Characterization of Amines by Fast Black K Salt in Thin-layer Chromatography

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Amines were characterized on a silica gel thin-layer chromatographic (TLC) plate with the diazonium reagent Fast Black K salt (FBK) and with subsequent novel procedures: acid treatment or treatment with *N*-(1-naphthyl)ethylenediamine in acid solution. The differentiation of primary, secondary and tertiary aliphatic and aromatic amines was demonstrated, with special attention to drug substances. By using the *N*-(1-naphthyl)ethylenediamine treatment a 5-fold improvement in the detection limits for aliphatic secondary amines was achieved compared with FBK alone, allowing detection of 0.01 μg of methamphetamine and 0.04 μg of methyl phenidate. The structures of the coloured reaction products were elucidated by spectroscopic and TLC methods. An unexpected reaction was observed with dialkylanilines, which reacted by *N*-coupling with various diazonium salts with cleavage of an alkyl group.

Keywords: *Fast Black K salt; thin-layer chromatography; amines; diazo coupling; forensic analysis*

Thin-layer chromatography (TLC) possesses a unique advantage among chromatographic techniques in allowing post-chromatographic derivatization of immobilized analytes. This makes possible the sequential use of a wide variety of derivatization reagents. A sample can also be run on several parallel tracks of the TLC plate and each track can then be treated with different reagents. The combined information obtained from a retardation factor (R_F) and from colour reactions provides a powerful means for analyte characterization that can be superior to more expensive instrumental techniques.

In a recent paper,[1] we showed the ability of a diazonium reagent, Fast Black K salt (FBK), to differentiate between sub-microgram amounts of aliphatic amines by colour and elucidated the structures of the coloured products. Primary amines form a violet and secondary amines an orange–red or red product, whereas tertiary amines do not react. However, differentiation of the respective types of arylamines and also aliphatic amines from arylamines was found to be more difficult.

This paper describes improved differentiation methods for aliphatic amines and also shows that aromatic amines and phenols are readily discerned by the novel FBK techniques. Drug substances are included to demonstrate the value of these methods in drug analysis, particularly in toxicological drug screening.

Experimental

Materials

Diazonium salts FBK [2,5-dimethoxy-4-(4-nitrophenylazo)-benzenediazonium chloride hemi(zinc chloride), purity 30%] (**1a**) and Fast Red B salt (2-methoxy-4-nitrobenzenediazonium tetrafluoroborate, 95%) (**1b**) were purchased from Aldrich (Milwaukee, WI, USA), and Fast Red GG salt (4-nitrobenzenediazonium tetrafluoroborate, 90%) (**1c**) was from Sigma (St. Louis, MO, USA). The other diazonium salts 4-nitrobenzenediazonium chloride (**1d**), benzenediazonium chloride (**1e**), 2-methoxybenzenediazonium chloride (**1f**), 4-methoxybenzenediazonium chloride (**1g**) and 2,5-dimethoxybenzenediazonium chloride (**1h**) were prepared by diazotization of the corresponding anilines as follows: to the cold solution of 0.3 mmol of the amine in 6 cm^3 of 0.2 mol dm^{-3} HCl (1.2 mmol), 207 mm^3 of cold 10% $NaNO_2$ (0.3 mmol) solution were added, and the diazonium reagent was used immediately.

N-(1-Naphthyl)ethylenediamine dihydrochloride (98%) (**2**) was from Merck (Darmstadt, Germany).

Coupling components (Table 1) **5a–b**, **6b**, **6d**, **6h**, **6k**, **7b**, **7e–f** and **8d** were from Aldrich (Steinheim, Germany, and Milwaukee, WI, USA), **6i** was from BDH (Poole, Dorset, UK), **6f–g** and **8e** were from Fluka (Buchs, Switzerland), **3c** and **6j** were from Merck, and **3d** was from Sigma. *N*-Ethyl-*p*-toluidine (**7c**) and *N*-ethyl-2,4,6-trimethylaniline (**7d**) were synthesized from the corresponding anilines,[2] separated from impurities by preparative TLC, with use of an automatic TLC sampler and the mobile phase I, and extracted from the sorbent into dichloromethane–methanol (1 + 1).

The other coupling components and chemicals used have been described previously.[1] All coupling components were of 97% or higher purity, except for **7b** (96%) and **8d** (95%). The drug substances (Table 1) **3e–h**, **4c–f**, **6l–o** and **7g** were obtained from various pharmaceutical companies and they were of pharmaceutical purity.

The TLC plates were of glass coated with a 0.25 mm layer of silica gel 60 F_{254} (Merck).

Apparatus

The automatic TLC sampler was an ATS III from Camag (Muttenz, Switzerland) and the scanning densitometer was a TLC Scanner II, also from Camag. The high-performance liquid chromatography (HPLC), ultraviolet/visible spectrophotometry (UV/VIS), proton nuclear magnetic resonance spectrometry (^{1}H NMR) and mass spectrometry (MS) instrumentation have been described previously.[1]

Thin-layer Chromatography

Sample preparation

Methanolic or aqueous methanolic solutions containing 2 mg cm^{-3} each of the coupling components were prepared. For the investigation of the colour reactions on the TLC plate (Table 1), 1 mm^3 of each solution was manually applied to the plate, and the visualization procedures were carried out without prior development. For the detection limit studies, dilutions of the 2 mg cm^{-3} solutions were carried out.

Visualization methods

The plates were sprayed lightly, using a Desaga (Heidelberg, Germany) test-tube atomizer, with a filtered 0.5% m/v

Table 1 Colour reactions of coupling components with Fast Black K salt on silica gel TLC plates*

Substance	FBK	FBK + NaOH	FBK + NaOH + HCl	FBK + NaOH + NEDA†/HCl
Aliphatic primary amines—				
3a Ethylamine hydrochloride	Light red	Violet	Ochre	Ochre
3b 2-Phenylethylamine hydrochloride	Light red	Violet	Ochre	Ochre
3c Ethylenediamine hydrochloride	Light red	Red–violet	Ochre	Ochre
3d Putrescine hydrochloride	Light red	Violet	Ochre	Ochre
3e Amantadine hydrochloride	Light red	Violet	Ochre	Ochre
3f Amphetamine sulfate	Light red	Violet	Ochre	Ochre
3g Phentermine hydrochloride	Light red	Violet	Ochre	Ochre
3h Tocainide hydrochloride	Light red	Violet	Ochre	Ochre
Aliphatic secondary amines—				
4a Diethylamine hydrochloride	Orange–red	Orange–red	Violet → cream	Blue
4b *N*-Methyl-2-phenylethylamine	Orange–red	Orange–red	Violet → cream	Blue
4c Cyclopentamine hydrochloride	Orange–red	Orange–red	Violet → cream	Blue
4d Fluoxetine hydrochloride	Orange–red	Orange–red	Violet → cream	Blue
4e Methamphetamine hydrochloride	Orange–red	Orange–red	Violet → cream	Blue
4f Prilocaine hydrochloride	Orange–red	Orange–red	Violet → cream	Blue
Poly(amines)—				
5a Spermidine	Red	Red	Ochre	Blue–green
5b Spermine	Red	Orange–red	Ochre	Blue
Aromatic primary amines—				
6a Aniline	Red	Red–violet	Ochre	Brown–violet
6b *m*-Toluidine	Red	Red–violet	Brown	Brown–violet
6c *p*-Toluidine	Red	Red–violet	Ochre	Brown–violet
6d 2,4-Dimethylaniline	Red	Red–violet	Ochre	Ochre
6e 2,4,6-Trimethylaniline	Red	Red–violet	Ochre	Ochre
6f *o*-Anisidine	Red	Red–violet	Brown	Brown
6g *p*-Anisidine	Red	Red–violet	Red–brown	Brown
6h 2,5-Dimethoxyaniline	Intense violet	Intense violet	Intense blue–violet	Intense blue–violet
6i 4-Nitroaniline	Yellow	Green–yellow	Violet → ochre	Brown–violet
6j 4-Aminobenzoic acid	Red	Red	Violet → ochre	Violet
6k Ethyl 4-aminobenzoate	Red	Red	Violet → ochre	Violet
6l Carbutamide	Light red	Red	Violet → ochre	Brown–violet
6m Nomifensine maleate	Light red	Red	Violet → ochre	Brown–violet
6n Procainamide hydrochloride	Red	Red	Violet → ochre	Brown–violet
6o Procaine hydrochloride	Red	Red	Violet → ochre	Brown–violet
Aromatic secondary amines—				
7a *N*-Ethylaniline	Orange–red	Orange–red	Violet → green	Blue
7b *N*-Ethyl-*m*-toluidine	Red–violet	Brown–violet	Violet → blue–violet	Blue
7c *N*-Ethyl-*p*-toluidine	Orange–red	Orange–red	Violet → cream	Blue
7d *N*-Ethyl-2,4,6-trimethylaniline	Orange–red	Orange–red	Violet → cream	Blue
7e *N*-Ethyl-4-nitroaniline	Yellow	Yellow	Yellow	Green
7f *N*-Methyl-4-aminobenzoic acid	Orange–red	Orange–red	Violet → cream	Blue
7g Amethocaine hydrochloride	Red	Red	Violet → cream	Blue
Aromatic tertiary amines—				
8a *N*,*N*-Dimethylaniline	Brown–red	Brown–red	Violet → green	Blue
8b *N*,*N*-Dimethyl-*p*-toluidine	Orange–red	Orange–red	Violet → cream	Blue
8c *N*,*N*-Dimethyl-2,4,6-trimethylaniline	Yellow	Yellow	Cream	Yellow
8d *N*-Ethyl-*N*-methylaniline	Brown–red	Brown–red	Violet → green	Blue
8e *N*,*N*-Diethylaniline	Violet	Violet	Blue–violet	Red–violet

* Amount of substance applied = 2 μg.
† *N*-(1-Naphthyl)ethylenediamine.

aqueous solution of **1a**, then dried briefly with a hot-air blower, sprayed generously with 0.5 mol dm^{-3} NaOH and dried thoroughly, prior to one of the following procedures.

Acid treatment. The plate was sprayed with 2 mol dm^{-3} HCl and dried.

Acid coupling treatment. The plate was sprayed with a 1% m/v solution of **2** in 0.5 mol dm^{-3} HCl and dried.

Structure elucidation by TLC

For the investigation of the **1a** derivatives formed on the plate, the TLC plate was developed directly after the following application sequence. With use of an automatic TLC sampler, 3 mm^3 each of solutions of the coupling components **3a–d**, **4a–b**, **5a–b**, **6a–k**, **7a–f** and **8a–e** were applied to separate tracks of the plate, followed by over-application with 2 mm^3 of 0.1 mol dm^{-3} NaOH and 5 mm^3 of the diazonium salt solution (with **1b–h**, only **8a–e** were studied). With **1a–c**, a 1% m/v filtered aqueous solution was used; with **1d–h** a 0.05 mol dm^{-3} solution (see above) was used.

The application was performed by spraying narrow bands of 6 mm length, and the plate was dried between the application stages. The development was carried out over a distance of 7 cm in a 20 × 10 cm double-trough developing chamber from Camag, with use of mobile phase I (Fig. 1). Separate plates were then submitted to the acid treatment and the acid coupling treatment (see above).

For the investigation of the acid or acid coupling products formed on the plate, the application procedure described above was continued by over-applying 5 mm^3 of 2 mol dm^{-3} HCl or 5 mm^3 of a 1% solution of **2** in 0.5 mol dm^{-3} HCl,

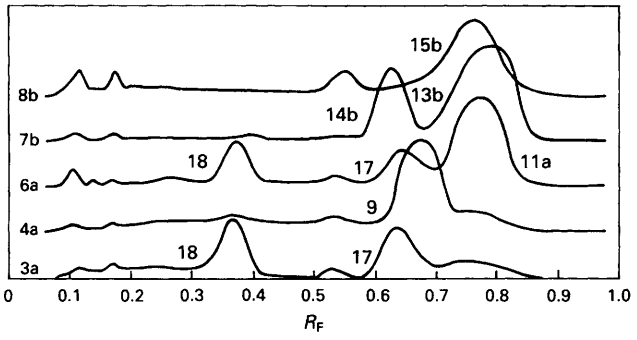


Fig. 1 Thin-layer chromatographic separation of FBK derivatives from aliphatic and aromatic amines. The pre-chromatographic reaction was carried out on a silica-gel plate by sequential application of a coupling component, NaOH and FBK, and developing with toluene–methanol (9 + 1). Absorbance detection was by reflectance at 500 nm

respectively, and the TLC plate was developed with mobile phase II. Additionally with **6a–k**, the spots obtained by spraying with the acid coupling reagent (Table 1) were scraped off, extracted with dichloromethane–methanol (1 + 1) and analysed with use of mobile phase II. After drying, the plate was sprayed with 2 mol dm^{-3} HCl to restore acidity for visual inspection.

The mobile phases were as follows:

Mobile phase I. Toluene–methanol (9 + 1).

Mobile phase II. Ethyl acetate–methanol–concentrated ammonia (80 + 15 + 5).

Structure Elucidation by Spectroscopy

Syntheses

For the preparation of **10**, 0.42 g of **1a** was dissolved in 20 cm^3 of water, and the filtered solution was added, with stirring, to a solution containing 1 mmol of **2** in 10 cm^3 of water. The precipitate formed was centrifuged, washed twice with 0.5 mol dm^{-3} HCl and suspended in 2 mol dm^{-3} NaOH. The suspension was extracted twice with dichloromethane, and the organic phase was evaporated to dryness. Yield: 134 mg.

The reaction between **1a** and the aromatic primary amines **6a–c**, the aromatic secondary amines **7a–b** and the aromatic tertiary amines **8a–b** was carried out in solution as follows: 0.42 g of **1a** was dissolved in 20 cm^3 of water, and the filtered solution was added, with stirring, to a solution containing 1 mmol of the amine in 2 cm^3 of 0.5 mol dm^{-3} HCl. Sodium hydroxide solution (2 mol dm^{-3}) was added dropwise, with stirring, until the solution was clearly alkaline (pH >9), and the reaction vessel was allowed to stand in ice for 10 min. The precipitate formed was centrifuged, washed twice with cold water and dried in air. Yields: 94 mg (from **6a**), 119 mg (**6b**), 110 mg (**6c**), 88 mg (**7a**), 124 mg (**7b**), 75 mg (**8a**) and 122 mg (**8b**).

For the preparation of **19**, 0.42 g of **1a** was dissolved in 20 cm^3 of water, and the filtered solution was made clearly alkaline (pH >9) with 2 mol dm^{-3} NaOH. The suspension formed was allowed to stand in an ultrasonic bath for 10 min, then it was centrifuged, and the supernatant phase was separated. Another basic fraction was obtained by suspending the precipitate in 2 mol dm^{-3} NaOH, sonicating and centrifuging. The combined supernatant phases were acidified with 2 mol dm^{-3} HCl, extracted twice with dichloromethane, and the organic phase was evaporated to dryness.

Purification

The products from **6–8** were separated and purified by preparative HPLC by collecting fractions from successive 0.5 cm^3 injections of 5 mg cm^{-3} solutions prepared in the mobile phase or in toluene–methanol (9 + 1). The mobile phase was toluene–methanol (99.9 + 0.1). The flow rate was 2.5 cm^3 min^{-1}, and the analytes were monitored at 480 nm.

The product **19** was separated from impurities by preparative TLC, with use of an automatic TLC sampler and mobile phase II, and extracted from the sorbent into dichloromethane–methanol (1 + 1).

The purified products were compared for identity with those formed on the TLC plate, by use of TLC with mobile phase I.

Spectroscopic analyses

Proton NMR spectra (200 MHz) were recorded in $CDCl_3$. Mass spectra were recorded with an electron energy of 70 eV, unless stated otherwise, *via* a direct inlet probe at 100–250 °C. The UV/VIS spectra were recorded in methanol.

N-{4-[2,5-*Dimethoxy*-4-(4-*nitrophenylazo*)*phenylazo*]-1-*naphthyl*}*ethylenediamine* (**10**). ^{1}H NMR: δ (ppm) 3.19 (2 H, quintet, CH_2NH_2), 3.47 (2 H, q, CH_2NH), 4.10 (3 H, s, CH_3O), 4.16 (3 H, s, CH_3O), 5.98 (1 H, t, NH), 6.69 (1 H, d, aromatic H *ortho* to $NHC_2H_4NH_2$, J = 8.9 Hz), 7.5–8.1 (4 H, m, aromatic H), 7.55 (1 H, s, aromatic H^a), 7.64 (1 H, s, aromatic H^b), 8.06 (2 H, d, aromatic H^c, J = 9.1 Hz), 8.15 (1 H, d, aromatic H *meta* to $NHC_2H_4NH_2$, J = 8.8 Hz), 8.37 (2 H, d, aromatic H^d, J = 9.1 Hz). MS (19 eV): *m/z* 499 (3%) [M^+], 456 (9) [$M - C_2H_3NH_2$], 426 (7), 322 (8), 302 (86), 272 (38), 195 (100).

4-[2,5-*Dimethoxy*-4-(4-*nitrophenylazo*)*phenylazo*]-N-*nitrosobenzenamine* (**19**). ^{1}H NMR: δ (ppm) 3.85 (3 H, s, CH_3O), 4.01 (3 H, s, CH_3O), 6.01 (1 H, s, aromatic H^a), 6.38 (1 H, s, aromatic H^b), 7.33 (2 H, d, aromatic H^c, J = 9.2 Hz), 8.26 (2 H, d, aromatic H^d, J = 9.2 Hz), 11.4 (1 H, br s, NH). MS: *m/z* 331 (4%) [M^+], 303 (100) [$M - N_2$], 181 (35), 151 (33), 138 (62), 123 (83).

The spectral data for **11a–c**, **12b**, **13a–b**, **14a–b**, **15a–b** and **16a** are presented in Tables 2 and 3.

Results and Discussion

Table 1 shows the behaviour of the coupling components (**3–8**) on the silica gel TLC plate in the FBK (**1a**) visualization and in the subsequent acid or acid coupling treatment. The colours obtained for various types of amines are summarized in Table 4. The acid coupling reaction with aliphatic secondary amines (**4**) is shown in Scheme 1. The structures of the synthesized reaction products from aromatic amines (**11–16**) are shown in Scheme 2. Fig. 1 demonstrates the TLC separation of the **1a** derivatives from selected coupling components after a reaction on the plate.

Aliphatic Primary Amines

Aliphatic primary amines (**3**) react with **1a** to produce violet spots on the TLC plate, the main coloured components being a violet 1,3-diaryltriazene (**17**) and a red primary arylamine (**18**), both derived exclusively from **1a**.[1]

Ar–N=N–NH–Ar
17

$Ar–NH_2$
18

Both the acid treatment and the acid coupling treatment produced an ochre colour. The colour change was due to **18**, which in acidic medium turned to ochre yellow, probably because of protonation of the amino and/or the azo group.[3] The compound was formed also from **17** with acid. In the acid coupling, some regeneration of **1a** from **17** and subsequent coupling with **2** with formation of a blue–green product (see below) was observed, but it did not have a contribution to the over-all ochre colour.

Table 2 ^{1}H NMR and MS data for triazenes from FBK and arylamines*

Compound No.	d	c	b	a	e	f	g	OMe	Ph–Me	NH, NH_2	Aliphatic	*m/z* (%)
11a†	8.35, 2 H, d, *J* = 9.1	7.99, 2 H, d, *J* = 9.1	7.49, 1 H, s	7.42, 1 H, s	7.62, 2 H, d, *J* = 8.2	7.33–7.52, 3 H, m		4.12, 3 H, s 3.97, 3 H, s		10.07 1 H, br s, NH		328(27), 302(48), 288(100), 151(29), 138(81), 122(40)
11b‡	8.36, 2 H, d *J* = 8.1	7.99, 2 H, d *J* = 8.2	7.49. 1 H, s	7.42, 1 H, s	7.12–7.54, 4 H, m			4.13, 3 H, s 3.97, 3 H, s	2.45 3 H, s	10.06 1 H, s, NH		420(3) [M^+], 392(75) [M − N_2], 362(21), 302(100), 137(43), 122(81)
11c§	8.35, 2 H, d, *J* = 8.9	7.98, 2 H, d, *J* = 8.9	7.49, 1 H, s	7.40, 1 H, s	7.54, 2 H, d, *J* = 8.3	7.26, 2 H, d, *J* = 8.2		4.12, 3 H, s 3.96 3 H, s	2.42, 3 H, s	10.01, 1 H, s, NH		405(18), 360(84), 302(57), 288(100), 195(82), 138(80)
13a¶	8.37, 2 H, d *J* = 9.1	8.03, 2 H, d *J* = 9.1			7.30–7.60, 7 H, m			4.08, 3 H, s and 3.99 3 H, s			4.45, 2 H, q, CH_2 and 1.39 3 H, t, CH_3	434(100) [M^+], 406(12) [M − N_2], 302(25), 148(73), 135(35) 120(92)
13b‖	8.32, 2 H, d, *J* = 9.0	7.98, 2 H, d, *J* = 9.0	7.45, 1 H, s	7.30, 1 H, s	7.21–7.26, 4 H, m			4.03, 3 H, s 3.94, 3 H, s	2.39, 3 H, s		4.40, 2 H, q, CH_2 and 1.33, 3 H, t CH_3	448(5) [M^+], 420(77) [M − N_2], 405(15), 314(42), 135(41), 120(100)
15a**	8.37, 2 H, d, *J* = 8.9	8.03, 2 H, d, *J* = 8.9		7.42–7.55, 4 H, m		7.23–7.31, 3 H, m		4.08, 3 H, s 4.01, 3 H, s			3.81, 3 H, s, CH_3	420(10) [M^+] 392(100) [M − N_2], 314(42), 287(52), 151(51), 122(86)
15b††	8.37, 2 H, d, *J* = 9.0	8.02, 2 H, d, *J* = 9.0	7.50, 1 H, s	7.29, 1 H, s	7.41, 2 H, d, *J* = 8.6	7.25, 2 H, d, *J* = 8.6		4.07, 3 H, s 4.01, 3 H, s	2.40, 3 H, s		3.79, 3 H, s, CH_3	434(3) [M^+], 406(100) [M − N_2], 302(27), 287(100), 151(85), 122(100)

* δ_H ppm, *J* = Hz.
† 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-phenyltriazene.
‡ 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-(*m*-tolyl)triazene.
§ 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-(*p*-tolyl)triazene.
¶ 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-ethyl-3-phenyltriazene.
‖ 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-ethyl-3-(*m*-tolyl)triazene.
** 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-methyl-3-phenyltriazene.
†† 1-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenyl]-3-methyl-(*p*-tolyl)triazene.

Aliphatic Secondary Amines

Aliphatic secondary amines (**4**) react with **1a** to produce orange–red 1-aryl-3,3-dialkyltriazenes (**9**)[1] (Scheme 1). The acid treatment generated a transient violet colour that soon turned to a cream colour. The colour was tentatively assigned to a yellow compound (**19**) (λ_{max} 447 nm), which can exhibit characteristics of a diazohydroxide and an *N*-nitrosamine.

$$\text{Ar–N=N–OH} \rightleftharpoons \text{Ar–NH–N=O}$$
19

The acid coupling treatment resulted in a blue product that formed in a few minutes. The colour, which was more blue–green than blue in weaker spots was found to be due to the 4-azo coupling product (**10**) (λ_{max} 588 nm) of **1a** and **2** (Scheme 1). The coupling component (**2**) has previously been

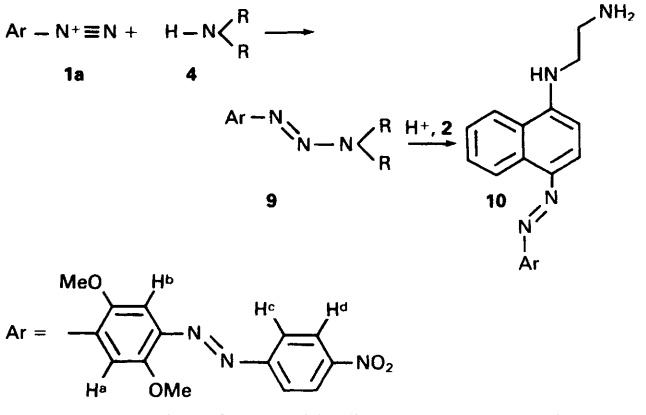
Table 3 ^{1}H NMR and MS data for aminoazo compounds from FBK and arylamines*

Compound No.	d	c	b	a	e	f	g	OMe	Ph–Me	NH, NH_2	Aliphatic	*m/z* (%)
12b†	8.38, 2H, d, J = 9.1	8.06, 2H, d, J = 9.2	7.52, 1H, s	7.44, 1H, s	7.76, 1H, d, J = 8.5	6.60, 1H, s	6.57, 1H, d, J = 8.5	4.08, 3H, s 4.06, 3H, s	2.72, 3H, s	4.12 2H, s, NH_2		420(75) [M^+], 390(21), 368(8), 134(47), 121(15), 106(100)
14a‡	8.39, 2H, d, J = 8.8	8.05, 2H, d, J = 8.8	7.51, 1H, s	7.47, 1H, s	7.92, 2H, d, J = 8.6	6.67, 2H, d, J = 8.8		4.10, 3H, s 4.06, 3H, s		4.25, 1H, br t, NH	3.30, 2H, quint, CH_2 and 1.33, 3H, t, CH_3	434(63) [M^+], 252(5), 207(5),, 148(73), 135(38), 120(100)
14b§	8.38, 2H, d, J = 8.0	8.05, 2H, d, J = 8.0	7.51, 1H, s	7.45, 1H, s	7.81, 1H, d, J = 9.0	6.49, 1H, s	6.46, 1H, d	4.08, 3H, s 4.06, 3H, s	2.73, 3H, s	4.20, 1H, br t, NH	3.28, 2H, quint CH_2 and 1.31, 3H, t, CH_3	448(88) [M^+], 418(34), 368(20), 314(23), 162(39), 134(100),
16a¶	8.38, 2H, d, J = 8.9	8.05, 2H, d, J = 8.9	7.52, 1H, s	7.49, 1H, s	7.96, 2H, d, J = 9.0	6.77, 2H, d, J = 9.0		4.11, 3H, s 4.06, 3H, s			3.14, 6H, s, $(CH_3)_2$	434(100) [M^+] 404(5), 217(6), 148(74), 135(35), 120(93)

* δ_H ppm, J = Hz.
† 4-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenylazo]-3-methylbenzenamine.
‡ 4-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenylazo]-*N*-ethylbenzenamine.
§ 4-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenylazo]-*N*-ethyl-3-methylbenzenamine.
¶ 4-[2,5-Dimethoxy-4-(4-nitrophenylazo)phenylazo]-*N*,*N*-dimethylbenzenamine.

Table 4 Simplified scheme of colours obtained for various types of amines with FBK and with the subsequent acid or acid coupling treatment

	FBK + NaOH	FBK + NaOH + HCl	FBK + NaOH + NEDA/HCl
Aliphatic primary amines	Violet	Ochre	Ochre
Aliphatic secondary amines	Orange–red	Cream	Blue
Aliphatic tertiary amines	No colour	—	—
Aromatic primary amines	Red–violet or red	Ochre or brown	Brown–violet
Aromatic secondary and tertiary amines	Orange–red	Cream or green	Blue



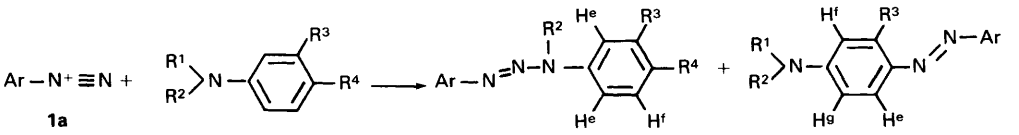
Scheme 1 Reaction of FBK with aliphatic secondary amines and subsequent coupling with *N*-(1-naphthyl)ethylenediamine (**2**)

used in the determination of sulfonamides and primary arylamines[4] and also of nitrite[5] by diazotization and coupling. On the other hand, the fact that 3,3-dialkyltriazenes in acidic medium behave like diazonium salts was already shown in the last century,[6] and this has been utilized in *Rapidogen*-type dyes.[7] However, the visualization of amines in TLC by use of the cleavage/coupling reaction is novel.

Poly(amines) showed characteristics of both primary and secondary amines as was expected. Visualization with **1a** and with the acid coupling treatment suggested a secondary amine structure, whereas the acid treatment suggested a primary amine structure.

Aromatic Primary Amines

Aromatic primary amines (**6**) were found to react with **1a** by *N*-coupling to produce red triazenes (**11**) (λ_{max} 485, 486 and 489 nm for **11a–c**, respectively) and by *C*-coupling to produce violet aminoazo compounds (**12**) (λ_{max} 535 nm for **12b**). *N*-Coupling was clearly favoured on the TLC plate, but in solution both *N*- and *C*-azo products formed. *C*-Coupling, predominantly at the *para*-position, was significant on the TLC plate only with compounds possessing ring-activating substituents in *meta*- or *ortho*-positions, especially with **6h**, but also with **6b** and to a lesser extent with **6f**. The compounds with ring-deactivating substituents (**6i–k**) reacted more weakly, and exclusively by *N*-coupling on the TLC plate. In



	R^1	R^2	R^3	R^4
6a	H	H	H	H
6b	H	H	Me	H
6c	H	H	H	Me
7a	H	Et	H	H
7b	H	Et	Me	H
8a	Me	Me	H	H
8b	Me	Me	H	Me

	R^2	R^3	R^4
11a	H	H^f	H^g
11b	H	Me	H^g
11c	H	H^f	Me
13a	Et	H^f	H^g
13b	Et	Me	H^g
15a	Me	H^f	H^g
15b	Me	H^f	Me

	R^1	R^2	R^3
12b	H	H	Me
14a	H	Et	H^e
14b	H	Et	Me
16a	Me	Me	H^e

Scheme 2 Reaction of FBK with arylamines

particular, the reactivity of the 4-nitro derivative (**6i**) was so weak that its native yellow colour remained dominant. Small amounts of **17** and **18** were formed from compounds with activating substituents (**6a–g**, but not **6h**).

The acid treatment produced, in most instances, an ochre colour, due to **18**, which formed in the degradation of the triazenes (**11**). The violet aminoazo derivatives (**12**) remained intact, but turned to blue–violet, which contributed to the colours from **6b**, **6f**, **6g** and **6h**. With compounds possessing ring-deactivating substituents, a violet colour developed first and turned then to ochre.

The acid coupling treatment produced variations of a brown–violet colour, consisting of an ochre yellow (**18**), a blue (**12**), and a blue–violet (from **6a–c**), a blue (from **6g**) or a violet (from **6j–k**) component. The latter components, which all had a yellow colour in basic medium, were probably from *C*-coupling between **2** and the diazonium salt from **6**. The compound **10** was not observed in the spots removed from the plate. However, if a pre-chromatographic derivatization procedure was used, **10** was observed because of an excess of intact **1a**.

Aromatic Secondary Amines

Aromatic secondary amines (**7**) were found to react with **1a** by *N*-coupling to produce red triazenes (**13**) (λ_{max} 482 and 489 nm for **13a–b**, respectively) and by *C*-coupling to produce violet aminoazo compounds (**14**) (λ_{max} 544 and 599 nm for **14a–b**, respectively). Again, *N*-coupling was favoured on the TLC plate, but in solution both *N*- and *C*-azo products formed. *C*-Coupling was significant on the TLC plate only with **7b**. The 4-nitro derivative (**7e**) was so unreactive that its native yellow colour remained dominant.

In the acid or acid coupling treatment the spots appeared similar to those obtained from aliphatic secondary amines. However, in the acid treatment, a different behaviour was observed with compounds capable of *C*-coupling. Because of small amounts of **14** present, a green shade (**7a**) or even a blue colour (**7b**) developed.

Aromatic Tertiary Amines

Aromatic tertiary amines (**8**) were found to react with **1a** by *C*-coupling to produce violet aminoazo compounds (**16**) (λ_{max} 546 nm for **16a**), as expected, but also by *N*-coupling, with a cleavage of an alkyl group, producing red triazenes (**15**) (λ_{max} 480 and 488 nm for **15a–b**, respectively). Again, *N*-coupling was favoured on the TLC plate, but in solution both *N*- and *C*-azo products formed.

This type of substitution reaction has previously been observed only under special conditions. Penton and Zollinger[8] observed the substitution of a methyl group of *N,N*-dimethylaniline by 4-methoxybenzenediazonium tetrafluoroborate in anhydrous acetonitrile. Colonna *et al.*[9] reported a similar mono-demethylation of 4-acetyl-*N,N*-dimethylaniline with 4-nitrobenzenediazonium chloride in aqueous solution, while no such reaction was observed with the other five *N,N*-dimethylanilines studied.

The acid or acid coupling treatment resulted in colours similar to those obtained with aromatic secondary amines, as was expected.

The *N*-coupling reaction of dialkylanilines on the TLC plate with different diazonium salts is of a general nature. *N,N*-Dimethyl-*p*-toluidine (**8b**) was the most reactive, followed by *N,N*-dimethylaniline (**8a**) and *N*-ethyl-*N*-methylaniline (**8d**). With **8d** it was the methyl group that was substituted. *N,N*-Diethylaniline (**8e**) reacted poorly, indicating that ethyl groups are not substituted as easily as methyl groups. *N,N*-Dimethyl-2,4,6-trimethylaniline (**8c**) was fairly unreactive, probably because of steric hindrance due to the *ortho*-methyl groups. Significant *C*-coupling was observed with **8e** and **8d**. An exact comparison of the reactivities of the diazo components (**1**) was not possible, but it appeared that the more electrophilic 4-nitro-substituted diazonium salts (**1a–d**) produced more triazenes than the others. The behaviour of **1c** and **1d** was identical. The colours of the *C*-azo dyes after the acid coupling treatment from **1a–h** were greenish blue, violet, violet, violet, blue–violet, blue, blue and blue, respectively.

Spectral Considerations

The UV/VIS maxima and the observed colours indicate a clear difference between the red triazenes and the violet aminoazo compounds. An exception is the triazene **17**, which has a violet colour[1] because of extensive conjugation. The highly conjugated aminoazo compound **10** has a distinctive greenish blue colour.

Triazenes and aminoazo compounds can be readily differentiated by their ^{1}H NMR spectra. The 1,3-diaryltriazenes showed an NH signal at about 10 ppm, and the 3-alkyl-1,3-diaryltriazenes showed a CH_2N or a CH_3N signal at 4.4 and 3.8 ppm, respectively. The 4-aminoazo compounds, on the other hand, showed two high-field aromatic protons.

The mass spectra of the 1,3-diaryltriazenes **11a** and **11c** revealed extensive fragmentation. These compounds were also unstable when allowed to dry on the silica gel plate. The *m*-tolyl-substituted compound (**11b**), however, was more stable and showed a molecular ion of low abundance and an abundant [M − N_2] ion. The 3-alkyl-1,3-diaryltriazenes showed both an M^+ and an [M − N_2] ion in various proportions. The aminoazo compounds showed an abundant M^+ without an [M − N_2], in contrast to the triazenes.

Phenols and Heterocyclic Compounds

Phenols, resorcinols and heterocyclic compounds usually produce violet colours with **1a**.[1,10] These colours are apparently due to *C*-azo coupling products and turn to blue–violet,

similarly to the aminoazo compounds, in the acid or acid coupling treatment. Compounds possessing a phenolic hydroxy and an aliphatic amino group can produce mixed colours with **1a**,[10] but behave like phenols in the acid or acid coupling treatment.

Drug Analysis

With the acid coupling treatment, the identification power of TLC drug screening procedures can be significantly enhanced compared with use of **1a** alone. In particular, the differentiation of aromatic primary amines and phenols from each other and from other amines is now possible. To take some specific examples from a screening of amphetamine derivatives,[10] the differentiation of previously poorly resolved drugs such as amphetamine from nomifensine, chlorphentermine from labetalol and metoclopramide, fencamfamin from oxypertine, methoxyphenamine from carbutamide, and 3,4,5-trimethoxyamphetamine from pindolol is now possible by colour. Fortunately, there are few aromatic secondary amine drugs and the aromatic tertiary amine structures encountered in drugs (*e.g.*, imipramine) are generally unreactive with **1a**.

An improvement can also be obtained in the detection sensitivity for secondary amines by using the acid coupling treatment. Following the TLC method described previously,[1] detection limits of 0.01 and 0.04 μg were obtained for methamphetamine and methyl phenidate, respectively. These values are five times lower than those obtained with **1a** alone.[1] To obtain a high sensitivity, however, the visualization procedure described above should be strictly followed in order to decompose the excess of FBK, which causes a blue background, with NaOH and heat.

Conclusions

The sequential use of **1a** and one of the detection procedures described allows rapid, sensitive and inexpensive characterization of amines on the TLC plate. In particular, the structures often encountered in drug substances, *i.e.*, aliphatic primary, secondary and tertiary amines, aromatic primary amines, and phenols, can in most instances be readily differentiated from each other by utilizing the acid coupling treatment. The reaction of dialkylanilines with cleavage of an alkyl group was found to be of a more general nature than has been previously supposed. Pre-chromatographic derivatization on the TLC plate, with use of an automatic TLC sampler and subsequent chromatography, was found to be an ideal way of studying the present type of reaction. This method, involving the acid or acid coupling treatment, can also be used for the characterization of pure unknown amines.

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