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Trace determination of aromatic amines or phenolic compounds in dyestuffs by high-performance liquid chromatography with on-line preconcentration

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Abstract

Aromatic amines or phenolic compounds at ppb levels in water were determined by on-line preconcentration with a precolumn followed by high-performance liquid chromatography with UV absorption detection. This method was applied to the determination of aromatic amines or phenolic compounds at ppm levels in commercial dyestuffs. The dyestuff was dissolved in water and precleaned with an SAX cartridge packed with an anion-exchange resin; the effluent was then analysed using the proposed on-line preconcentration and determination method.

1. Introduction

The isolation and identification of phenolic compounds is of great importance owing to their wide applicability in pharmaceuticals, dyes, pesticides and foods. The impact of phenolic compounds on the environment is subject to increasing attention. The US Environmental Protection Agency (EPA) lists eleven substituted phenols as "priority pollutants" [1]. Much research is devoted to these phenolic substances [2–7] and many chromatographic separation and detection systems have been tested; however, the separation and determination of other toxic substituted phenols [8] have received little attention.

The carcinogenic activity of benzidine and several aromatic amines is well known [9]. These compounds were widely used as intermediates in the production of azo dyes and pigments [10,11].

Several methods have been developed for the determination of benzidine and related congeners [12–17]. High-performance liquid chromatography (HPLC) is generally regarded as the best technique for the determination of aromatic amines. A method frequently employed to determine aromatic amines in water-soluble dyes involves extraction of amines with chloroform, followed by diazotization of amines and coupling of diazonium salts with a reagent (R-salt or pyrazolone-T) to form a mixture of coloured products [18–20]. The products are then separated by HPLC and determined with UV absorption detection at 254 and 510 nm. However, the protracted and complicated procedures are not only tedious, which limits the number of samples that can be analysed, but also susceptible to contamination and loss of aromatic amines. Hence a simple and specific method is needed to determine aromatic amines in direct dyes.

In this work, a precolumn was used for the

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on-line enrichment of aromatic amines or phenolic compounds at ppb levels from water, with subsequent determination by HPLC with UV absorption detection. Simple methods for determining aromatic amines or phenolic compounds at ppm levels in dyestuff samples, based on off-line clean-up with an SAX cartridge packed with anion-exchange resin, followed by on-line preconcentration (with a precolumn and the column switching technique) and determination by HPLC were developed.

2. Experimental

2.1. Materials

Analytical-reagent grade 3-diethylamino-phenol (DEAP), 2,4-dinitro-1-hydroxynaphthalene (2,4-DNHNA), 5-hydroxy-1,4-naphthoquinone (5-HNQ), 4,4'-dihydroxybiphenyl (4,4'-DHBP) and 4-aminoazobenzene (4-AAB) were obtained from Tokyo Chemical Industry (Tokyo, Japan), 2,7-dihydroxynaphthalene (2,7-DHNA) from Merck (Darmstadt, Germany), 2-hydroxynaphthalene (2-HNA) and 1-hydroxynaphthalene (1-HNA) from Janssen Chemical (Beerse, Belgium) and benzidine (Bz), 3,3'-dimethylbenzidine (DMBz), 4-aminobiphenyl (4-ABP), 3,3'-dichlorobenzidine (DCBz) and 2-naphthylamine (2-NA) from Sigma (St. Louis, MO, USA) and were used as received. LC-grade sodium acetate and acetonitrile were obtained from Fisher Scientific (Fairlawn, NJ, USA). Deionized water was purified in a Milli-Q filtration system (Millipore, Bedford, MA, USA) to obtain LC-grade water for preparing mobile phases and standard solutions.

2.2. Preparation of standard solutions

Stock standard solutions were prepared by weighing the aromatic amines or the phenolic compounds and dissolving them in methanol. A working composite standard solution was prepared by combining an aliquot of each of the stock standard solutions and diluting the mixture with water. These solutions were stored in dark glass bottles at 4°C.

2.3. Apparatus

The HPLC system, assembled from modular components (Waters), consisted of a Model 600E pump, a Model 486 UV detector and a Model 715 automatic sampler. A Millennium workstation (Waters) was utilized to control the system and for acquisition and analysis of data.

2.4. On-line preconcentration of aromatic amines or phenolic compounds from water

For on-line enrichment of aromatic amines, the sample loader (Waters Model 170) was used to introduce the aqueous sample (5 ml) at 0.9 ml/min on to a precolumn (IEC CM-825, 75 mm × 8 mm I.D.) (Showa Denko, Shodex). The packing material of this precolumn was carboxymethyl-bonded silica. The mobile phase, acetonitrile–acetate buffer (pH 4.66) (40:60, v/v) was used to desorb analytes from the precolumn and subsequent separation on the analytical column (Nova-Pak C₁₈, 15 cm × 3.9 mm I.D.) (Waters). The UV detector was set at 280 nm.

For on-line preconcentration of phenolic compounds, the precolumn (LiChrosorb RP-18, 25 mm × 4 mm I.D.) (Merck) and the analytical column (Nova-Pak phenyl, 75 mm × 3.9 mm) (Waters) were used. The mobile phase was acetonitrile–0.1 M acetate buffer (pH 4.66) (17:83, v/v). The UV detector was set at 254 nm.

The flow-rate of the mobile phase was kept at 1 ml/min throughout the experiments. Back-flushing was used.

2.5. Procedures for the determination of aromatic amines or phenolic compounds in dyestuff samples

Dyestuff samples of four kinds (Direct Blue 6, amaranth, Sunset Yellow FCF and D&C Orange No. 4) were used (Tokyo Chemical Industry). Dyestuff (0.1 g) was weighed into a volumetric flask (100 ml), water (ca. 50 ml) was added and the solid was dissolved completely with ultrasonic vibration. For recovery tests, a suitable aliquot of the standard solution of the aromatic amines or the phenolic compounds was added to the dyestuff solution, the pH was adjusted to 5

(for phenolic compounds) or 6 (for aromatic amines) with 0.01 M HCl and water was added to volume. The solution (exactly 10 ml) was passed through the SAX cartridge and the effluent collected. By this means, whereas aromatic amines or phenolic compounds passed unretained through the SAX cartridge, dyestuff components were adsorbed. To minimize losses of aromatic amines or phenolic compounds caused by partial adsorption on the SAX cartridge, the cartridge was then eluted with 4 ml of 0.1 M acetate buffer (pH 4.66) and the eluent was collected in a tube. The effluent and eluent were combined and mixed. An aliquot of the mixed solution (5 ml) was then enriched with an on-line device and analysed in the manner described above.

3. Results and discussion

3.1. On-line preconcentration of aromatic amines from water with a CM-825 precolumn

The recovery data for two concentrations are given in Table 1. Aromatic amines were recovered in the range 87–102%; the recoveries were approximately the same at both spiking levels. A plot of aromatic amine concentration (range 10–100 ng/ml) vs. integrated area gave linear regression lines having correlation coefficients >0.993. The method detection limits for these aromatic amines were 0.1–0.6 ng/ml.

Table 1
Recoveries of selected aromatic amines from water by on-line enrichment on a CM-825 precolumn^a

Compound	Recovery ^b (%)	
	2 ppb	100 ppb
Bz	89 ± 2	94 ± 1
DMBz	93 ± 6	96 ± 1
2-NA	98 ± 2	101 ± 0
4-ABP	96 ± 6	97 ± 0
DCBz	102 ± 10	87 ± 1
4-AAB	101 ± 5	102 ± 1

^a Aqueous sample (5 ml) was pumped at 0.9 ml/min through the CM-825 precolumn.

^b Average value and standard deviation of triplicate runs.

Table 2
Recoveries of selected phenolic compounds from water by on-line enrichment on a LiChrosorb RP-18 precolumn^a

Compound	Recovery ^b (%)	
	5 ppb	100 ppb
DEAP	89 ± 3	105 ± 3
2,7-DHNA	107 ± 2	100 ± 1
4,4'-DHBP	96 ± 2	98 ± 1
2,4-DNHNA	92 ± 10	98 ± 2
5-HNQ	86 ± 1	103 ± 3
2-HNA	102 ± 4	98 ± 1
1-HNA	99 ± 4	97 ± 3

^a Aqueous sample (5 ml) was pumped at 0.9 ml/min through the LiChrosorb RP-18 precolumn.

^b Average value and standard deviation of triplicate runs.

These results are comparable to those obtained using an RP-18 precolumn [21].

3.2. On-line preconcentration of phenolic compounds from water with a LiChrosorb RP-18 precolumn

As shown in Table 2, phenolic compounds were recovered in the range 86–107% for the two concentrations specified; the recoveries were approximately the same at both spiking levels. The linearity of the method was tested by analysing phenolic compound standards at four concentrations in the range 10–100 ng/ml. The

Table 3
Recoveries of selected aromatic amines added to commercial dyes^a

Compound	Recovery ^b (%)		
	Sunset Yellow FCF		Amaranth 100 µg/g
	10 µg/g	100 µg/g	
Bz	95 ± 7	94 ± 2	99 ± 2
DMBz	83 ± 3	87 ± 3	85 ± 4
2-NA	88 ± 8	93 ± 9	100 ± 1
4-ABP	94 ± 6	91 ± 2	88 ± 2
DCBz	96 ± 5	85 ± 4	101 ± 3
4-AAB	86 ± 3	85 ± 2	81 ± 4

^a The dyestuff solutions (0.1 g per 100 ml) were processed as described under Experimental.

^b Average value and standard deviation of triplicate runs.

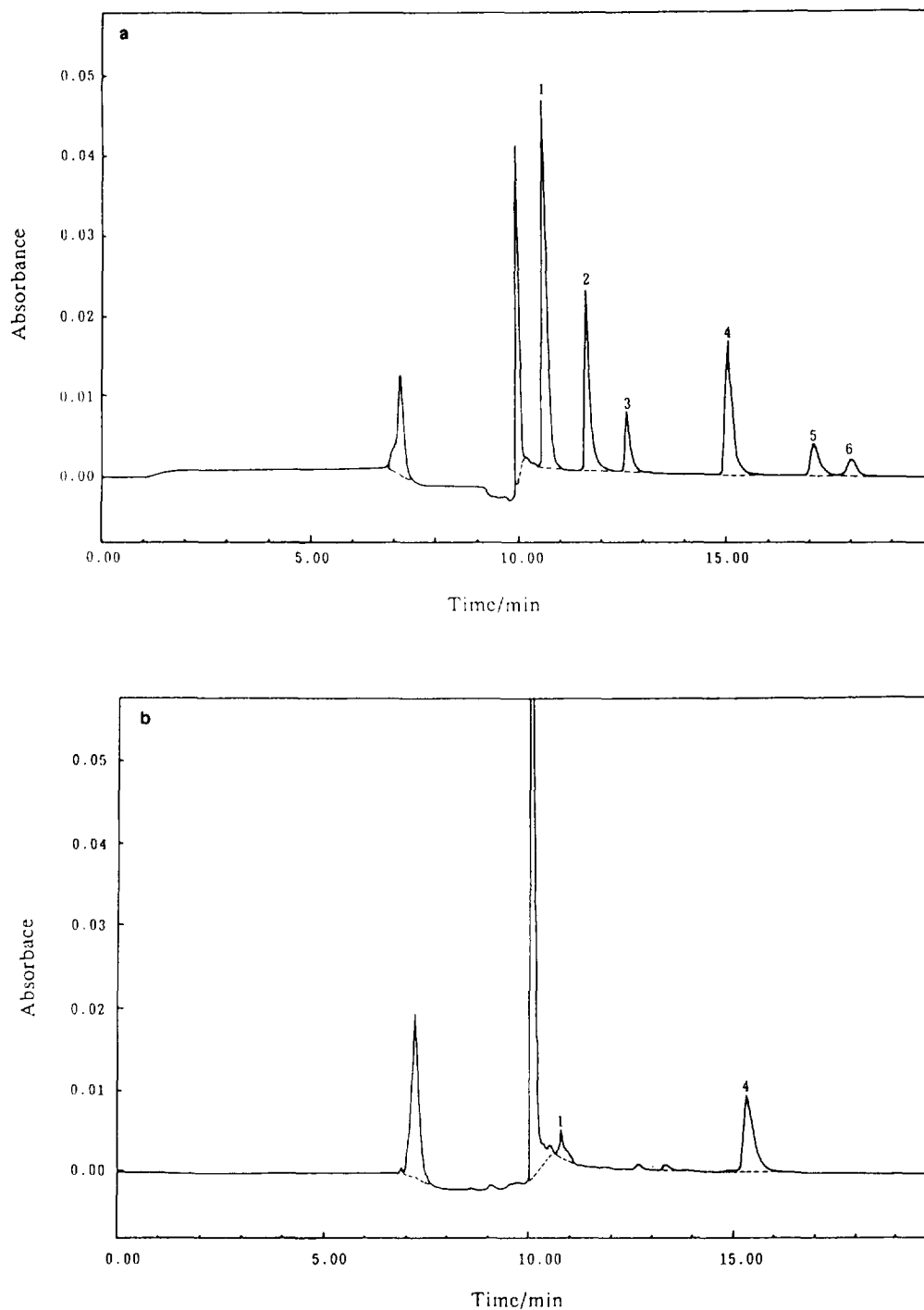


Fig. 1. Chromatograms of (a) a 10 ng/ml aromatic amine standard chromatogram and (b) a dye sample (Direct Blue 6) after clean-up on an SAX cartridge, on-line enrichment with a CM-825 precolumn and separation on a C_{18} column. Mobile phase, acetonitrile–0.1 M acetate buffer (pH 4.66) (40:60, v/v). For other experimental conditions, see text. Peaks: 1 = Bz; 2 = DMBz; 3 = 2-NA; 4 = 4-ABP; 5 = DCBz; 6 = 4-AAB.

relationship between peak area and concentration was linear over the entire range for phenolic compounds (the correlation coefficient of the regression lines exceeded 0.998). The method detection limits for these phenolic compounds were 0.1–1.5 ng/ml.

3.3. Determination of aromatic amines in dyestuff samples

A disposable SAX cartridge was used as an off-line clean-up filter, followed by on-line pre-concentration (with a CM-825 precolumn) and determination by HPLC. The recovery of aromatic amines from “spiked” dyestuffs was excellent (Sunset Yellow FCF 83–96%, amaranth 81–101%; Table 3). The standard deviation of the mean was less than 9%. No aromatic amine was found in the unspiked dyes.

The method detection limits of this procedure were Bz 0.6, DMBz 0.9, 2-NA 1.8, 4-ABP 0.5, DCBz 1.7 and 4-AAB 1.6 $\mu\text{g/g}$. The detection limits were measured with a concentration equivalent to three times the standard deviation of replicated measurements ($n = 7$) of the analytes in the dyestuff sample (Sunset Yellow FCF). The Occupational Safety and Health Administration [22] states that the concentrations of these compounds in various matrices to which workers may be exposed must not exceed 0.1%. The content of aromatic amines in synthetic food dyestuffs is limited to 0.01% by European colour additive specifications [23]. Therefore, the proposed method is capable of determining aromatic amines at concentrations much lower than these two regulatory limits.

The utility of the method was demonstrated by the analysis of Direct Blue 6. Representative chromatograms resulting from analysis of the sample and standard (10 $\mu\text{g/l}$) are shown in Fig. 1a and, b, respectively. The baseline exhibits a slight background because of the efficient removal of co-existing interferents on the SAX cartridge. As the aromatic amines are well isolated from other dyestuff components, the presence of a peak with the retention time of an aromatic amine gave a presumptive test for its presence. The results indicate that certain amounts of

carcinogenic aromatic amines may be present in this dye, namely $1.0 \pm 0.2 \mu\text{g/g}$ of benzidine and $6.5 \pm 0.2 \mu\text{g/g}$ of 4-aminobiphenyl. These values were obtained from triplicate determinations according to a calibration graph. The concentrations of benzidine and 4-aminobiphenyl in this dye, determined by the method of standard additions, were 0.9 and 6.6 $\mu\text{g/g}$, respectively.

3.4. Determination of phenolic compounds in dyestuff samples

A disposable SAX cartridge was found to be suitable as a clean-up filter; it can trap the anionic dyestuff from water without affecting the trace enrichment of phenolic compounds on the LiChrosorbRP-18 column except for 2,4-dinitro-1-hydroxynaphthalene (2,4-DNHNA). After the clean-up step, the dyestuff aqueous samples were processed by on-line pre-concentration (with the LiChrosorb RP-18 precolumn) followed by HPLC determination. The recoveries of phenolic compounds added to the dyestuff (Sunset Yellow FCF) at concentrations of 10 and 100 $\mu\text{g/g}$ were excellent (81–102%; Table 4). The standard deviation from the mean was less than 6%. The recoveries of all phenolic compounds indicated that the proposed clean-up and on-line enrichment procedure is highly specific and efficient with no interference from other components of the dyestuff sample.

The method detection limits ($n = 7$) of this

Table 4
Recoveries of selected phenolic compounds added to Sunset Yellow FCF^a

Compound	Recovery ^b (%)	
	10 $\mu\text{g/g}$	100 $\mu\text{g/g}$
DEAP	88 \pm 3	93 \pm 6
2,7-DHNA	97 \pm 6	99 \pm 1
4,4'-DHBP	102 \pm 3	101 \pm 5
5-HNQ	91 \pm 1	85 \pm 5
2-HNA	99 \pm 1	95 \pm 4
1-HNA	81 \pm 2	84 \pm 5

^a The dyestuff solutions (0.1 g per 100 ml) were processed as described under Experimental.

^b Average value and standard deviation of triplicate runs.

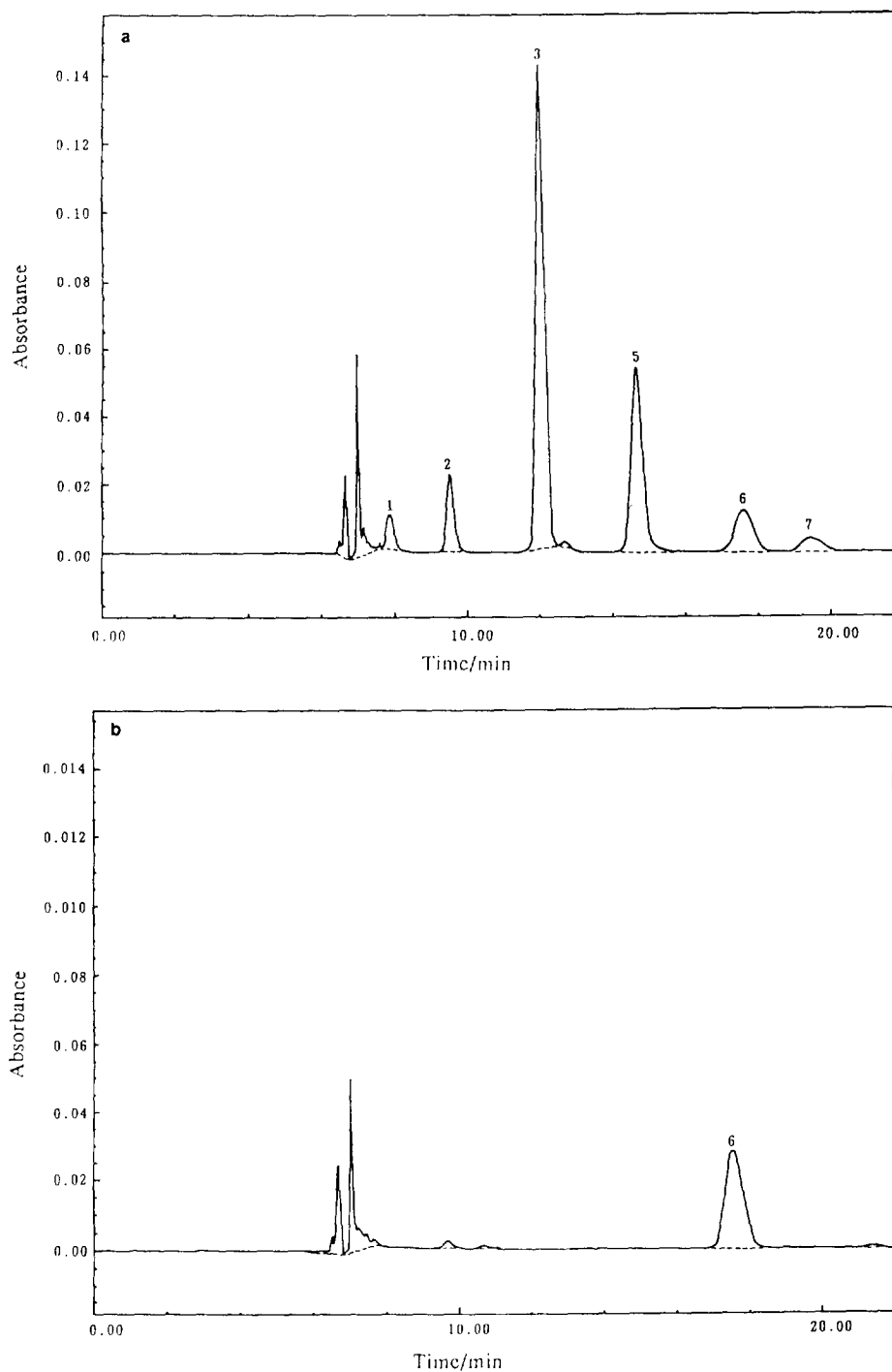


Fig. 2. Chromatograms of (a) a phenolic compound standard (100 ng/ml) and (b) a dye sample (D&C Orange No. 4) after clean-up on an SAX cartridge, on-line enrichment with an RP-18 precolumn and separation with a phenyl column. Mobile phase, acetonitrile–0.1 M acetate buffer (pH 4.66) (17:83, v/v). For other experimental conditions, see text. Peaks: 1 = DEAP; 2 = 2,7-DHNA; 3 = 4,4'-DHBP; 5 = 5-HNQ; 6 = 2-HNA; 7 = 1-HNA.

procedure for the individual components were DEAP 1.80, 2,7-DHNA 0.87, 4,4'-DHBP 1.50, 5-HNQ 0.42, 2-HNA 0.90 and 1-HNA 1.26 $\mu\text{g/g}$. The proportion of phenolic compounds in dyestuffs for drug and cosmetic use is limited to 0.2% according to the USA Code of Federal Regulations [24]. The proposed method is capable of determining phenolic compounds at concentrations of 0.1% of the regulatory limit.

The utility of the method was clarified by the analysis of D&C Orange No. 4. Representative chromatograms obtained from analytes of the sample and a 100 ppb standard are shown in Fig. 2a and b, respectively. The baseline contains a slight background as a consequence of the efficient removal of co-existing interferents left on the SAX cartridge. As phenolic compounds were well isolated from other dyestuff components, the presence of a peak with the retention time of a phenolic compound gave a presumptive test of its presence. The results (using the calibration graph method) indicate that $215 \pm 3 \mu\text{g/g}$ of 2-HNA may be present in this dye. The concentration of 2-HNA in this dye determined by the standard additions method was $216 \mu\text{g/g}$, which is identical with the value obtained from the calibration graph.

4. Conclusions

Aromatic amines or phenolic compounds in water at ppb levels can be determined by on-line preconcentration with a small precolumn followed by HPLC with UV absorption detection.

This method was applied to the determination of aromatic amines and phenolic compounds at ppm levels in commercial dyestuffs. Because of the small capacity of the precolumn and the presence of dyestuff components at relatively high concentrations in the commercial dye samples tested, it was necessary to introduce a clean-up step to remove dyestuff compounds from aqueous samples. An anion-exchange cartridge (SAX) was used for the clean-up of aqueous dyestuff samples prior to the on-line preconcentration and determination procedures. The proposed method allows the simple, rapid and

efficient clean-up and on-line enrichment and simultaneous determination of aromatic amines and phenolic compounds, and is expected to be suitable for routine analyses for these compounds in commercial dyestuffs.

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