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HPLC Analysis of Printing Inks

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ABSTRACT: The high pressure liquid chromatography (HPLC) method for analyzing printing inks extracted from documents is described. The procedure is sufficiently sensitive for analysis of small areas of printing inks. The analysis can distinguish between different production lots from the same manufacturer. The method was applied on two forensic cases involving forged documents.

KEYWORDS: questioned documents, forged documents, printing inks, high pressure liquid chromatography, criminalistics

Forensic science laboratories often deal with cases involving forged banknotes and forged documents. Forgeries are usually identified by various optical methods in combination with the expertise of the investigator in the field of printing and copying methods. In some instances, chemical analysis of printing inks should be useful to answer the question whether two or more documents (forgeries) could have the same origin. Successful chemical analyses of photocopy toners have been reported by several authors [*1–4*]. However, forensic analysis of printing inks has not attracted much interest. Nondestructive optical techniques such as UV-fluorescence and IR-luminescence are commonly used for this purpose. The use of microspectrophotometry [*5*] and X-ray emission analysis [*6*] of printing inks has been reported.

In forensic science, it is desirable to apply nondestructive methods to the material investigated. Destructive methods, such as various chromatographic techniques give more information about the chemical composition of various materials analyzed. This is important, when a common origin of these materials has to be established.

In this study we have used high pressure liquid chromatography (HPLC) to separate and detect various components in printing inks extracted from documents. This method was optimized and applied to two cases involving forged payments from the Social Insurance Office of Sweden and forged forms for transactions through the Postal Giro method of payment.

Composition of Printing Inks

Printing ink consists normally of three major components [*7*]—coloring matter, oil or resin, and solvent. The oil or resin together with the solvent provide the liquid portion of the ink, which is called the vehicle. The coloring matter is either dye (soluble in the

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vehicle) or pigment (insoluble in the vehicle). Dyes are divided into different classes depending on their properties, such as, acid dyes, basic dyes, solvent dyes, etc.

Experimental

Materials

All the solvents for HPLC were of HPLC grade—methanol, acetonitrile, dichloromethane, and hexane. Potassium perchlorate, tetrabutylammonium hydrogen sulphate and pyridine were of p. a. purity; 1-heptane sulphonic acid sodium salt was purchased from Kodak and used without further purification.

Methods

Extraction of Printing Inks from Documents—The extraction of printing inks from documents was done as follows. A piece of paper containing the ink was placed in a 5 mL beaker. About 1 mL dichloromethane was added and the mixture was heated for a few minutes. The extract was removed and the paper extracted further with 2% hydrochloric acid in methanol. The acetic methanol extract was combined with the dichloromethane extract. Any residues of printing ink on the paper were finally extracted by heating the sample at 80°C with pyridine for at least 5 min. The combined pyridine, acidic methanol, and dichloromethane extracts were gently evaporated to a volume that could be transferred to a 200 μL Reacti-Vial (Pierce). The solvent was then removed by directing a stream of dry nitrogen onto the solution. The dry extract (the colored residue) was dissolved in methanol and analyzed by HPLC (10 μL aliquots of the extract were injected onto the HPLC column).

HPLC Analysis—Equipment—The chromatographic system used consisted of a Varian 5000 Liquid Chromatograph equipped with three detectors, connected in series. A variable wavelength detector (Varian UV-100) operated at visible light range, the wavelength was chosen according to the shade of printing ink (510 nm for red and brown inks, 570 nm for blue inks). This procedure was necessary because a diode array detector was not available. Also chosen was a fixed wavelength detector (Waters Model 440) operated at 254 nm and a Perkin Elmer fluorescence detector (LC 240) worked with the excitation wavelength of 350 nm and the emission wavelength of 550 nm. The fluorescence detector was connected to a Perkin Elmer LCI-100 integrator. The signals from the UV-VIS detectors were recorded with an Omega Data system from Perkin Elmer.

The HPLC column used was a 20 cm 5 μm Nucleosil C_{18} with a 4.6 mm inside diameter (Scandinaviska GeneTec AB). Some analyses were carried out using a 20 cm 5 μm Nucleosil Si 50 normal phase column. The samples were injected into a 10 μL loop (Valco Valves).

Conditions—Both ionic (anionic and cationic) and nonionic compounds are present in printing inks. Therefore, different kinds of ion-pairing reagents in combination with different pH values of the mobile phase were tested to achieve optimal separation. The choice of mobile phase and experimental conditions was based on the successful separations of cosmetic dyes [*8*,*9*] and fiber dyes [*10*], reported in the literature. Four different mobile phases for reversed phase HPLC were tested. Each mobile phase consisted of two solvents, A and B. The samples were eluated using a linear gradient from A to B in 20 min, at a flow rate of 1 mL/min. The final composition (B) was maintained for 15 min, followed by a reversed gradient for 5 min, and re-equilibration of the column by maintaining the initial composition (A) for 10 min.

Mobile phase 1: A—50% methanol + 50% water containing 10 mM potassium perchlorate, pH adjusted to 3.0 with perchloric acid,

B—100% methanol

Mobile phase 2: A—50% methanol + 50% water containing 5mM heptanesulphonic acid, pH 3.0,

B—100% methanol

Mobile phase 3: A—50% methanol + 50% water containing 10 mM tetrabutylammonium hydrogen sulphate, pH adjusted to 5.5 with ammonia,

B—100% methanol

Mobile phase 4: A—30% acetonitrile + 70% water containing 10 mM $KClO_4$, pH adjusted to 3.0 with perchloric acid,

B—100% acetonitrile

In some experiments, normal phase HPLC analyses were carried out. The mobile phase used was: A—50/50 dichloromethane/hexane, B—90/10 dichloromethane/methanol.

All the solvents were degassed for 10 min by ultrasonic treatment just before use.

Colorants and Reference Inks—Reference samples of pure colorants known to appear in, for example, printing inks were obtained from various suppliers. Some pigments and dyes were obtained from AB G-man, Trelleborg, Sweden. Three samples of red printing inks of the same shade but produced by different manufacturers were received from PrintCom AB, Linköping, Sweden.

The samples of pure colorants were dissolved in methanol and mixed in several reference solutions in order to try and identify some of the colored components of printing inks.

From about 15 various documents, forms and leaflets containing printing inks with either red or blue shadows, small pieces were cut out and the printing inks analyzed. The two samples denoted "red inks" and "blue inks" in the text were used to test the separation efficiency of the mobile phases on real samples.

Samples of printing inks and other samples not directly soluble in methanol were extracted according to the procedure described previously.

Results and Discussion

Extraction of Inks

Most of the samples showed satisfactory solubility when using the two first extraction steps (dichloromethane and acidic methanol). The use of pyridine as the extraction solvent was found necessary only for blue printing inks. Some of the blue-shadowed inks could not be removed from the documents completely by the extraction procedure used. The unextractable residue was presumably caused by various phthalocyanines, which are insoluble in solvents, are heat resistant and have an excellent fastness to light [7].

HPLC Analysis

Reversed Phase Separations—All four mobile phases employed in this study were useful for separation of printing inks into their components. The tests were performed using the mixtures of red inks and blue inks (see previous section) as well as various mixtures of pure colorants. Mobile phase 3 was less successful in separating the inks into individual components. This phase gave broader peaks and also some nearby unretained peaks in the visible range. The combination of low pH and the use of perchlorate as ion-pairing

agent in the mobile phase gave the best separations with well resolved peaks and a broad variation in retention times. The use of acetonitrile instead of methanol improved the separation efficiency and also decreased the changes in baseline owing to gradient elution. Thus the mobile phase 4 was chosen for the analyses of printing inks reported here.

Figures 1 and 2 show the chromatograms obtained for the mixture of red and blue inks, respectively, using the mobile phase 4. The visible light detection was at 510 nm for red inks and 570 nm for blue inks. One of the peaks in Fig. 1 (visible light) with a retention time of about 6 minutes is not well-shaped, otherwise the separation is good. Figure 1 also reveals that many constituents of red printing inks exhibit fluorescence under the experimental conditions. The use of sensitive fluorescence detection, although influenced by the possible presence of interfering compounds from paper, might be useful when trace amounts of the ink are available.

The reproducibility of the method—the extraction and HPLC separation—was evaluated by replicate analyses of red colored printing ink from a single document. An area of approximately 4 mm^2 was cut out from the document and the ink was extracted and analyzed. The procedure was repeated three times using the same document. The results are depicted in Fig. 3, which shows the chromatograms detected at 510 nm. The three chromatograms show good agreement. The reproducibility between the chromatograms detected at 254 nm and by fluorescence detection was also very good.

An analysis of the mixture of 10 colorants, known to appear in printing inks, is shown in Fig. 4. The amount of each of the colorants injected was about 50 to 100 ng. The identity of some of the detected peaks is noted in this figure. The detection limit of the HPLC analysis varies between the different colorants, being generally below 50 ng. For colorants exhibiting fluorescence the sensitivity is much higher. Lacking the access to an diode array detector, we have not tried to identify the various peaks in Figs. 1 and 2.

In an actual case submitted to our laboratory, a series of forged payments from the

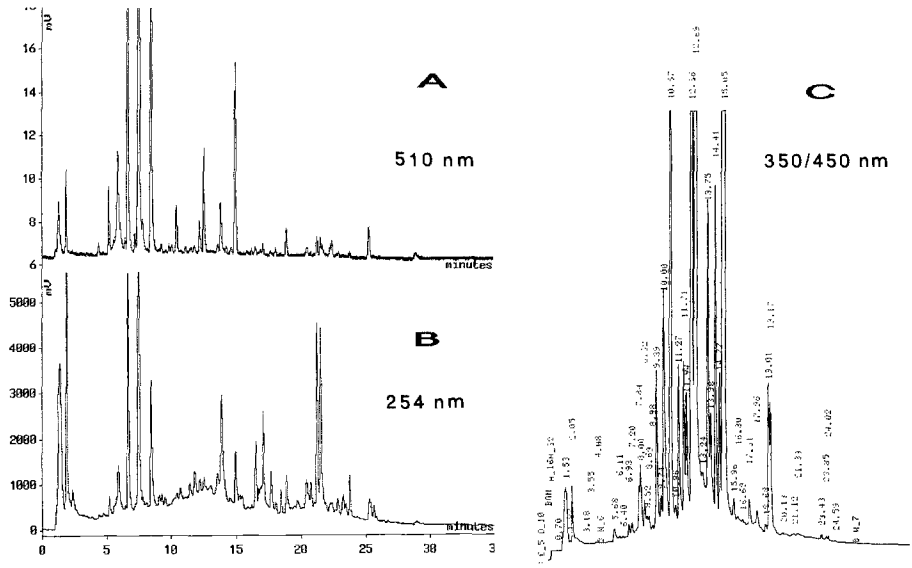

FIG. 1—*HPLC chromatograms (reversed phase separation) obtained for a mixture of red-colored printing inks extracted from various documents. The detection wavelength was 510 nm for the chromatogram marked A and 254 nm for chromatogram B. The chromatogram C is from the fluorescence detector, operating at 350 nm for the excitation and at 550 nm for the emission. Mobile phase 4 was used for the eluation.*

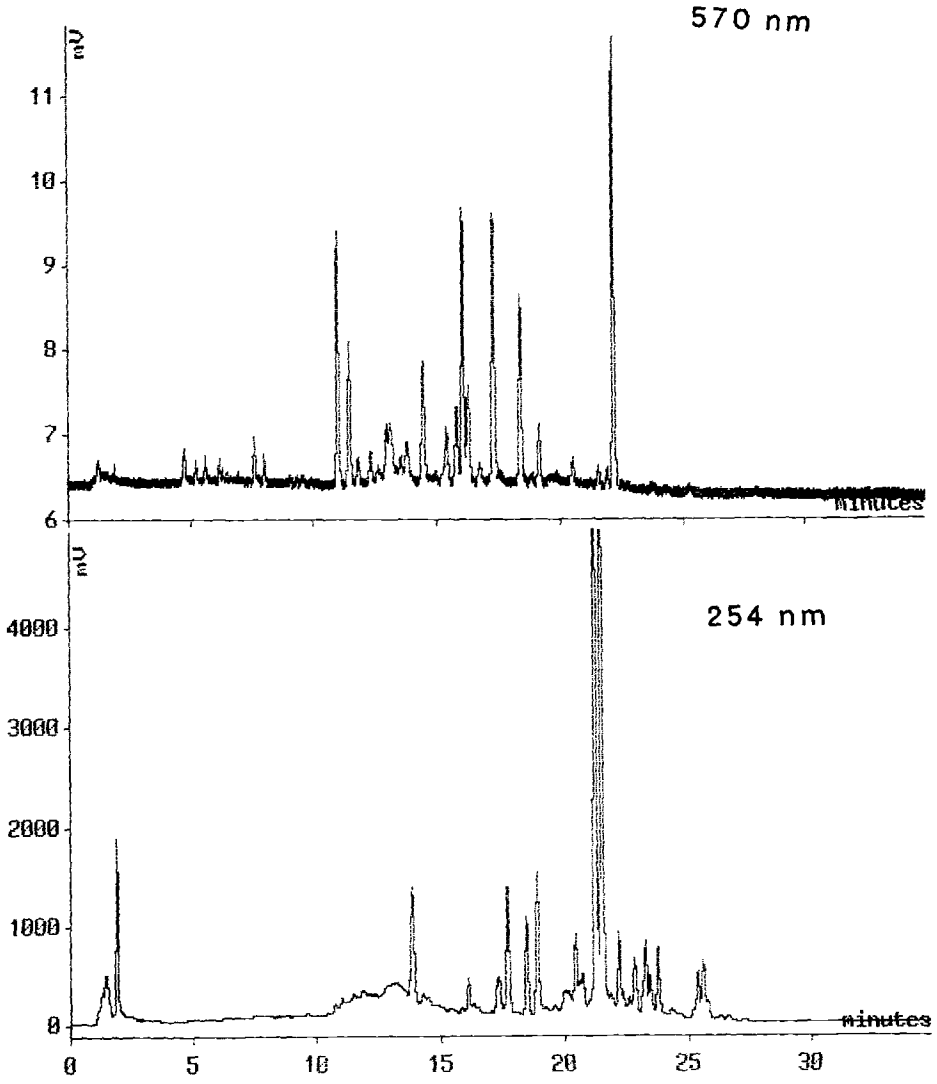

FIG. 2—*HPLC chromatograms detected at 570 nm (upper trace) and 254 nm (lower trace) for a mixture of blue-colored printing inks extracted from various documents. Other experimental conditions were the same as in Fig. 1.*

Social Insurance Office of Sweden and several forged Postal Giro payment forms were investigated. Both types of documents contain printing inks with red and brown shades. Comparison was made with the analyses of corresponding inks on the genuine documents. The composition of printing inks on the suspect documents differed qualitatively from those of the genuine ones. Figure 5 illustrates differences in HPLC chromatograms obtained at 510 nm for red-colored inks on genuine respective suspect checks from the Social Insurance Office.

The analyses carried out on several suspect documents showed that the composition of inks of the same shade on the same kind of document was indistinguishable. These results indicate that the whole series of forged document have the same origin. There

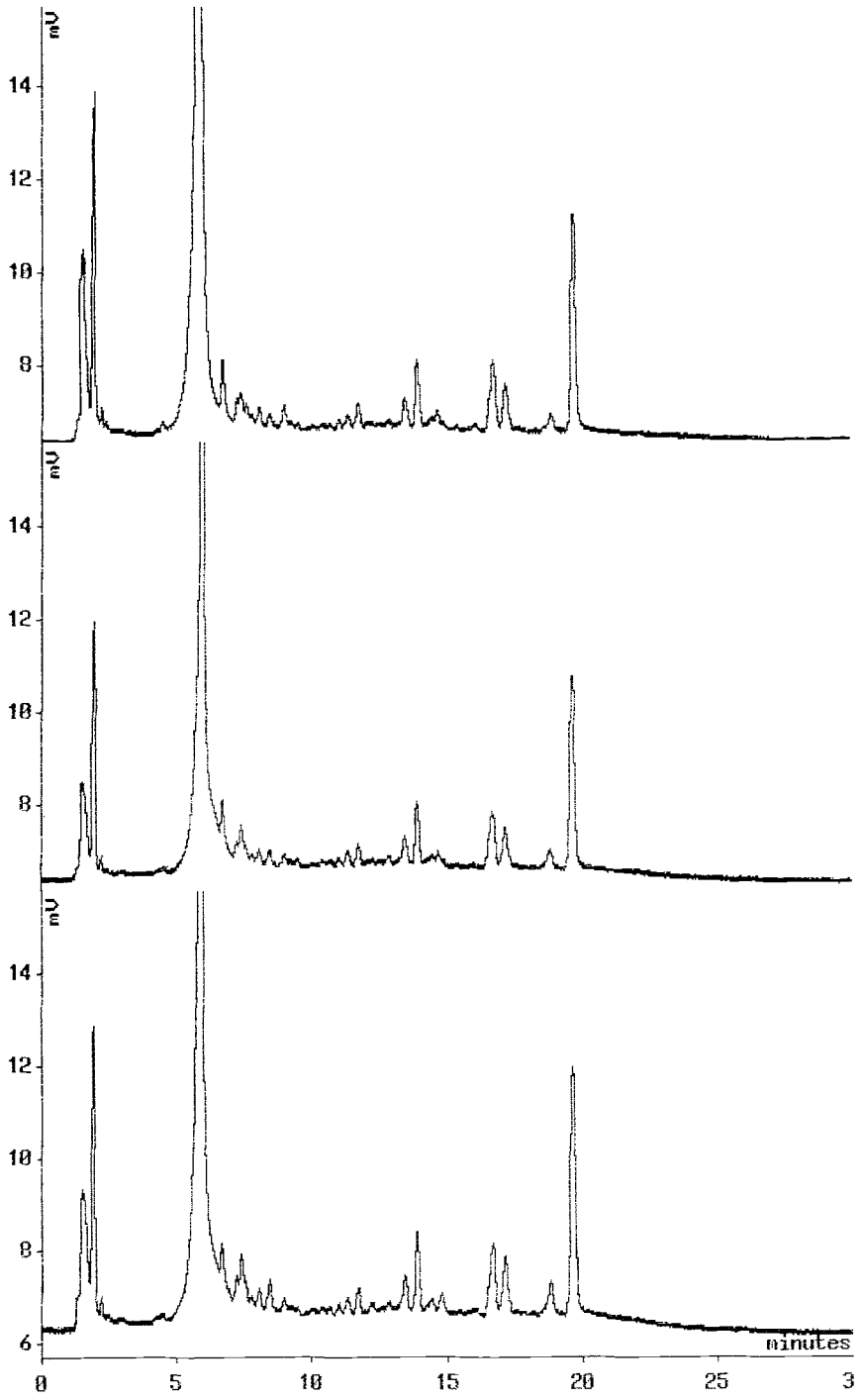

FIG. 3—*Three replicate analyses of red-colored inks from the same document. Analytical conditions as in Fig. 1. The detection wavelength was 510 nm.*

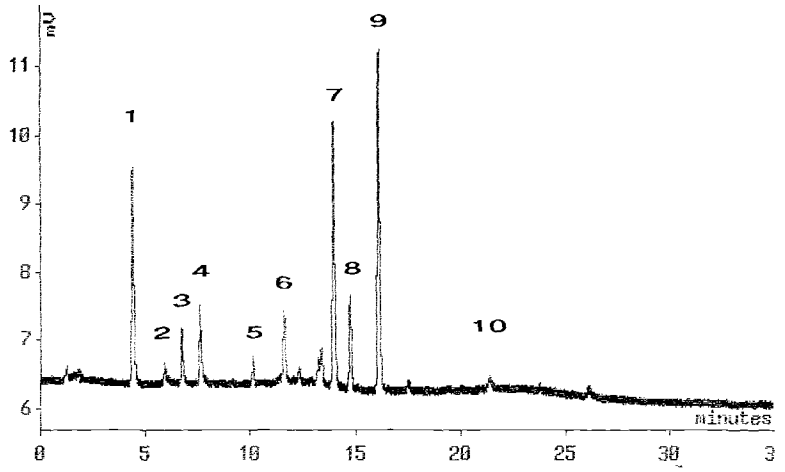

FIG. 4—*HPLC separation of the mixture of 10 colorant of red or violet shade. The detection wavelength was 510 nm. About 50–100 ng of each compound was injected. Some peaks were identified (tentatively, by their retention times) as Orange II, CI 15510 (1), Lithol Red, CI 15630 (2), Lithol Rubine 4B, CI 15850 (3), Lake Red C, CI 15585:1 (4), Eosine G, CI 45380 (5), Phloxine B, CI 45410 (6), Rhodamine B, CI 45170 (7), Methyl Violet, CI 42535 (8), Crystal Violet, CI 42555 (9) and Sudan III, CI 26100 (10).*

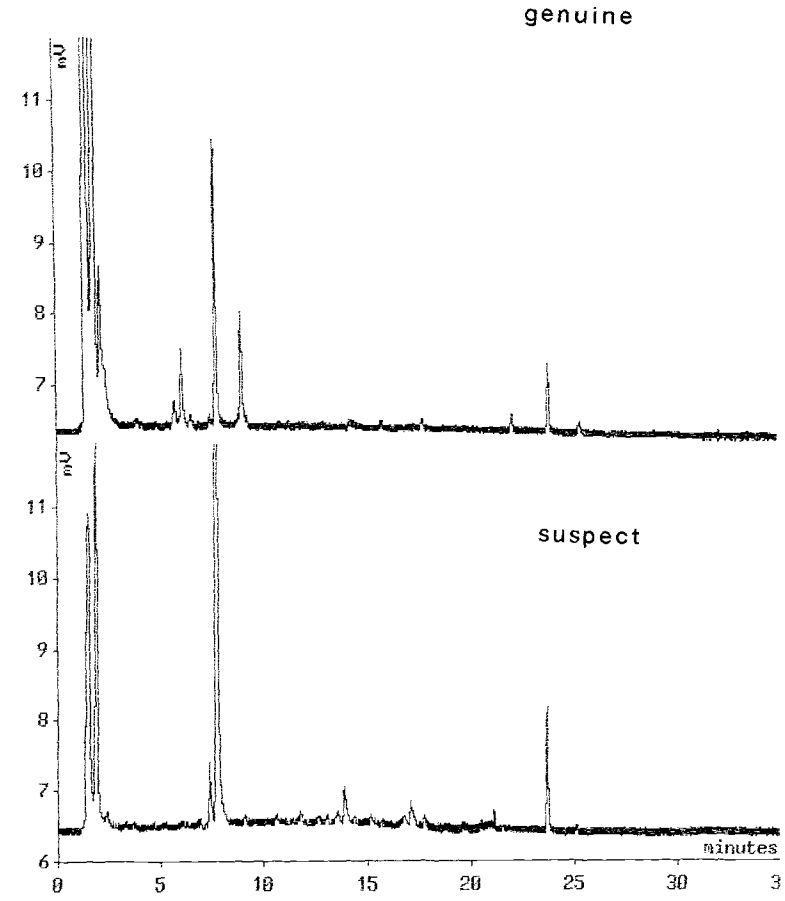

FIG. 5—*HPLC chromatograms obtained at 510 nm for red-colored inks on genuine (upper trace), respectively suspect checks from the Social Insurance Office of Sweden.*

were, however, clear differences in composition between similar shade inks on Postal Giro forms and Social Insurance checks, respectively.

The HPLC analysis can detect the differences in composition of inks from various production lots. Figure 6 demonstrates the differences between two batches of red-colored inks from the same manufacturer (the time of production is not known). When this method is applied for forensic comparison between suspect and genuine documents, care must be taken to ensure that the reference material is correctly chosen. We found clear differences in composition of red-colored inks on Postal Giro order forms printed in 1989 and 1991, respectively. It is not known to us whether these differences depended on differences between production runs of the same manufacturer or if the inks were received from different suppliers.

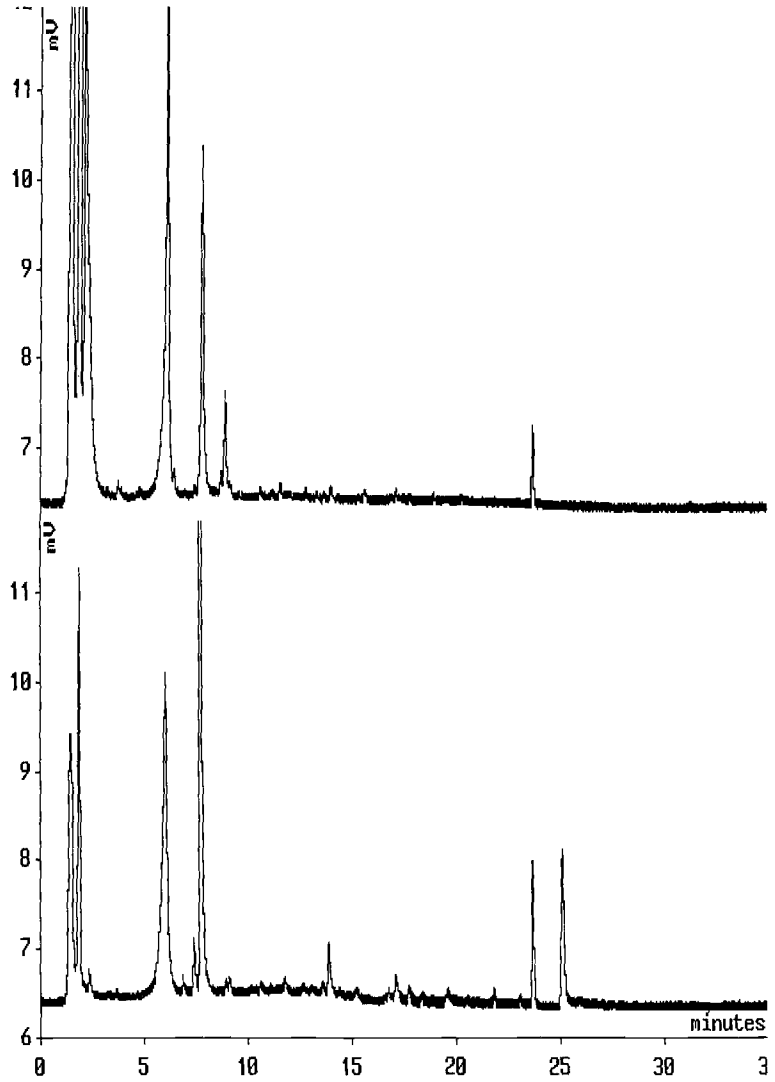

FIG. 6—*HPLC chromatograms obtained at 510 nm for two different production runs of red-colored inks from the same manufacturer.*

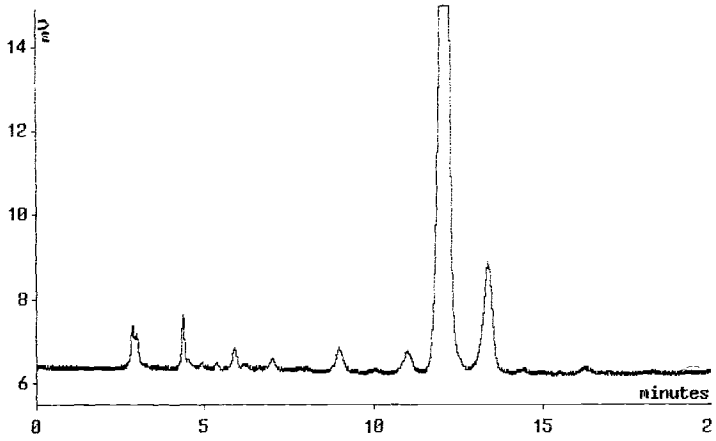

FIG. 7—*Normal phase separation of the mixture of red-colored inks (the same mixture as in Fig. 1). The detection wavelength—510 nm.*

Normal Phase Separations—Some of the components in printing inks are soluble in dichloromethane but slightly soluble or insoluble in methanol. These components will be removed from the documents in the first extraction step but cannot be analyzed by reversed phase chromatography. Therefore, some experiments were performed using normal phase HPLC columns. Figure 7 shows the chromatogram obtained for the mixture of red inks analyzed previously in Fig. 1. The detection wavelength was 510 nm. Only a limited number of peaks are detected by normal phase chromatography. This method might be useful in cases when the solubility of the ink in a mobile phase used for the reversed phase separations is unsufficient.

Conclusions

The method for analyzing printing inks from documents described in this study is destructive to the sample. The ink must be extracted from the document. The standard technique for extraction of inks from documents is described.

The entire analytical procedure has shown good reproducibility.

The sensitivity is sufficient for analyses of small areas of thin layers of printing inks, for example, single letters.

The analysis can detect differences in the composition of inks beween different production lots from the same manufacturer. This makes it suitable for evaluating whether two or more documents contain inks having the same origin.

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