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High Pressure Liquid Chromatographic Separation of Dyes Encountered in Illicit Heroin Samples

Over the last several years an increasingly large number of illicit heroin samples of the brown variety have contained dyes. STRIDE² data analysis for the time period from August 1974 to December 1975 indicates that coloring matter was detected in 26% of the brown heroin submissions to the Southwest Regional Laboratory in San Diego. Apparently a formulation of dyes is used in the heroin dilution process to disguise the addition of adulterants that are normally white in their natural state. The resultant mixture maintains a relatively dark color and the diluted product is more easily sold as a high quality brown heroin.

Preliminary thin-layer chromatographic data [1] have indicated that the coloring agents are often combinations of acid water-soluble colors, such as Red Color Index (C.I.) 16255 and the Food, Drug, and Cosmetic Act colors Red No. 2, Yellow No. 5, Yellow No. 6, and Blue No. 1.³ It was thought that useful intelligence information might be obtained if a more definitive separation could be achieved that would allow a comparison of chromatograms, showing the relative ratios of dyes present in samples exhibiting similar dye patterns. To this end, the use of paired ion chromatography in conjunction with high pressure liquid chromatography (HPLC) was investigated.

Experimental Procedures

Initially, liquid chromatographic separations with a conventional reverse-phase system were attempted; however, the dyes in question were poorly retained on the column and little, if any, separation was obtained. The application of paired ion chromatography in a reverse-phase system was found to give quite adequate separation in a reasonable elution time.

Paired ion chromatography, as described in Ref 2, involves using a counter-ion in the mobile phase that forms an ion pair with, in this case, the ionized dye. The resultant ion pair then has a markedly increased retention in the reverse-phase system and a separation of the dye mixture is possible. In this study, tetrabutylammonium hydroxide was used to provide the counter-ion.

The mobile phase that resulted in the optimum separation was a mixture of methanol and water in a ratio of 58:42. Each solvent contained a 0.005M concentration of tetra-

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²System to retrieve information from drug evidence.

³Food, Drug, and Cosmetic Act (FD&C) colors are certifiable by the U.S. Food and Drug Administration for use in coloring foods, drugs, and cosmetics. They consist largely of sulfonated dye derivatives, which as a group are generally referred to as acid synthetic dyes.

butylammonium hydroxide, with acetic acid added to each solvent until the pH was adjusted to between 7 and 8. The final acetic acid concentration ranged from 0.03 to 0.04% (v/v). Studies showed that both the ratio of methanol to water and the pH were critical factors. As the methanol concentration was increased, even to 59%, the fastest eluting peaks, Yellow No. 5 and Red No. 2, began to run together. Conversely, as the methanol concentration was decreased to approach 57%, the slower eluting Yellow No. 6 was no longer separated from Red No. 2. It was also found that a pH outside the range of 7 to 8 resulted in inadequate separation.

The liquid chromatograph employed in this study was a Waters Model 6000 equipped with a Model 440 ultraviolet (254-nm) detector. The column packing used was an octadecylsilane material chemically bonded to a solid support having a particle size of about 10 μm . The support material was packed in a stainless steel 4-mm inside diameter column about 30 cm in length.⁴

Sample preparation was generally limited to dissolving the sample of interest in an aliquot from the methanol/water solution used as a mobile phase and filtering the solution through a 0.5- μm membrane filter. Procaine and certain unidentified alkaloidal constituents of heroin will elute in the area of the dyes. Fortunately, procaine was present in very few of the samples and the minor alkaloidal constituents were generally not present in sufficient quantity to cause interference with the dyes. Interference, when encountered, was eliminated by using a wool dyeing extraction procedure, which takes advantage of the acidic nature of the dyes to effect their isolation. Details of the wool extraction procedure are as follows. Approximately 0.5 g of sample is dissolved in dilute acetic acid. A 51-mm (2-in.) square of wool felt is placed in the solution and allowed to stand overnight. The wool felt, with the absorbed dyes, is then removed from solution and rinsed with warm water. The wool felt square is then gently heated in dilute ammoniacal solution to dissociate the absorbed dyes from the wool.

Results

Separation of Standard Dyes

Screening the heroin samples for dyes by thin-layer chromatography had shown that the most commonly encountered dyes were the FD&C colors Yellow No. 5, Yellow No. 6, Red No. 2, Blue No. 1, and C.I. 16255; therefore, a concentrated effort was made to separate these five dyes. Very early in the study it was found that the red dye C.I. 16255 and FD&C Blue No. 1 could not be adequately separated under the chromatographic conditions listed above. However, the detectability of Blue No. 1 relative to the other FD&C dyes was greatly reduced because of its significantly lower molar absorptivity at 254 nm. Consequently, we did not include Blue No. 1 in our study.

As a matter of practical consideration based on the above limitations, our primary concern was directed toward achieving a suitable separation of the following four most commonly encountered dyes: (1) FD&C Yellow No. 5, (2) FD&C Red No. 2, (3) FD&C Yellow No. 6, and (4) C.I. 16255 (an isomer of Red No. 2). In addition, the following three dyes, less often encountered, were separated from the other four: (5) FD&C Red No. 40, (6) D&C Red No. 10, and (7) FD&C Red No. 4. The separation achieved is reproduced as Fig. 1 and shows the elution order as listed above.

The elution order of these azo-sulfonic acid dyes can be predicted to some degree by the number of ionizable sites on the molecule (see Table 1). Among dyes with an equivalent number of ionization sites, the elution order appears to be secondarily influenced by

⁴ Commercially available prepacked from Waters Associates as a micro "Bondapak" C₁₈ column.

ELUTION ORDER

- 1) FD&C Yellow No. 5
- 2) FD&C Red No. 2
- 3) FD&C Yellow No. 6
- 4) C.I. 16255
- 5) FD&C Red No. 40
- 6) D&C Red No. 10
- 7) FD&C Red No. 4

COLUMN: μ BONDAPAK C₁₈
4 mm ID x 30 cm

SOLVENT: A) MeOH/H₂O (58/42)
with 0.005 M Tetrabutyl-
ammonium Hydroxide
(TBA) ca. 0.03% Acetic
Acid (to pH 7-8)

DETECTOR: UV: 0.5 AUFS, 254 nm

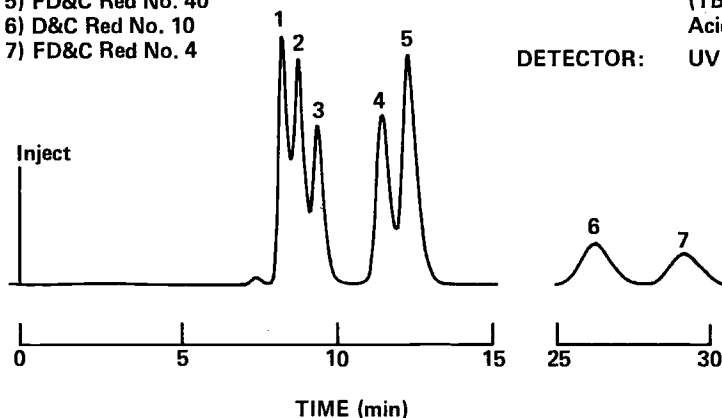


FIG. 1—Mixed dye standards.

TABLE 1—Correlation of elution order with number of ionizable sites.

Elution Order	Official Name	Dissociable Sodium Ions	Molecular Weight
1	FD&C Yellow No. 5	3	534
2	FD&C Red No. 2	3	604
3	FD&C Yellow No. 6	2	452
4	C.I. 16255	3	604
5	FD&C Red No. 40	2	496
6	D&C Red No. 10	1	400
7	FD&C Red No. 4	2	480

molecular weight. The elution of C.I. 16255 after Yellow No. 6 is one exception to this general observation; the former has three ionizable sites while the latter has only two.

Also, the elution times of FD&C Red No. 40 and Red No. 4 might have been expected to coincide more closely considering their charge equivalency and similar molecular weight. These observations indicate that the number of ionizable sites and the molecular weight were not the sole factors in influencing elution order.

Separation and Chromatographic Comparison of Dyes

Figure 2 illustrates the usefulness of HPLC in determining whether a particular dyed diluent had been used in the preparation of a heroin sample. Figure 2a is the chromatogram of the heroin sample; no cleanup, other than filtration, was performed prior to injection. Figure 2b is the chromatogram of some diluent seized with, but packaged separately from, the heroin. The presence of the diluent in the heroin is clearly demonstrated by the coincidence in dye peaks and the similar relative peak heights. It should be noted that the unidentified subsidiary peak in both chromatograms is thought to be a higher sulfonated dye associated with Yellow No. 6.

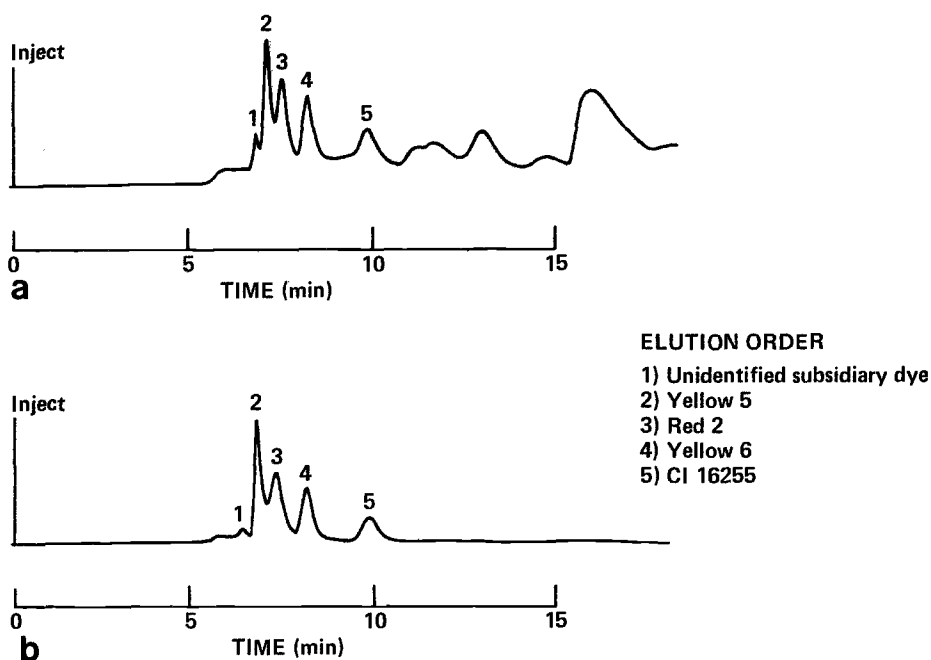


FIG. 2—(a) Chromatogram of heroin sample containing dyes. (b) Chromatogram of dye-impregnated diluent seized in conjunction with the heroin of (a). Presence of the diluent in the heroin is clearly demonstrated by similarity in dye profiles.

Forensic comparative examination of diluents seized with contraband heroin can sometimes yield useful information when examination of the heroin itself, for comparative purposes, is inconclusive. An example of this situation occurred recently when two heroin exhibits of exceptionally high purity (70%) were seized in the San Diego area within a month of each other. As is the custom of late, each seizure was accompanied by material packaged separately from the heroin; this material was labeled "corte" and was intended for later use as a diluent. In-depth comparative examination of the alkaloids present in the portion of the two exhibits that contained heroin revealed very few points of analytical similarity. However, the comparative chromatograms (Fig. 3) of the associated diluents reveal marked similarity between the dye profiles. While the question of common origin cannot be resolved on the basis of dye profiles alone, it is readily apparent that dye analysis can make an important contribution.

Another example showing comparative chromatograms of dyes in two otherwise unrelated heroin samples is shown in Fig. 4. Again, note the similarity in dye profiles.

Figure 5 is the chromatographic dye profile of a heroin-associated diluent seized in the Los Angeles area. The presence of a previously unencountered red dye (Red No. 40) made this sample noteworthy.

Figures 6 and 7 are further examples showing the similarity in dye profiles of different heroin exhibits. Each of these four samples was subjected to the wool dyeing extraction process mentioned previously.

Conclusion

The forensic characterization of dyes found in brown heroin preparations offers yet another diagnostic tool to aid in comparison studies or to support intelligence programs. It

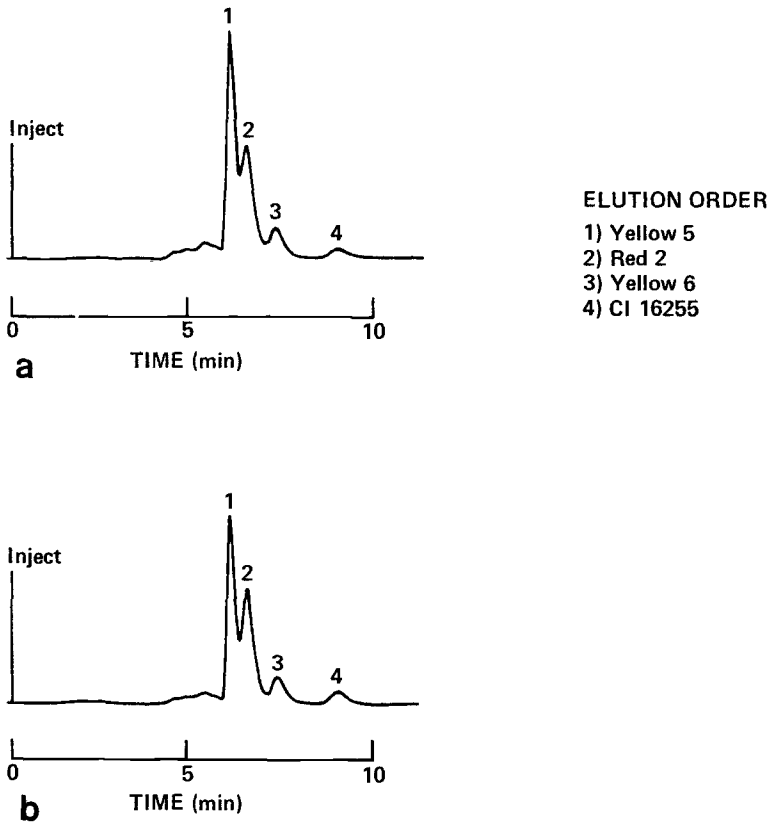


FIG. 3—(a) Chromatogram of untreated dyed diluent seized with exceptionally pure brown heroin (70%) in the San Diego area. (b) Chromatogram of a similar untreated diluent, again seized with high-grade brown heroin at the port of entry, San Ysidro, Calif. This seizure followed that of (a) by a month.

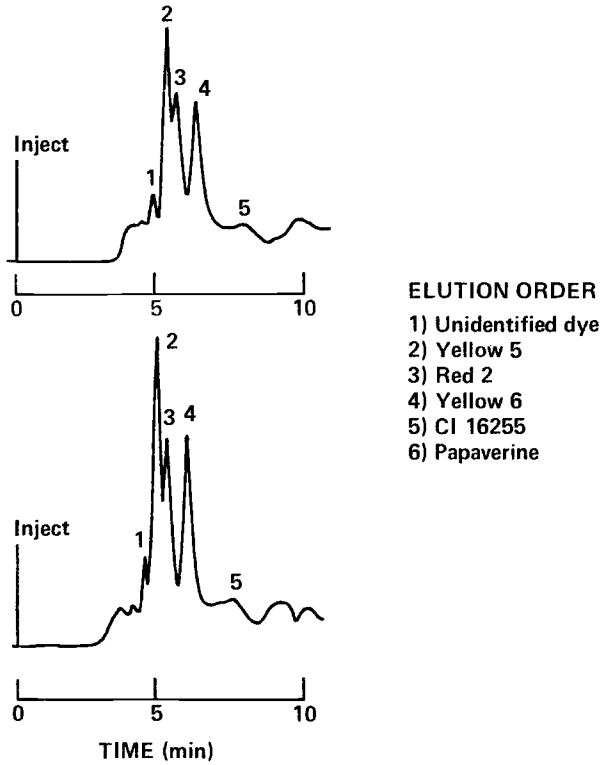


FIG. 4—Comparative HPLC examination of two unrelated heroin samples with similar dye profiles.

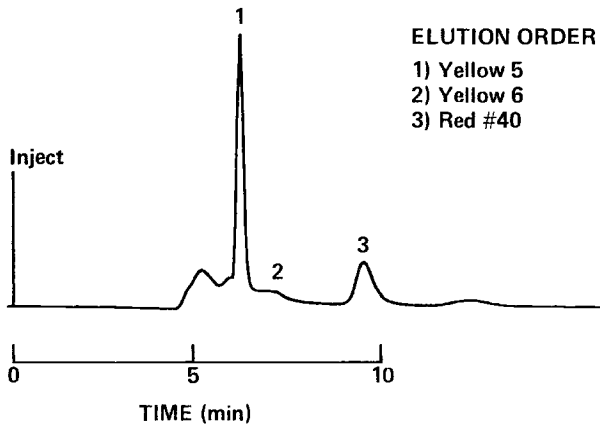


FIG. 5—Chromatogram of untreated heroin diluent seized in the Los Angeles area. Presence of Red No. 40 was confirmed by thin-layer chromatography.

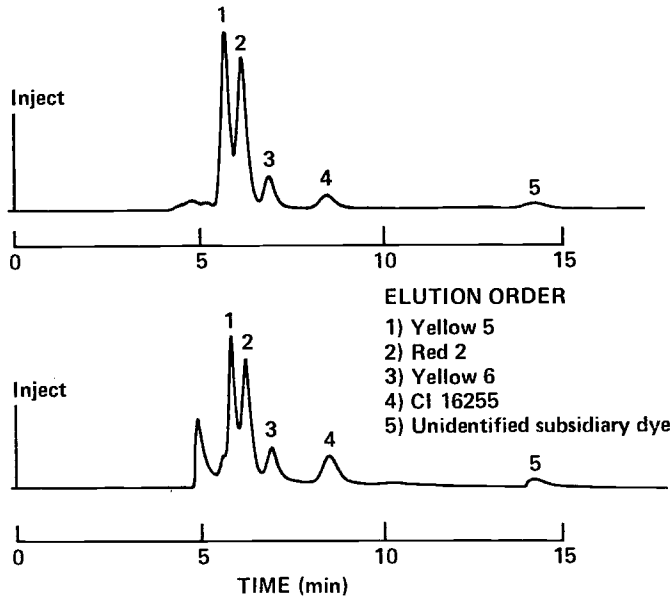


FIG. 6—Chromatographic dye profiles of heroin samples, after wool extraction, seized at the port of entry, San Ysidro, Calif.

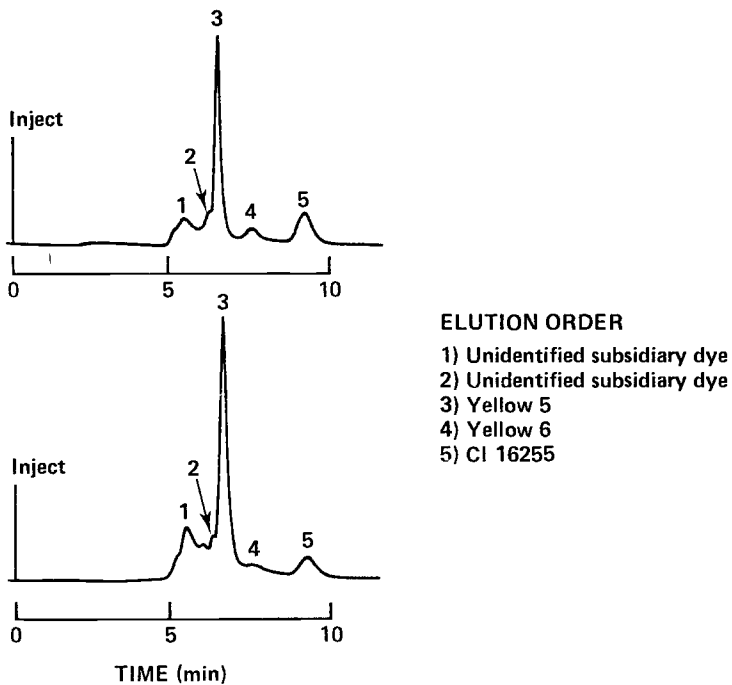


FIG. 7—Chromatographic dye profiles of heroin samples, after wool extraction, of two heroin samples seized in different locales.

is hoped that continued comparative examinations of dye patterns via HPLC will enable the laboratory to take a more active role in providing analytical proof in support of other information obtained by enforcement officers or in initiating intelligence information where none previously existed.

Acknowledgment

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References

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