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Characterization of Alkali Blue Pigment in Counterfeit Currency by High Performance Liquid Chromatography

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ABSTRACT: A method for the forensic science characterization of black printing inks is presented. High performance liquid chromatographic techniques have been developed for the semiquantitative analysis of pigment components and manufacturing artifacts present in alkali blue pigment, which is commonly found in black letterpress and offset inks. These analyses have shown that differences can be demonstrated in the component makeup of alkali blue from different sources, and that these differences may be useful in showing relationships or a lack thereof between evidentiary ink samples.

KEYWORDS: forensic science, inks, chromatographic analysis, ink analysis, pigment, counterfeit currency, high performance liquid chromatography, alkali blue

The forensic science characterization of printing inks has been used in the Bureau of Engraving & Printing Laboratory as a tool in associating counterfeit government obligations with each other, with inks used in their production, and with counterfeiters. Court testimony has been given on numerous occasions as a result of correlations that have been found between counterfeit Federal Reserve notes and offset printing inks recovered from illicit printing operations. These correlations have generally been made on the basis of similar class printing ink characteristics, including color, spectral reflectance, major element composition, and pigment composition. It is the purpose of this study to improve upon the class characterization of printing inks by investigating soluble pigment components as parameters that may be useful in individualizing an ink sample to a particular manufacturer or manufacturer's batch.

Previous work in the association of inks with manufacturers involved the characterization of writing inks (ball pen, fiber tip marker, fountain pen, and so forth) by high resolution thin-layer chromatography [*1–3*], electrophoresis [*4,5*], and high performance liquid chromatography (HPLC) [*6*]. These studies were successful because of the high degree of recipe variation among writing ink manufacturers and the solubilities of writing ink vehicles and components. Printing inks generally differ from writing inks in that they have fairly standard compositions and contain mostly insoluble components that become locked up within a highly cross-linked vehicle once the ink dries. As a result, the few soluble components that may be recoverable from printed ink films must be scrutinized very closely to ascertain similarities or differences which may be applied in a comparative analysis. A search of the

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literature has failed to produce any previous study involving the association of recoverable printing ink components with manufacturers.

Analysis of Alkali Blue

Alkali blue (Pigment Blue 19, Colour Index #42750:1, or Pigment Blue 61, Colour Index #42765:1) [7] is a synthetic organic pigment that is generally incorporated into black letterpress and offset inks to reduce the brownish undertone of the primary pigment, carbon black. Most of the counterfeit currency notes we have analyzed contain alkali blue as an ingredient in the face (black) ink. The solubility, or tendency to bleed, of alkali blue in alcohol makes it one of the few black printing ink components that can be readily recovered from a dried ink film and characterized. The complexity of the mixture of pigment components (unreacted intermediates, side reaction products, contaminants, and so forth) formed in the manufacture of alkali blue also provides it with the potential for exhibiting individualizing batch characteristics. This study focuses on the analysis of alkali blue to determine the feasibility of distinguishing between different manufacturers on the basis of the makeup of the extractable components.

An analytical HPLC technique was developed and used for characterizing alkali blue from three major manufacturers. Ten samples were taken from a single pigment batch from each manufacturer, and three repetitive separations were performed on each sample. The semiquantitative peak area data for each sample was taken as the average for the three separations, giving a total of ten data points per manufacturer per chromatographic peak.

All three of the pigments analyzed were of the respective manufacturer's "Alkali Blue-Red Shade" designation.

Equipment, Materials, and Reagents

The HPLC system consisted of:

(1) two Waters Associates M-6000A H.P.L.C. pumps;
(2) Waters Associates U6K sample injector;
(3) Waters Associates Micro Bondapak C-18 reverse phase column, 30-cm by 4-mm inside diameter;
(4) Varian Associates Varichrome ultraviolet/visible variable wavelength detector;
(5) Waters Associates model 720 system controller; and
(6) Waters Associates model 721 data module.

A 25-μL injection syringe (Hamilton #802) was used along with an electronic microbalance (Cahn model 26) and screw cap vials (15 by 45 mm).

The solvents were methanol, liquid chromatography grade (Waters Associates) and water, liquid chromatography grade (prepared in-house).

Experimental Procedure

Sample Preparation

For two of the manufacturers, approximately 0.5-mg dry pigment powder was weighed out and transferred to each of ten glass vials. Three millilitres of methanol were added to each vial 30 min before analysis, and the sample was filtered on a fine fritted glass funnel about 5 min before the first injection.

The third manufacturer's batch was in the form of a flushed base (pigment dispersed in vehicle) which would not readily dissolve in methanol. These samples were mulled with an equal volume of tung oil (to reduce viscosity) and drawn down with an ink knife onto filter

paper. This process greatly increased the surface area of the sample and thereby allowed extraction of the alkali blue. Ten small sections were cut at random from a total of four separate filter paper drawdowns, placed into glass vials, and extracted and filtered the same as for the powder samples.

It was found during analysis that the areas of some chromatographic peaks would either increase or decrease as a function of the time that had elapsed between sample dissolution and analysis. These changes took effect over a period of hours and were presumably a result of reactions occurring between the solvated components. To keep the sample-to-sample changes minimal, a constant interval of 30 min was taken between the addition of methanol and the first injection, with the sample being stored in the dark except for filtration and injection.

Liquid Chromatographic Analysis

The HPLC method developed for this analysis incorporates the separation parameters listed in Table 1. Three 25-μL injections were made per sample, with the second and third injections occurring at 30-min intervals to allow ample time for column reequilibration after gradient regeneration. Typical chromatograms for the three manufacturers are shown in Figs. 1 to 3. A blank reference gradient was run by injecting a methanol extract of filter paper, and is shown in Fig. 4. An increase in detector attenuation by a factor of 2 (for Manufacturer B) and by a factor of 4 (for Manufacturers A and C) was necessary just prior to the elution of Peak 6 to keep it within the output range of the detector. The attenuation was decreased back to 0.05 after the elution of Peak 7. These attenuation changes can be seen as slight baseline shifts on the chromatograms; however, they had minimal effect on the peak area data as a result of the use of the valley-to-valley peak integration mode.

Table 2 gives the mean normalized peak areas and standard deviations obtained for each manufacturer. Normalized peak areas were obtained for each separation by dividing the area for each peak by the total area for all eight peaks. Normalized peak areas are, for the most part, independent of injection volume or concentration, and make direct comparison of one manufacturer with another possible without having to use an internal standard. A comparison of the data given in Table 2 shows that the "fingerprint" formed by the eight peak areas enables the pigments to be readily distinguished from one another.

TABLE 1—*Liquid chromatographic separation parameters.*

Column:	Waters Associates Micro Bondapak C-18 Reverse Phase, 30-cm by 4-mm inside diameter
Eluant:	Solvent A: methanol:water 1:2 v/v
	Solvent B: 100% methanol
	Gradient: 10% B/90% A to 100% B in 15 min, linear
	Delay time: 0
	Gradient duration: 15 min
	Final hold: 5 min
	Regeneration: 2 min, linear
	Reequilibration: 8 min
	Flow rate: 2.0 mL/min
Detector:	Visible absorbance at 590 nm
	Attenuation: 0.05/0.10 (Manufacturer B) and 0.05/0.20 (Manufacturers A and C) absorbance units full scale
Data module:	Integration mode: valley-to-valley
	Anticipated peak width: 10 s
	Noise rejection: 75
	Area rejection: 10
	Chart speed: 1 cm/min
Injection:	Volume, 25 μL
	Concentration, about 0.1 to 0.2 mg/mL

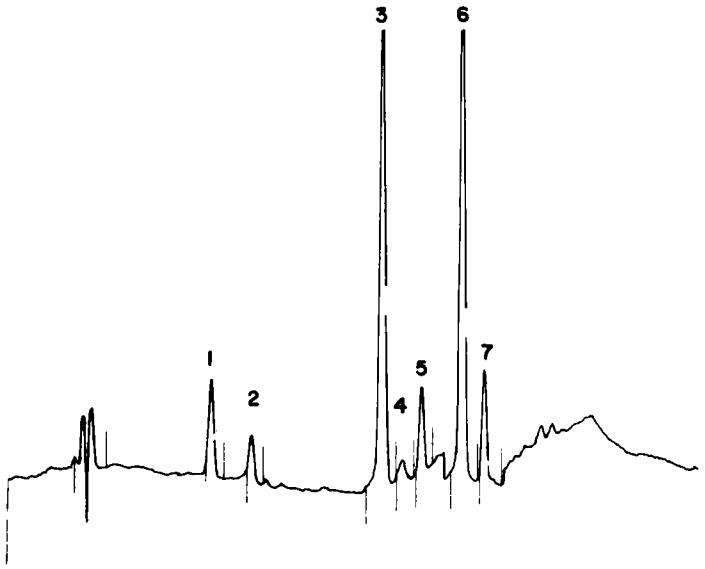


FIG. 1—*HPLC separation of alkali blue from Manufacturer A.*

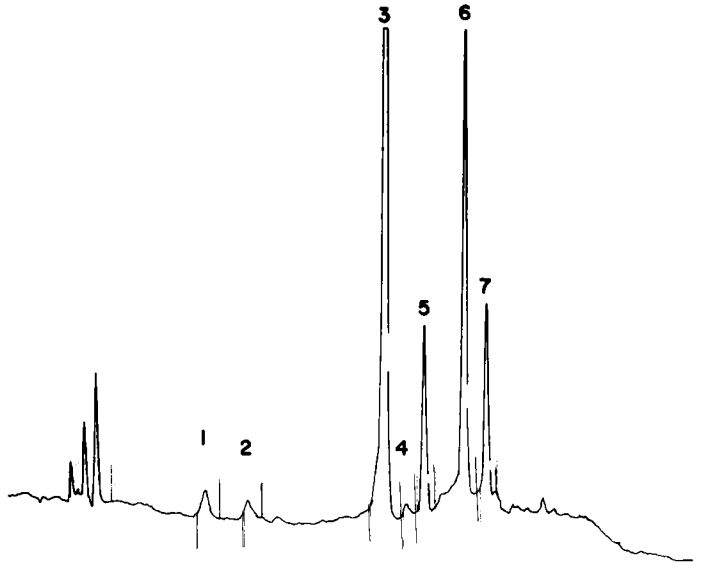


FIG. 2—*HPLC separation of alkali blue from Manufacturer B.*

Statistical Analysis of the Data

One-Way Analysis of Variance

One-way analyses of variance (statistical *F*-tests) [*8,9*] were performed on normalized peak areas to determine whether adequate sampling procedures were being followed. The *F*-test is designed to show whether greater variability exists within samples (injection-to-injection) or between samples. The normalized area data for Peak 7 was chosen for this test.

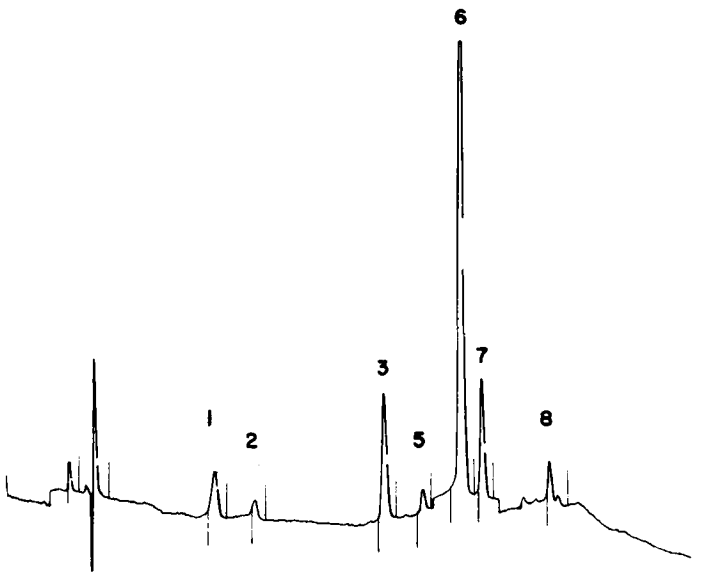


FIG. 3—*HPLC separation of alkali blue from Manufacturer C.*

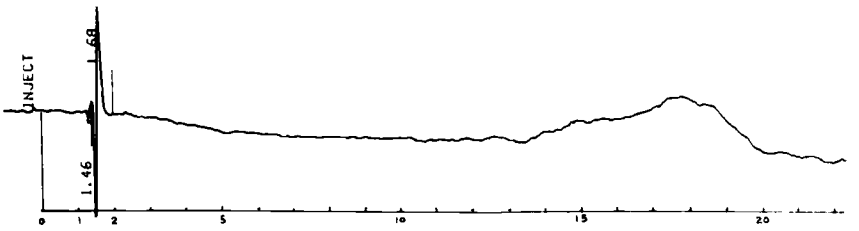


FIG. 4—*Reference HPLC run (filter paper extract).*

TABLE 2—*Normalized peak areas.* [a]

	Manufacturer A		Manufacturer B		Manfacturer C	
Peak No.	Area, %	S.D.[b]	Area, %	S.D.[b]	Area, %	S.D.[b]
1	3.75	0.24	1.08	0.21	1.32	0.36
2	1.30	0.10	0.82	0.08	0.50	0.14
3	15.25	0.22	26.75	0.83	4.19	0.17
4	0.59	0.08	0.58	0.10	0	...
5	2.25	0.11	5.11	0.22	0.70	0.10
6	64.94	0.41	55.06	0.87	77.95	1.20
7	11.88	0.21	10.59	0.27	14.04	0.68
8	0	...	0	...	1.35	0.31
	99.96		99.99		100.05	

[a] Number of points for all data = ten (each point = average of three injections).
[b] S.D. = Standard deviation.

Table 3 lists the results for all three manufacturers. Manufacturers A and B show greater variability within samples than between; this indicates that the liquid chromatography is less reproducible than is the sampling and preparation procedure. For these samples, many separations on a few samples would be expected to give the same results as a few separations (per sample) on many samples. Manufacturer C shows the opposite effect to that for Manufacturers A and B. Here, the relatively large between sample variability indicates that the sample preparation for the flushed base is not as reproducible as is that for the powders (A and B). For Manufacturer C, making a few separations per sample on many samples (as in the present case) is preferable to making many separations on a few samples.

Two-Sided Tolerance Intervals for Retention Times

Table 4 lists two-sided retention time tolerance intervals that would be expected, at the 95% confidence level, to contain the given chromatographic component in 95 out of 100 separations of alkali blue. The number of data points used in these calculations is equal to the number of separations in which the given peak appeared (Peak 4 did not appear for Manufacturer C, and Peak 8 did not appear for Manufacturers A or B). These tolerance intervals can be considered as a measure of the reproducibility of the chromatographic separation, including solvent preparation, gradient formation, and column degeneration. The absence of any overlap in these intervals indicates that the eluting peaks can be unambiguously (that is, with 95% confidence) identified by their retention times in a sample of alkali blue 95% of the time.

Analysis of Peak Area Data

Statistic analyses (such as Student's t-test) for the purpose of comparing normalized peak areas among the three manufacturers were not performed because, in the present case, such

TABLE 3—*Components of variance for chromatographic Peak 7.*[a]

Manufacturer	Within Samples	Between Samples
A	0.250	0.154
B	0.411	0.138
C	0.438	0.669

[a] Number of samples = ten and number of analyses within each sample = three.

TABLE 4—*Two-sided tolerance intervals*[a] *for peak retention times.*[b]

Peak No.	N	RT	S.D.	Lower Limit < (RT) > Upper Limit	
1	30	5.67	0.147	5.29	6.04
2	30	7.06	0.118	6.76	7.36
3	30	11.41	0.093	11.17	11.65
4	20	12.10	0.106	11.81	12.39
5	30	12.75	0.088	12.52	12.97
6	30	14.12	0.082	13.91	14.33
7	30	14.88	0.078	14.68	15.08
8	10	17.22	0.038	17.09	17.35

[a] 95% confidence that the numbered peak will elute within the respective tolerance interval in 95 out of 100 analyses.

[b] N = number of data points (Peak 4 was present for two manufacturers; Peak 8 was present for only 1), RT = mean retention time, and S.D. = standard deviation.

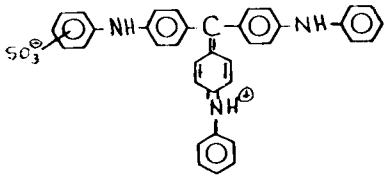
analyses were unnecessary. The ability to distinguish between all three manufacturers on the basis of peak areas is obvious; for Peaks 3, 5, and 6 alone, the observed area differences are on the order of ten standard deviation units for those respective peaks (see Table 2). Differences of this magnitude are easily discernable and require no more than a single sample per manufacturer to detect.

Discussion

Manufacture of Alkali Blue

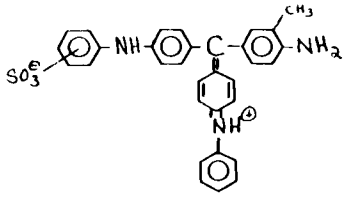
Forensically significant differences can be detected between samples of alkali blue from three different sources. None of the chromatographic components have been structurally identified, making it impossible to explain the observed differences in terms of chemistry. A glance at the manufacturing process may provide reasons as to why the observed differences exist.

A chemical structure for alkali blue [*10*] is shown in Fig. 5 (top). This structure is referred to by the Colour Index as Pigment Blue 61. A similar structure Fig. 5 (middle) is also known

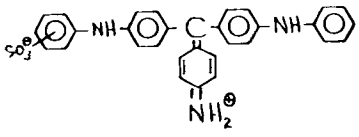


Pigment Blue 61 (C.I. #42765:1)

(Alkali Blue Green Shade)



Pigment Blue 19 (C.I. #42750:1)



Alkali Blue Red Shade

FIG. 5—*Structures for alkali blue:* (top) *pigment Blue 61 (C.I. #42765:1) (alkali blue green shade),* (middle) *Pigment Blue 19 (C.I. #42750:1), and* (bottom) *alkali blue red shade.*

as alkali blue and is referred to by the Colour Index as Pigment Blue 19. In addition to these is a third structure that is offered by manufacturers under the designation "red shade," which is shown in Fig. 5 (bottom). The second and third structures are basically lower phenylated homologs of the first structure, with the exception that the second structure also contains a methyl group.

A generalized manufacturing process for alkali blue begins with aniline and formaldehyde and proceeds through five steps [*10*], as shown in Fig. 6. The final product is a mixture of different structures, depending on the degree to which the carbinol base is phenylated. Generally, the higher the percentage of lower phenylated products in the final mixture, the redder the shade of pigment. The red shade of alkali blue is more widely used in the industry than is the green shade, probably because of its color and its higher tinctorial strength. Therefore, manufacturers strive to control the degree of phenylation, which is affected by "variations in time and temperature and the amount and type of catalyst used" [*10*, p. 622]. These variations may be related to the differences seen in the chromatographic separations of alkali blue from different manufactures.

Side reaction products may be another reason for differences in the final makeup of alkali blue. Because of the number of manufacturing steps involved, the commercial pigment is a complex mixture of "compounds of the triphenyl methane type, varying amounts of acridines, and products related to the oxidation and condensation of aniline" [*10*, p. 617]. In addition, the degree of sulfonation in the final manufacturing step may also lead to variations that become apparent through chromatographic analysis.

As a result, it appears that the number of different products formed during manufacture and variations in process control exercised by the manufacturer can lead to highly individualized pigment batches. Is it not surprising, therefore, that forensically significant differences can be easily detected in alkali blue from different sources.

Application of Analytical Procedure in an Actual Case

Testimony concerning the results of alkali blue analyses was given in Federal Court for the Western District of Texas, San Antonio, in June 1981 [*11*]. As far as can be determined, this was the first time that the use of high performance liquid chromatography for the analysis of printing ink has been offered and accepted in court. The case involved the association of counterfeit $20 Federal Reserve notes with manufacturing paraphernalia recovered from an illicit printing operation. One of these associations was made between alkali blue extracted from a counterfeit note face impression and alkali blue from black ink on an offset press cleaning mat. The chromatograms for these two samples are shown in Figs. 7 and 8, respectively. This evidence was offered as a demonstration of similar class characteristics between the counterfeit note and the ink on the mat. These similarities can easily be seen by a peak-for-peak comparison of the two chromatograms (the large spike to the left in Fig. 7 is an injection solvent front and not a sample component peak). Because of the lack of adequate background analyses on alkali blue from all manufacturers, however, we were unable to formulate an opinion as to the probability that these samples were indeed of common origin. This paper can be considered as a first step in the establishment of this necessary background information.

Conclusions

Alkali blue, a pigment commonly incorporated into black printing inks, has been shown to possess unique manufacturing characteristics that are discernable through HPLC analysis. Statistical analyses of the liquid chromatographic analytical techniques that were developed for this purpose have shown that these techniques afford an unambigous comparison between three manufacturers' pigments, and that the differences found between manufacturers were much larger than any caused by experimental or systematic error.

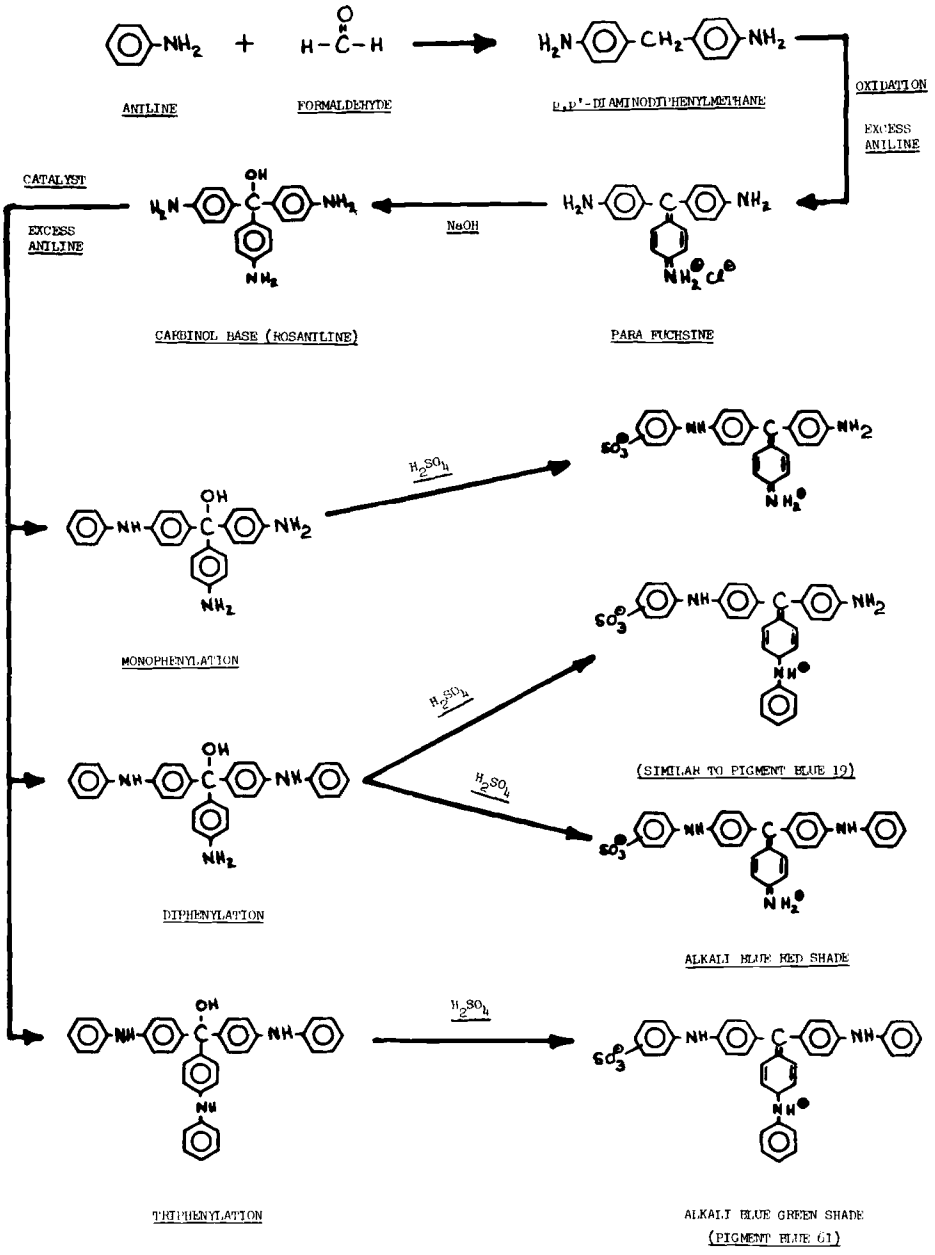


FIG. 6—*Manufacture of alkali blue.*

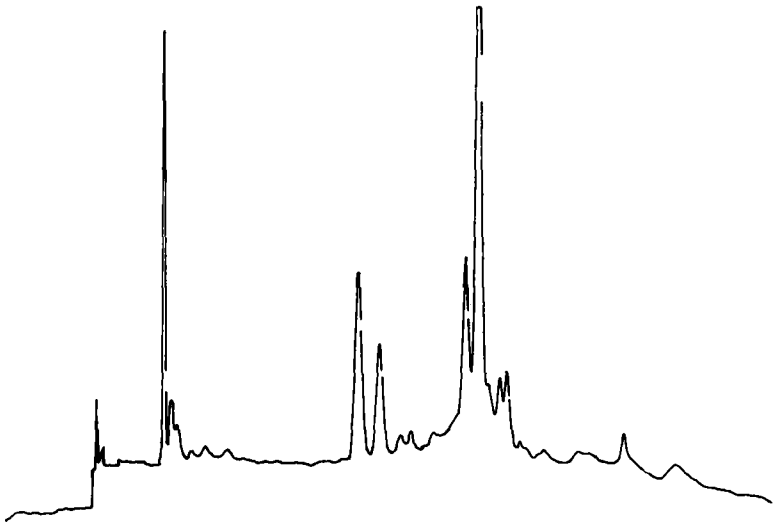


FIG. 7—*Alkali blue from counterfeit $20 Federal Reserve note.*

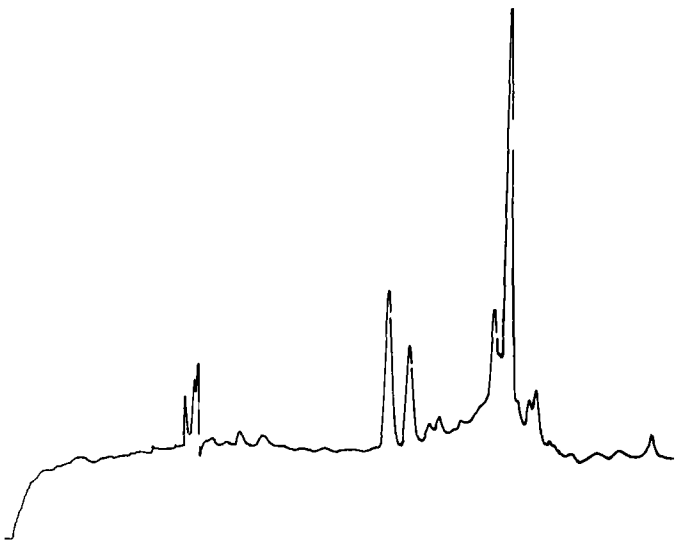


FIG. 8—*Alkali blue from press cleaning mat.*

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