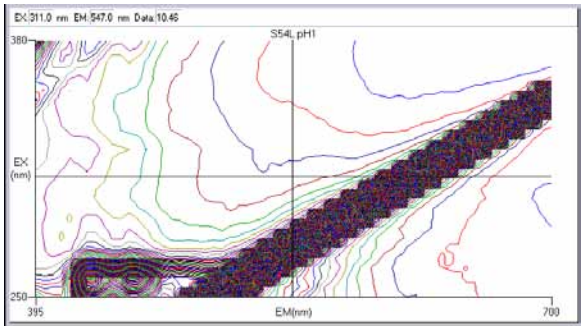
wool

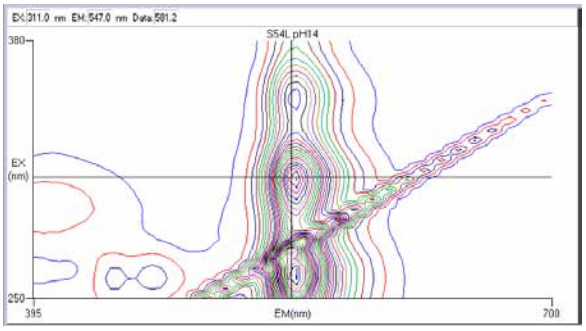




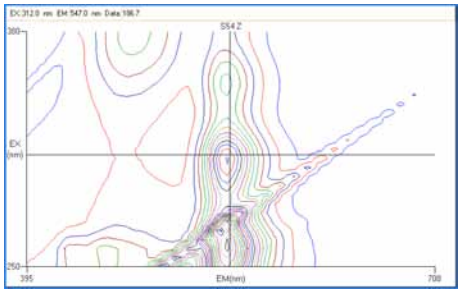
Continued

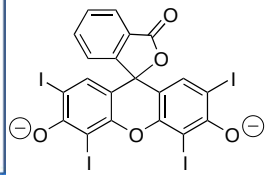
vial





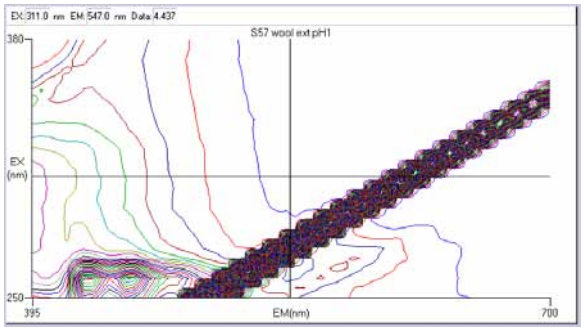
Sdb

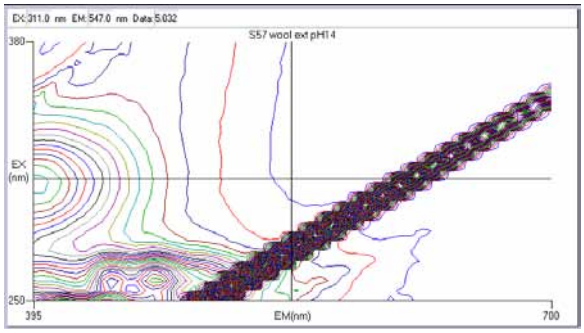




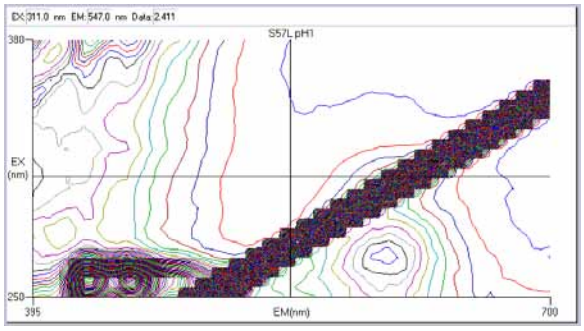
S 57	**[CI 50245 Mauveine]**

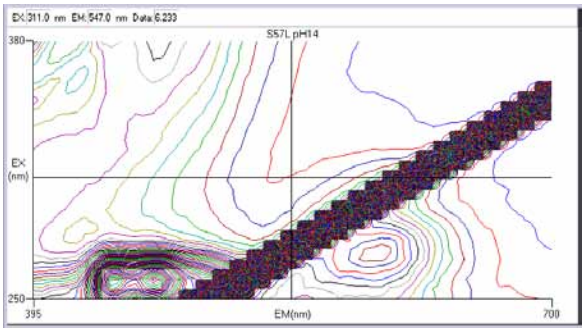
wool



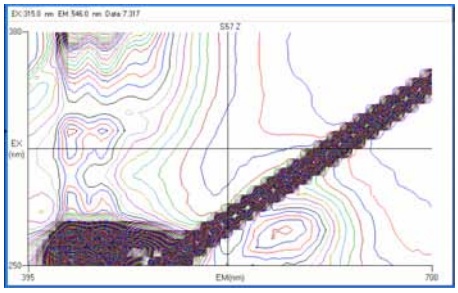


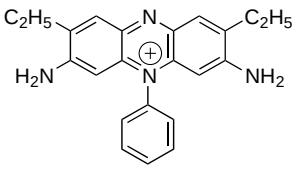
vial





Sdb





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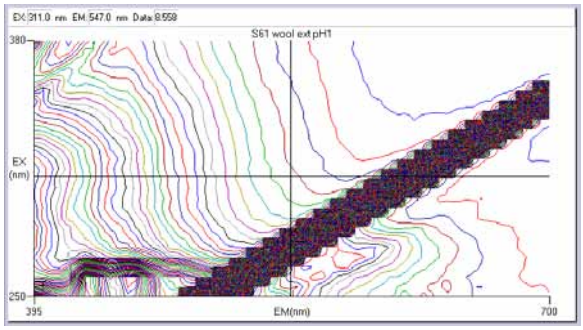
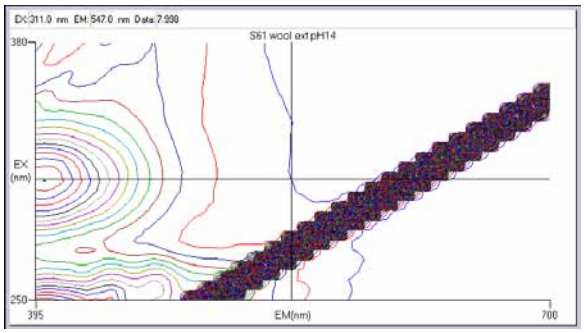
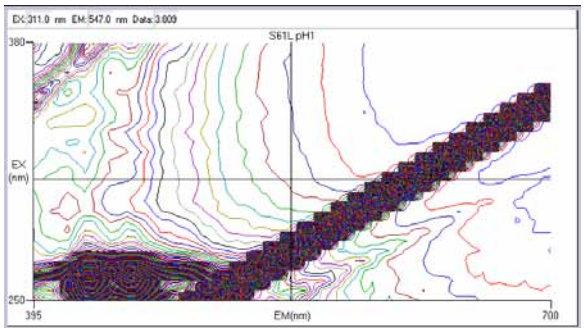
S 61	[CI 58000 Mordant Red 11]

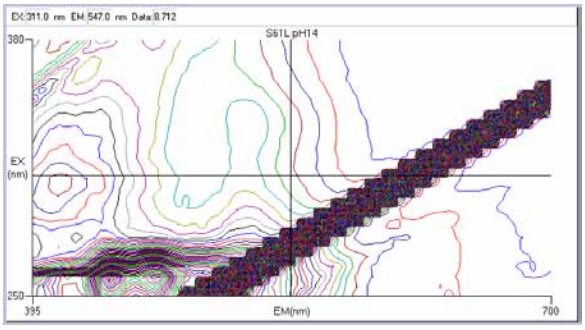


wool

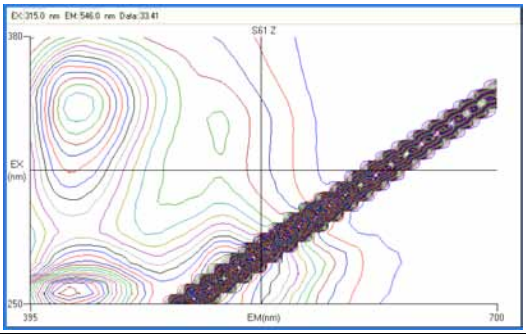


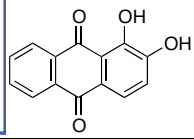


vial



Sdb





because of insufficient extraction.

4. Conclusions

Over 75% of the 65 synthetic organic dyes in the Schweppe collection can be successfully extracted from wool yarn under mild conditions and identified from their 3D fluorescence spectra at pH 1 and/or pH 14. Only 2 mg of wool is a sufficient sample size. There are three pairs of structurally similar dyes for which the spectra are too similar to allow differentiation (S 19/S 20, S 23/S 24 and S 25/S 26); in these cases, MALDI-TOF mass spectra would resolve the identity of the dyes [12].

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