Asian Journal of Chemistry Vol. 21, No. 8 (2009), 6156-6166

Development of Thin Layer and High Performance Liquid Chromatographic Methods for The Analysis of Colourings Substances Used in the Printing Inks

SEMIHA YENIDOGAN*, MEHMET OKTAV and MERT ULGEN‡
Department of Printing, Technical Education Faculty, Marmara University, 34722 Göztepe, Istanbul, Turkey
Tel: (90)(216)3365770; E-mail: semihayavuz@marmara.edu.tr

In the present study, a thin layer chromatography (TLC) and a reverse phase high performance liquid chromatographic (HPLC) separation methods were developed for the commercially available azo derivative pigments ie **PR1** (pigment red 1), **PR2** (pigment red 2), **PR5** (pigment red 5), **PO5** (pigment orange 5) and **PY1** (pigment yellow 1) and also for the azo derivative dyes *i.e.*, 1-[(4-chlorophenyl)azo]-2-naphthalenol (**D1**), ethyl 4-[2-hydroxy-1-naphthyl)azo]benzoate (**D2**) and 1-[(2,4,6-tribromophenyl)azo]-2-naphthalenol (**D3**) together with their potential reduction/degradation products, aromatic primary amines. In addition, three different commercial ink preparations contanining **PR2**, **PR5** and **PO5** were analyzed by using these developed HPLC system following solvent extraction. Compounds were separated by a thin layer chromatography system employing plastic backed silica gel adsorbents and various solvent system combinations consisted of petroleum ether, acetone, chloroform, dichloromethane and methanol. The reverse phase HPLC system was consisted of a mobile phase of acetonitrile:water (67:33, v/v) and a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm-long) and it was delivered at a flow rate of 1 mL/min. The detection was carried out at a maximum wavelength of 220 nm.

Key Words: Azo dyes, Printing pigments, TLC, RP-HPLC, Ink analysis.

INTRODUCTION

Azo-based dyes and last many pigments have been used in a number of applications by the printing industry for years[1-3]. They are rarely pure initially because of the presence of impurities in the reactants and the occurance of side reactions during manufacturing[4]. It has also been shown that synthetic precursor, intermediates and degradation products of these dyes could be potential health hazards owing to both their toxicity and their carcinogenicity[5-9]. Biological reduction of an azo dye is responsible for the possible presence of toxic amines in the organism. The reduction can also occur non-enzymatically[10].

†Faculty of Pharmacy, Marmara University, 81010 Haydarpasa, Istanbul, Turkey.

The use of selective chromatographic separation techniques is necessary for the identification of individual substances, especially for the detection of toxic degradation products such as aromatic amines from azo compounds. TLC is one of the most popular chromatographic methods because of its ease of use and its ability to quickly generate qualitative information[11,12]. Although TLC is quite cost effective and sufficent for differentation of many writing inks, much research has been devoted to exploring applications of high performance liquid chromatography (HPLC). HPLC offers more resolving power and may generate quantitative information on coloured and uncoloured components[13,14]. Chromatography has been explored as potential method for differentiation of ink samples. In recent years, several papers have described the separation of azo dyes and pigments. Most of these studies were employed reversed-phase ion pair chromatography. HPLC has successfully differentiated many ink samples in distinguishable by TLC. HPLC is also a useful tool to identify in impurities in azo dyes. The present report describes a simple, fast and reliable TLC and HPLC system.

EXPERIMENTAL

High performance liquid chromatograph was consisted of a HP-Agilent 1100 solvent delivery system, a Zorbax Eclipse XDB C8 (3.9 × 150 mm). HPLC column, a Rheodyne Model 7125i Injector, an HP-Diode-Array (G1315A) Detector and HP-Agilent ChemStation Integration Software.

Pigments and dyes: Pigment red 2 (**PR2**), pigment red 5 (**PR5**), pigment yellow 1 (**PY1**) and pigment orange 5 (**PO5**) were kindly obtained from DYO Pigment and Dye Cooporation, Izmir, Turkey as a gift. Pigment red 1 (**PR1**) and azo dyes *i.e.*, **D1**, **D2**, **D3** were previously prepared in our laboratory and their melting points were uncorrected[15]. The solvent based cerigraphy ink with **PR5**, the water based flexo ink with **PR2** and the solvent based flexo ink with **PO5** were also obtained from DYO. The chemical structures of these dyes and pigments were given in Table-1.

Other chemicals used in the study were purchased as follows: Acetone, diethylether, toluene, ethyl acetate, cyclohexane, dichloromethane, chloroform, methanol, petroleum ether (40-60 °C), acetone, sulphanilic acid, benzocaine, acetonitrile (HPLC grade) and plastic backed silica gel plates (Kieselgel 60 F254) (Merek); *p*-chloroaniline, 2,4,6-tribromoaniline, 2,6-dichloroaniline and 4-nitroaniline (Aldrich); *p*-nitroaniline (BDH); 4-methyl-2-nitroaniline (Lanchester).

Thin layer chromatography (TLC): The samples from stock solutions of compounds in acetonitrile were applied into plastic plates and these were developed in the solvent systems *i.e.*, **S1**: petroleum ether:acetone (70:30,v/v); **S2**: petroleum ether:ethyl acetate (80:20 v/v); **S3**: petroleum ether:chloroform (50:50, v/v) and **S4**: dichloromethane. The compounds were visualized under 254 nm UV light and their $R_f \times 100$ values were recorded (Table-2). All pigments and dyes produced yellow to orange colours under the visible light. A TLC chromatogram of dyes and pigments and their potential degradation/reduction products, primary aromatic amines is represented in Fig. 1.

TABLE-1
CHEMICAL STRUCTURES OF PIGMENTS AND DYES STUDIED

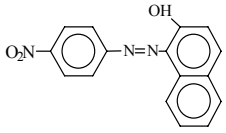
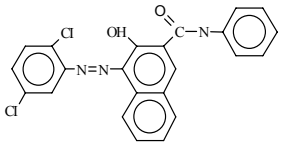
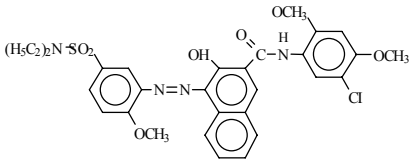
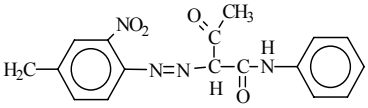
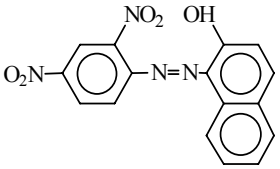
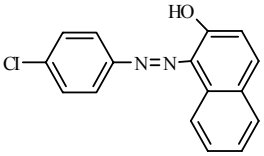
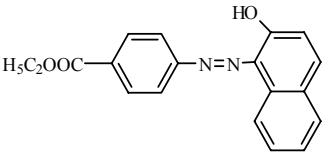
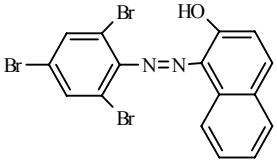
Pigment or dye (Abbreviation)	Chemical name	Chemical structure
Pigment Red 1 (**PR1**)	1-[(4-Nitrophenyl)azo]-2-naphthalenol	
Pigment Red 2 (**PR2**)	4-[(2,5-Dichlorophenyl)-azo]-3-hydroxy-N-phenyl-2-naphthalencarboxamide	
Pigment Red 5 (**PR5**)	N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxy-2-naphthalene-carboxamide	
Pigment Yellow 1 (**PY1**)	2-[(4-Methyl-2-nitro-phenyl)azo]-3-oxo-N-phenylbutanamide	
Pigment Orange 5 (**PO5**)	1-[(2,4-Dinitrophenyl)azo]-2-naphthalenol	
(**D1**)	1-[(4-Chlorophenyl)azo]-2-naphthalenol	
(**D2**)	Ethyl 4-[2-hydroxy-1-naphthyl)azo]benzoate	
(**D3**)	1-[(2,4,6-Tribromophenyl)-azo]-2-naphthalenol	

TABLE-2
CHROMATOGRAPHIC PROPERTIES OF PIGMENT AND DYES AND THEIR DEGRADATION / REDUCTION PRODUCTS (SEE TEXT FOR THE SOLVENT SYSTEMS)

Compound (abbreviation)	TLC (R_f × 100)				HPLC (Rt)
	S1	S2	S3	S4	
1-[(4-Chlorophenyl)azo]-2-naphthalenol (**D1**)	83.47	62.85	56.48	88	11.22
4-Chloroaniline (**4CA**)	51.78	23.81	18.51	64	2.51
1-[(4-Nitrophenyl)azo]-2-naphthalenol (**PR1**)	60.86	37.14	29.62	78.66	5.85
4-Nitroaniline (**4NA**)	39.13	11.42	12.03	66.66	2.02
Ethyl 4-[2-Hydroxy-1-naphthyl)azo]benzoate (**D2**)	70.43	46.66	31.48	57.33	8.27
Benzocaine (**BZK**)	46.08	19.04	12.03	33.33	2.19
1-[(2,4,6-Tribromophenyl)azo]-2-naphthalenol (**D3**)	84.34	72.38	57.40	95.89	28
2,4,6-Tribromoaniline (**TBA**)	80.87	68.57	68.52	94.52	6.03
2-[(4-Methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutanamide (**PY1**)	60.87	39.04	37.96	87.67	7.80
4-Methyl-2-nitroaniline (**MNA**)	54.78	33.33	29.63	78.08	2.61
N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxy-2-naphthalencarboxamide (**PR5**)	-	-	4.63	73.97	3.95
4-[(2,5-Diclorophenyl)azo]-3-hydroxy-N-phenyl-2-naphthalencarboxamide (**PR2**)	67.82	47.62	38.89	68.49	3.85
1-[(2,4-Dinitrophenyl)azo]-2-naphthalenole (**PO5**)	59.13	39.04	30.55	42.46	5.11

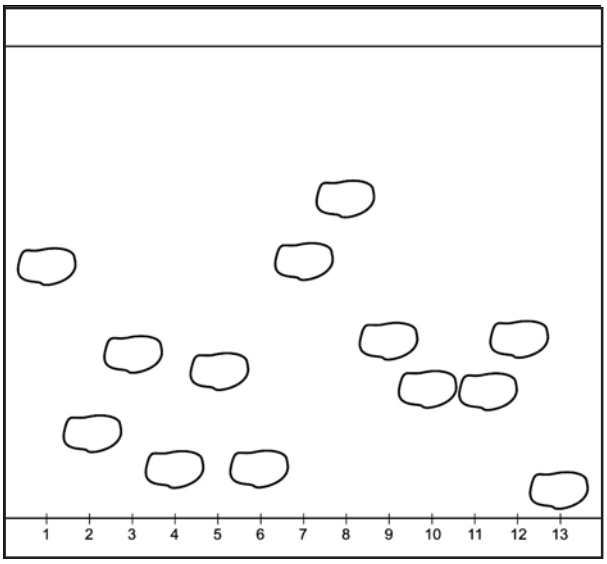
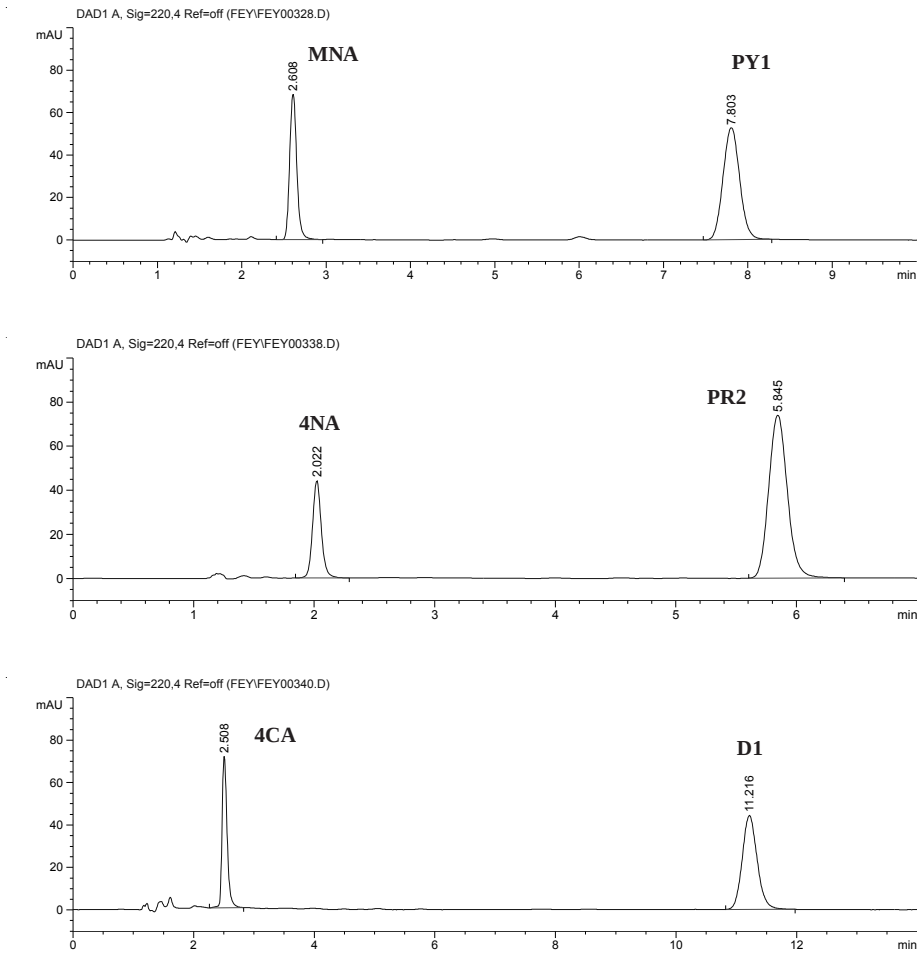


Fig. 1. A TLC chromatogram of dyes and pigments and their potential degradation/reduction products, primary aromatic amines (solvent system: S3; see Table-2 and tex for abbreviations and solvent system) (1 = **D1**; 2 = **4CA**; 3 = **PR1**; 4 = **4NA**; 5 = **D2**; 6 = **BZK**; 7 = **D3**; 8 = **TBA**; 9 = **PY1**; 10 = **MNA**; 11 = **PO5**; 12 = **PR2**; 13 = **PR5**)

High performance liquid chromatography (HPLC) of dyes and pigments: An HPLC method in which **D1**, **D2**, **D3, PR1** and **PY1** were separated from their corresponding potential degradation/reduction products, primary aromatic amines *i.e.*, **4CA**, **BZK**, **TBA**, **4NA** and **MNA** were developed. All compounds used in the present study were detected in a reverse phase isocratic HPLC system consisted of a mobile phase of acetonitrile:water (67:33 v/v) and a Zorbax Eclipse XDB-V8 HPLC column (4.6 × 150 mm). The flow rate was 1 mL/min. The detection was performed at a maximum wavelength of 220 nm.

The stock solutions for all dyes and pigments were prepared in acetonitrile. From these solutions, the concentrations of 28 ng/µL for **PY1**; 4 ng/µL for **MNA**; 9.3 ng/µL for **PR1**; 5 ng/µL for **4NA**; 10 ng/µL for **D1**; 25 ng/µL for **4CA**; 26.7 ng/µL for **D2**; 5 ng/µL for **BZK**; 50 ng/µL for **D3** and 7.5 ng/µL for TBA were injected into HPLC in 20 µL amounts. The chromatograms are demonstrated in Fig. 2.



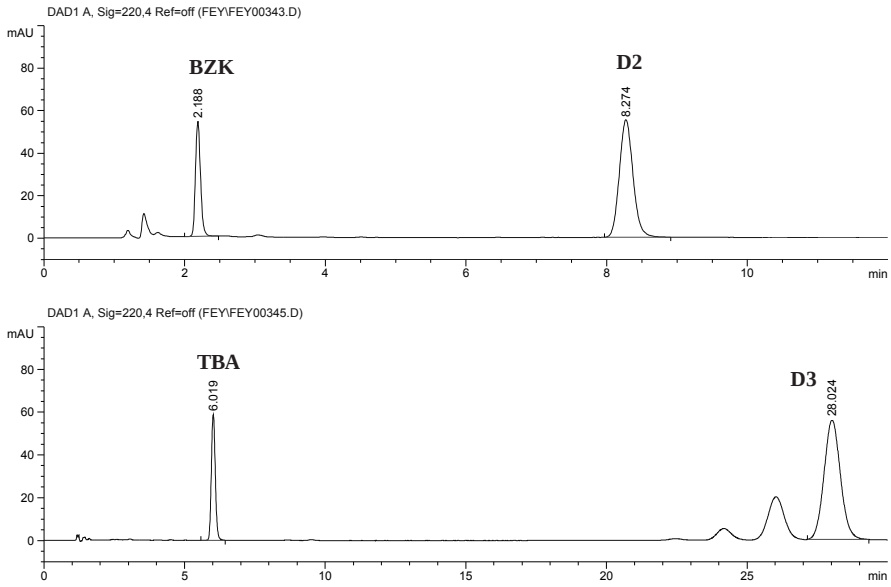


Fig. 2. HPLC chromatograms representing the separation of **PY1**, **PR1**, **D1**, **D2**, **D3** and their potential degradation/reduction products **MNA**, **4NA**, **4CA**, **BZK** and **TBA**, respectively

HPLC Analysis of ink samples with PR5, PR2 and PO5: For the HPLC analysis of ink samples, the ink (1 g) was washed three times with the suitable solvents (10 mL) with the solvent order of increasing solvent power[2] as shown in the Fig. 3. The experiments were done duplicate. At each extraction stage, the mixture was vortexed and then mixed in a tube shaker for 10 min and finally centrifuged for 5 min at 4000 rpm to separate the solvent from the insoluble matter. Every time, the extraction solvent (supernatant) was carefully decanted and the solid part was manipulated with the following solvent. In the final stage, acetone was evaporated and the residue (the extract) was dried, weighed (50 mg) and reconstituted with acetonitrile (1 mL). These final sample was centrifuged and the supernatant was applied to both TLC and HPLC (20 µL). The resulted chromatograms are shown in Figs. 4-7. The UV spectra obtained from authentic dye and pigments are presented in Fig. 8. The UV spectra obtained following the extraction of ink samples were the same as the authentic compounds (Figs. 4-8).

In the Fig. 2, HPLC chromatograms representing the separation of **PY1**, **PR1**, **D1**, **D2**, **D3** and their potential degradation / reduction products **MNA**, **4NA**, **4CA**, **BZK** and **TBA,** respectively (Table-1).

Figs. 4-6 shows the chromatograms from **PR5**, **PR2** and **PO5** standards together with the extracts obtained with following solvent extraction of commercial inks prepared with **PR5**, **PR2** and **PO5**. The similarity of UV sperctral data between the standards and extraction materials confirm the structure of pigments.

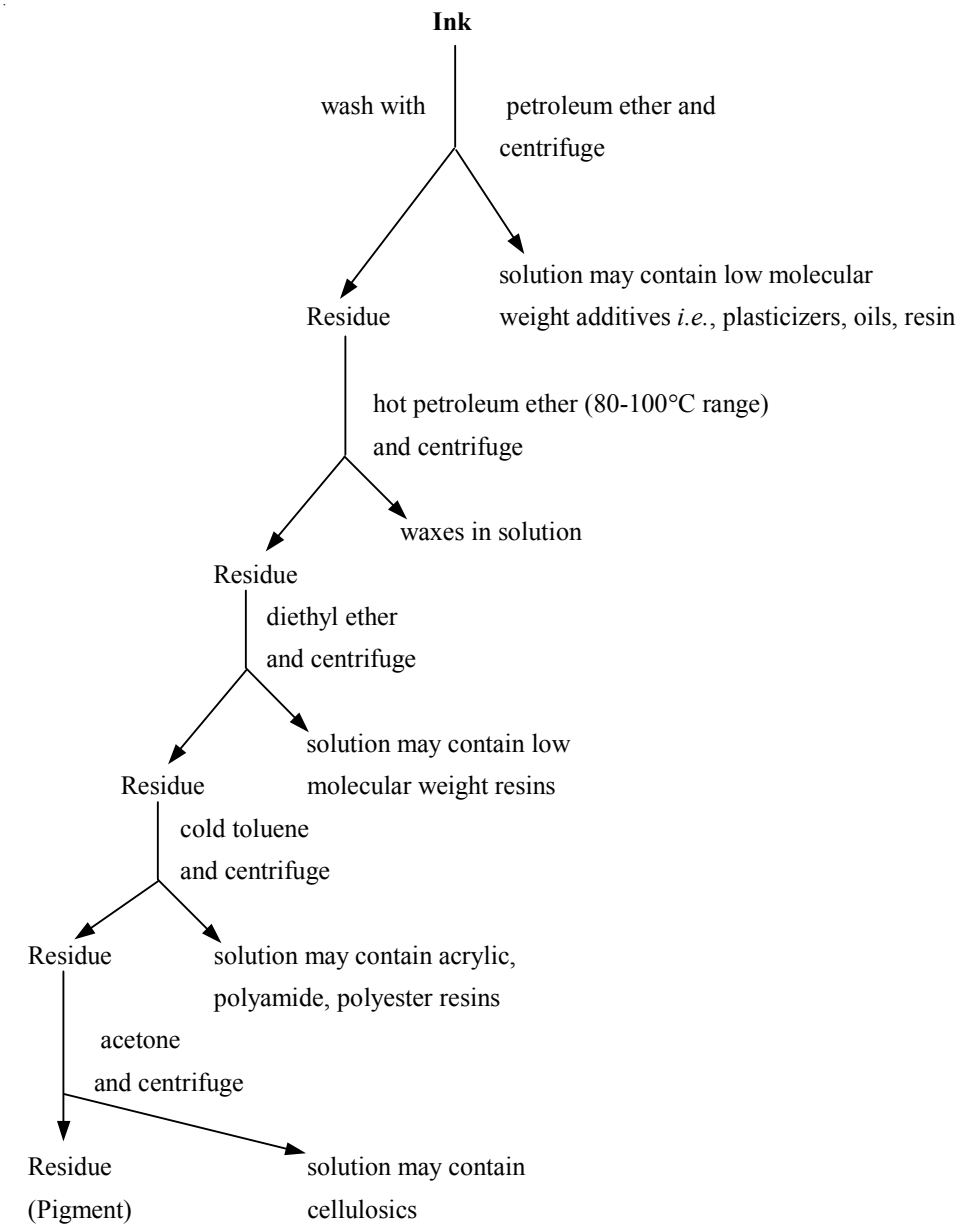


Fig. 3. Procedure applied for the pigment extraction from ink formulations with **PR2**, **PR5** and **PO5** (obtained from reference 2)

A TLC chromatogram which belongs to the same extracts is presented in Fig. 7. As shown, the pigments in ink samples can be isolated with these methods.

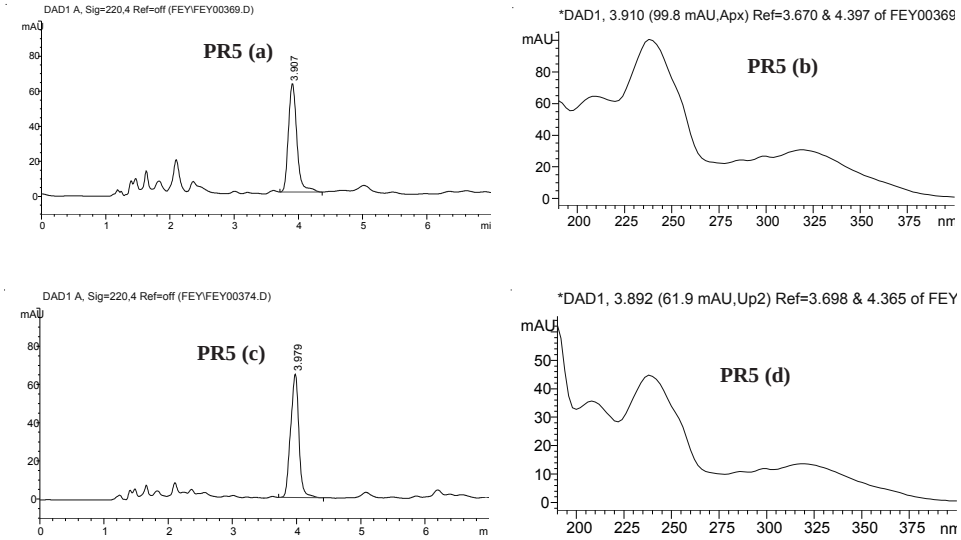


Fig. 4. (a) An HPLC chromatogram of authentic **PR5** and (b) its UV spectrum (c) A chromatogram obtained following extraction from a solvent based serigraphy ink with **PR5** (d) its UV spectrum

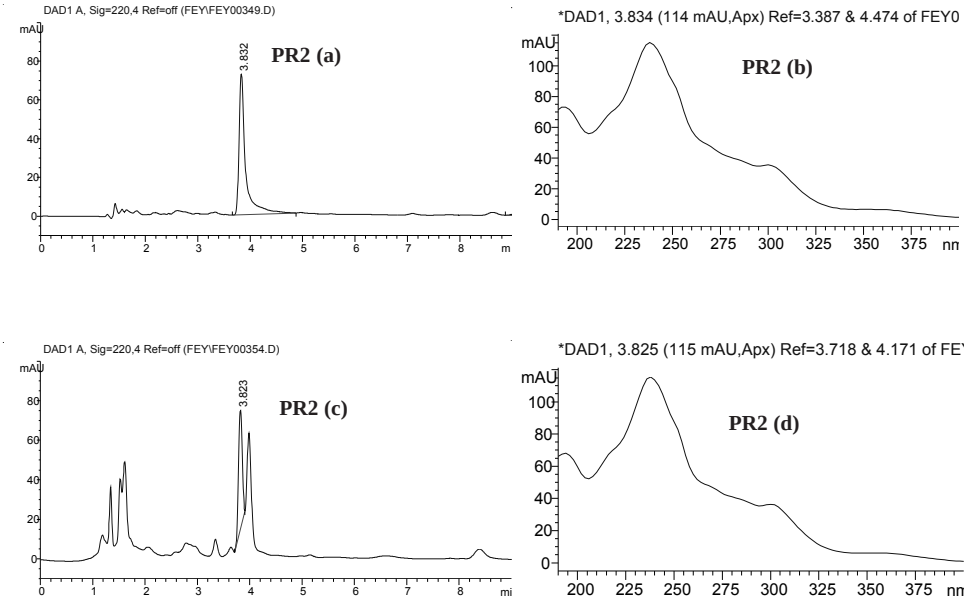


Fig. 5. (a) An HPLC chromatogram of authentic **PR2** and (b) its UV spectrum (c) A chromatogram obtained following extraction from a water based flexo ink with **PR2** (d) its UV spectrum

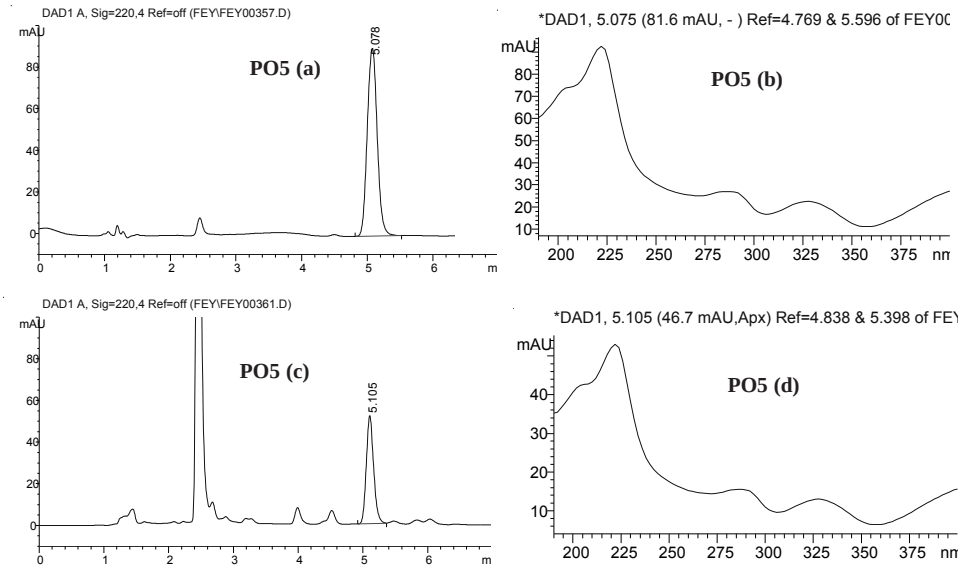


Fig. 6. (a) An HPLC chromatogram of authentic **PO5** and (b) its UV spectrum (c) A chromatogram obtained following extraction from a solvent based flexo ink with PO5 (d) its UV spectrum

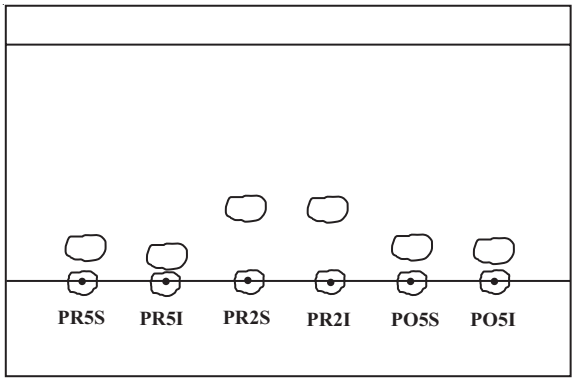


Fig. 7. TLC chromatogram of authentic **PR5**, **PR2**, **PO5** and following extraction from ink samples with **PO5** (d) its UV spectrum **PR5**, **PR2** and **PO5** (S = standard; I = ink; Solvent system: S3)

RESULTS AND DISCUSSION

In studies reported in the literature, reverse-phase HPLC systems and mobile phases including buffer solutions with the gradient elution were mostly used for the analysis of azo dyes[15-20]. In the present study, all the dyes and pigments and their reduction/degradation products were separated using a simple isocratic reverse-phase HPLC system with a mobile phase without any buffer solution. The compounds were eluted in reasonable retention times.

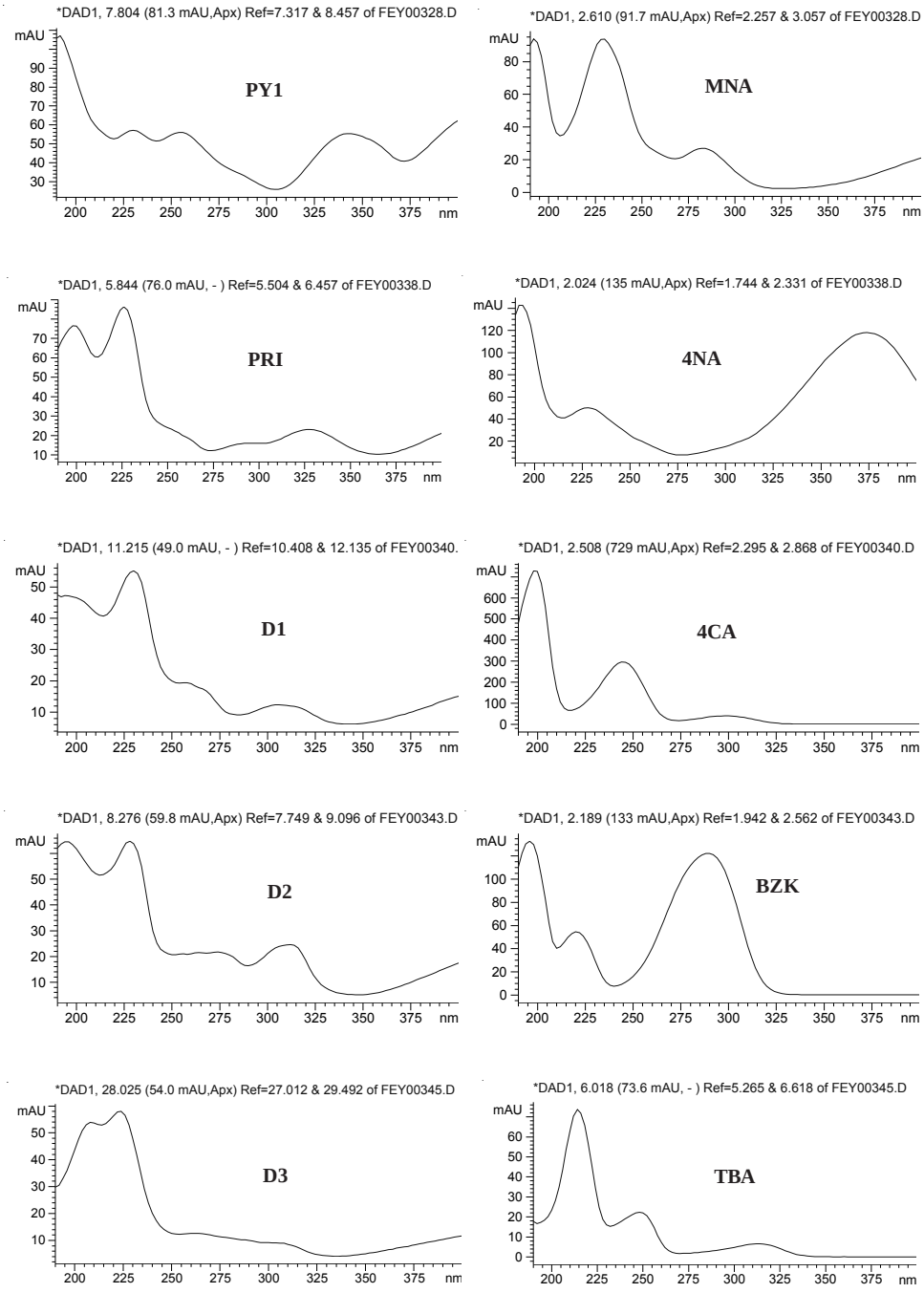


Fig. 8. UV spectra of **PY1**, **PR1**, **D1**, **D2**, **D3** and their potential degradation products **MNA**, **4NA**, **4CA**, **BZK** and **TBA**

In the studies reported previously, the HPLC analyses of azo dyes were carried out using gradient HPLC systems with the buffered mobil phases[15-20]. In the present study, a very fast isocratic method without any buffer system was developed for the dyes and pigments. On the other hand, all compounds were recorded with reasonable TLC $R_f \times 100$ values and HPLC retention times (Table-2). In addition, these compounds were able to be separated from their potential degradation/reduction products, primary aromatic amines (where the authentic standards were available). The recovered pigments by solvent extraction from commercially available 3 ink samples (**PR2**, **PR5** and **PO5**) were also detected by these systems.

When the potential risks caused by azo group of dyes and pigment in the organism or in the production stage and on the shelf are considered, the importance of investigation of these rapid, sensitive and reliable detection methods for such compounds could be understood. The present separation methods provide compositional information and these can easily be used for ink analysis in the forensic identifications.

REFERENCES

1. C.W. Maynard Jr., in ed.: J.A. Kent, Dye Application, Manufacture of Dye Intermediates and Dyes', Riegel's Handbook of Industrial Chemistry, Van Nostcand Reinhold, New York, pp. 809-61 (1983).
2. M. Clayton, in eds.: R.H. Leach, C. Armstrong, J.F. Brown, M.J. Mackezie, L. Randall and H.G. Smith, Raw materials, The Printing Ink Manual, VNR International, Berkshire, England, edn. 4, Ch. 4, pp. 109-281 (1988).
3. NPIRI Raw Materials Data Handbook, Vol. 4, Pigments, 3-19 National Printing Ink Research Institute (1983).
4. M. Chen, D. Moir, F.M. Benoit and C . Kubwabo, *J. Chromatogr.*, **825**, 37 (1998).
5. K.T. Chung, *Mutation Res.*, **114**, 269 (1983).
6. W.G. Levine, *Drug Metabol. Rev.*, **23**, 253 (1991).
7. P. Moller and H. Wallin, *Mutation Res.*, **462**, 13 (2000).
8. S. Tsuda, N. Matsusaka, H. Madarame, S. Ueno, N. Suna, K. Ishida, N. Kawamu, K. Sekihashi and Y.F. Sasaki, *Mutation Res.*, **465**, 11 (2000).
9. L. Sztandera, A. Garg, S. Hayik K.L. Bhat and C.W. Bock, *Dyes Pigments*, **59**, 117 (2003).
10. S. Nam and V. Renganathan, *Chemosphere*, **40**, 351 (2000).
11. B. Renger, Thin Layer (Planar) Chromatography' In: Encyclopedia of Separation Science (ed) I:D: Wilson, Vol. 8, pp. 3754-60 (2001).
12. A.C. Moffat, Thin-Layer Chromatography' In: Clarke's Isolation and Identification of Drugs, Second Edition-Edits.: A.C., pp. 160-7 (1988).
13. S. Lindsay, High Performance Liquid Chromatography Analytical Chemistry by Open Learning, John Wiley & Sons, Chichester (1987).
14. R. Gill, High Pressure Liquid Chromatography, In: Clarke's Isolation and Identification of Drugs in Pharmaceutticals, Body Fluids and Post-Mortem Material.edits, pp. 201-20 (1986).
15. J.A. Zlotnick and F.P. Smith, *J. Chromatogr.*, **733**, 265 (1999).
16. M. Perez-Urquiza, M.D. Prat and J.L. Beltran, *J. Chromatogr. A*, **871**, 227 (2000).
17. E. Seclaman, A. Sallo, F. Elenes, C. Crasmareanu, C. Wikete, S. Timofei and Z. Simon, *Dyes Pigments*, **55**, 69 (2002).
18. A. Rehorek, K. Urbig, R. Meurer, C. Schafer, A. Plum and G. Braun, *J. Chromatogr. A*, **949**, 263 (2002).
19. C. Baiocchi, M.C. Brussino, E. Pramauro, A.B. Prevot, L. Palmisano and G. Marci, *Int. J. Mass Spectrom.*, **214**, 247 (2002).
20. A. Pielesz, I. Baranowska, A. Rybak and A. Wlochowicz, *Ecotoxicol. Environ. Safety*, **53**, 42 (2002).

(*Received*: 1 November 2008; *Accepted*: 23 May 2009) AJC-7593