Some validation aspects on the analytical method for assaying carcinogenic amines from textile dyes

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REZUMAT – ABSTRACT

Aspecte privind validarea metodei analitice de determinare a aminelor cancerigene derivate din coloranți specifici industriei textile

Controlul siguranței chimice, precum și proprietățile ecologice au devenit o prioritate pentru industria textilă cu scopul de a evita efectele negative ale substanțelor chimice asupra oamenilor și asupra mediului înconjurător. Interesul crescut pentru toxicologia produselor textile este determinat de prezența compușilor periculoși în haine generați în urma proceselor de vopsire și de finisare. Pentru a proteja sănătatea umană, regulamentele europene, precum Oeko Tex Standard 100 și REACH, limitează prezența substanțelor chimice periculoase, cum ar fi aminele aromatice generate prin scindarea reductivă a coloranților azoici, la cel mult 30 mg/kg de material textil. Scopul principal al acestei lucrări de cercetare a fost elaborarea și validarea metodei cromatografice HPLC/MWD pentru identificarea și cuantificarea precisă și sigură a aminelor aromatice cancerigene derivate din coloranți azo specifici industriei textile. Determinarea simultană a 24 de amine aromatice reglementate a fost efectuată prin două metode cromatografice conform SR EN ISO 14362-1: 2017 pentru a evita interferențele matricei și erorile în identificarea compușilor din cauza prezenței izomerilor structurali. Analizele preliminare pentru a stabili lungimea de undă corespunzătoare absorbției maxime a fiecărei soluții standard de amine aromatice s-au efectuat simultan la patru lungimi de undă, 240, 280, 305 și 380 nm. Cu scopul de a demonstra siguranța, fiabilitatea și precizia datelor analizate, s-au validat atât metoda cromatografiei de lichide, cât și cea a cromatografiei de gaze. Au fost evaluați parametri ca: selectivitatea, precizia, limita de detecție și limita de cuantificare a celor două metode analitice. Certitudinea determinărilor a fost dovedită și de rezultatele testelor interlaboratoare efectuate de Institutul de Studii Interlaboratoare din Olanda asupra coloranților azoici din textile.

Cuvinte cheie: testarea competențelor, amine cancerigene, textile vopsite, textile ecologice, HPLC, GC-MS, validare

Some validation aspects on the analytical method for assaying carcinogenic amines from textile dyes

Chemicals safety control and ecological properties have become a priority for the textile industry in order to avoid the negative effects on humans and environment. The increasing interest for toxicology of textiles is determined by the presence of dangerous compounds in clothes generated from dyeing and finishing processes. In order to protect human health, European Regulations as Oeko Tex Standard 100 and REACH Regulation limit the presence of dangerous chemicals, such as aromatic amines, generated by reductive cleavage of azo dyes, by no more than 30 mg/kg of textile material. The main goal of this research work was to develop and validate a HPLC/MWD method for precise and reliable identification and quantification of carcinogenic aromatic amines derived from banned azo dye specific to the textile industry. The simultaneous determination of 24 regulated aromatic amines has been conducted by two chromatographic methods according to SR EN ISO 14362-1:2017 in order to avoid matrix interferences and compounds misidentification due to the presence of structural isomers. Preliminary analyses to establish the maximum absorption wavelength of each standard solution of aromatic amine were performed simultaneously at four wavelengths, 240, 280, 305 and 380 nm. With the scope of demonstrating the consistency, reliability and accuracy of the analysed data, both liquid and gas chromatographic method were validated. Parameters as selectivity, precision, limit of detection and limit of quantification of the analytical methods were evaluated. The certainty of the determinations was also proved by the results of proficiency testing conducted by IIS Netherlands on azo dyes in textiles.

Keywords: proficiency testing, carcinogenic amines, textile dyes, textile ecology, HPLC, GC-MS, validation

INTRODUCTION

Azo colourants are used to colour textile fibres, leather, plastics, papers, hair, mineral oils, waxes, foodstuffs and cosmetics [1–2]. 'Azo dye' is the collective term used to describe a group of synthetic that rose to prominence in the 1880s and are now comprise 70% of all organic commercial dyes [1]. The word 'Azo' signifies the presence of a chemical azo group (–N=N–) in the dye. Today, they are produced for the most part in China and India, followed by Korea, China and Argentina [1]. Azo dyes are popularly used, because they dye cloth at 60°C, while Azo-free dyes require a temperature of 100°C. Also, Azo dyes offer an extensive range of colours, better colour fastness and four times the intensity of the closest alternatives, making them invaluable to the textile industry.

In specific conditions, azo dyes produce by in vivo reductive cleavage of the azo groups primary aromatic amines (PAAs), that are considered by the international authorities to be toxic, and have mutagenic and carcinogenic effect. These specific reductive conditions are met in the digestive tracts and some organs of animals, including humans [3]. The main responsible for their toxicity is represented by the amino group bound to the aromatic system. 24 aromatic amines have been confirmed as or implicated to be, carcinogens in humans, and as many as 5% of Azo dyes can cleave to form these dangerous compounds [2]. They can be present in dyed product and in the environment due to incomplete synthesis or degradation of azo dyes.

There are three main routes of exposure to azo dyes: a) ingestion, mainly by babies and children, b) dermal absorption, the largest concern both for people wearing dyed clothing and for the staff from factories producing dyes and c) dye inhalation worrying for workers from factories but also for handling freshly dyed materials with azo dyes [1].

In one German dye plant, 100% of workers (15 people) involved in distilling 2-naphthylamine developed bladder cancer [4]. Aromatic amines are also present in tobacco smoke, which may explain why smoking seems to elevate the risk of bladder cancer. EU restricted aromatic amines have also been linked to splenic sarcomas and hepatocarcinomas [6].

Many strict government regulations worldwide limit the usage of azo dyes in textile and leather products. 22 aromatic amines are classified by the EU Commission as proven or suspected human carcinogens, and their concentration in textile materials is limited at 30 mg/kg [1]. Oeko-Tex Label, designed to protect the consumers is a voluntary label adopted by an increasing number of textile manufacturers, and regulates 24 aromatic amines, of which 22 overlap with the European legislation. The maximum amount of carcinogenic aromatic amines specified in Oeko Tex is 20 mg/kg [2].

The increasing need for less harmful chemicals used in textile industry, that have minimum of no harmful effects on human health justify the necessity for fast and accurate methods to test and quantify aromatic amines derived from azo dyes extracted from textile materials [2].

EXPERIMENTAL

Reagents and standards

Acetonitrile and methanol gradient grade from Merck KGaA (Germany), water for chromatography (resistivity min. 18.2 MΩxcm, TOC max. 50 ppb).

Analytical standards of 24 aromatic amines from Sigma-Aldrich and Dr. Ehrenstorfer GmbH (Germany).

Instrumentation

HPLC separation was performed on Agilent 1100 LC System using an Agilent Zorbax Eclipse XDB C18 column and MWD detector. GC separation was performed on Agilent 6890 GC System coupled with Agilent 5973N transmission quadrupole mass spectrometer (table 1).

Table 1

Agilent 6890 GC/5973N MS Operating Conditions	
Capillary Column	DB-35MS (J&W), 35 m × 0.25 mm × 0.25 µm;
Injector System	splitless
Injector Temp.	260°C
Carrier gas	helium
Flow (mL/min)	1 mL/min
Temp. programme	100°C (2 min), 100°C – 310°C (15°C/min), 310°C (2 min)
Injection Volume	1.0 µl
Detection	MS / Full Scan
Acquisition Parameters	EI Positive Ion Mode, 70 eV

Agilent 1100 HPLC/MWD Operating Conditions		
Analytical Column	Zorbax Eclipse XDB C18 150 mm x 4,6 mm x 3.5µm	
Column Temp.	32°C	
Injection Volume	5.0 µl	
Mobile Phase	Eluent 1: methanol	
	Eluent 2: 0.68 g potassium dihydrogen phosphate in 1000 mL water, 150 mL methanol	
Run time	35 min	
Flow rate	0,6–2,0 mL/min (gradient)	
Quantification	at 240 nm, 280 nm, 305 nm and 380 nm	
Gradient profile	Time (minutes)	Gradient (% Eluent 1)
	0.00	10.0
	22.50	55.0
	27.50	100.0
	28.50	100.0
	28.51	100.0
	29.00	100.0
	29.01	10.0
	31.0	10.0
	35.00	10.0

Sample preparation

Stock solutions of each amine (according to – SR EN ISO 14362-1:2017 [2]) with the concentration of 300 µg/mL in ACN were prepared. From these stock solutions, 8 solutions for calibration curve were prepared, with the following concentration: 3, 4.5, 6, 12, 24, 28, 30, 36 µg/mL.

Selection of maximum absorption wavelength for each of 22 aromatic amines

Each standard solution of the target amines in concentration of 50 µg/mL was analysed using spectrophotometric detection simultaneously at four wavelengths, 240, 280, 305 and 380 nm. Thus, a classification of the 24 amines depending on the

Table 2

240 nm	280 nm	305 nm	380 nm
Amines 3, 4, 6, 7, 8, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21*	Amines 2, 12, 1, 10	Amines 11	Amines 5, 22

* according to aromatic amines numbering from table 1 – ISO/FDIS 14362-1:2016(E) [8]

specific wavelength at which absorption is maximal was obtained (table 2).

The overlaid chromatograms of amines 3, 2, 11, and 22, solutions with concentration of 50 mg/L are shown in figures 1–4. Detection has been performed at 240, 280, 305 and 380 nm and the overlaid chromatograms indicate the detection wavelength for each amine.

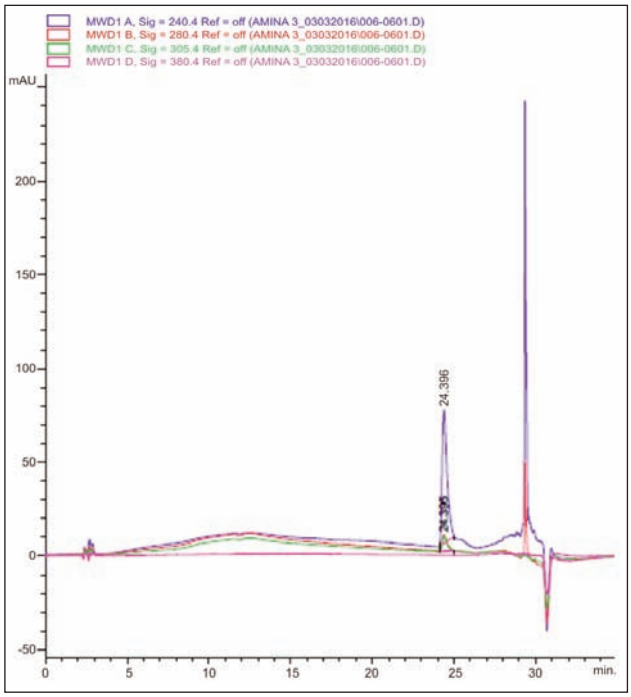

Fig. 1. Overlaid chromatograms of 4-chloro-o-toluidine, 50 mg/L, maximum absorption wavelength at 240 nm

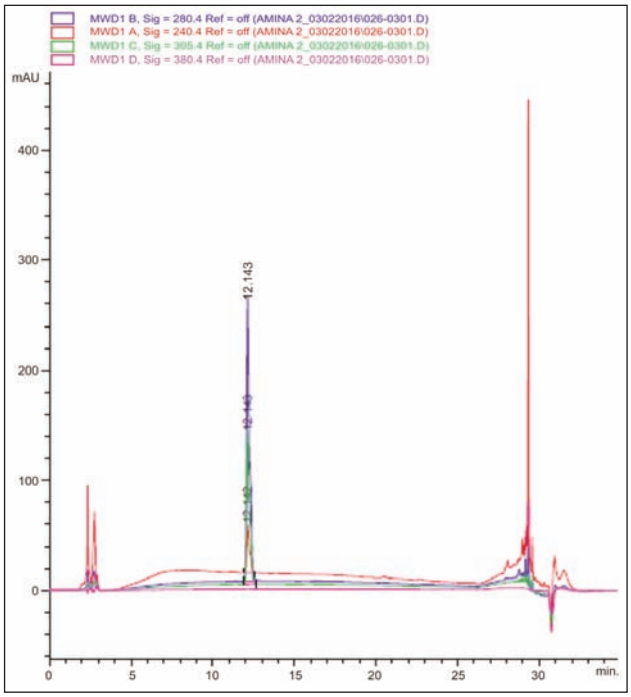

Fig. 2. Overlaid chromatograms of benzidine, 50 mg/L, maximum absorption wavelength at 280 nm

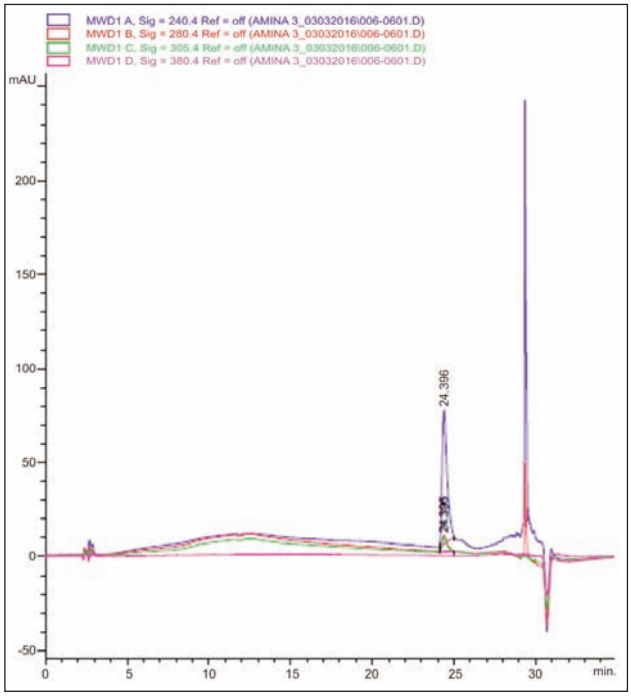

Fig. 3. Overlaid chromatograms of o-dianisidine, 50 mg/L, maximum absorption wavelength at 305 nm

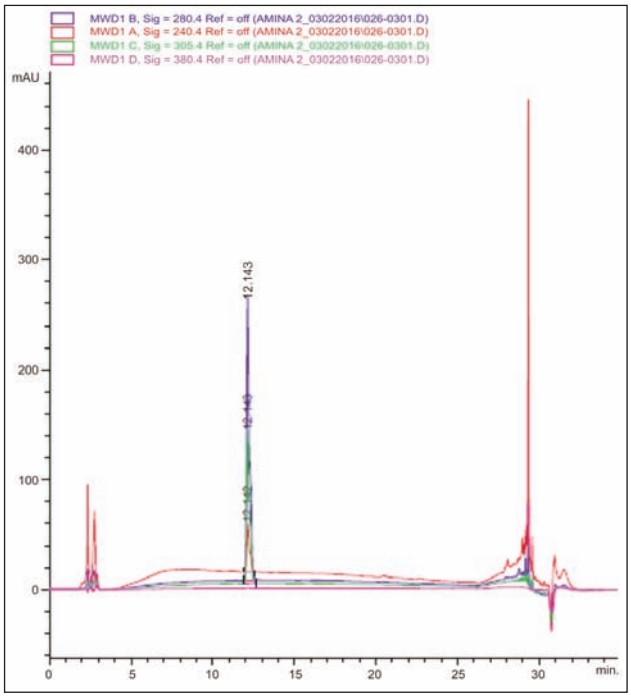

Fig. Overlaid chromatograms of 4-aminoazobenzene, 50 mg/L, maximum absorption wavelength at 380 nm

Performance characteristics of the analytical method

Selectivity

Selectivity is the ability of the analytical method to measure and differentiate analytes in the presence of components that are expected to be present in the sample. In the case of an HPLC method it must be demonstrated that the analyte of interest was very well separated from the other compounds in the sample and that the peak of interest did not overlap with other interfering peaks.

In order to demonstrate the method selectivity, apparent resolution and relative standard deviation (RSD %) of retention time between each two analytes with consecutive elution have been determined (table 3). 6 chromatographic separation of mixture of 24 amines by HPLC-MWD and GC-MS techniques have been performed. For calculation of relative standard deviation, average values of retention times were used.

As can be seen in table 3, the chromatographic resolutions between each 2 consecutive compounds exceed in all cases the value of 1.5 and in many cases have increased values (amine 12: R_s = 47.128), indicating very good separation capacity of the amine mixture on the Zorbax Eclipse XDB C18 column.

The relative standard percentage deviation calculated for average retention time is in all cases less or very close to the value of 1, showing a good selectivity of the separation method of aromatic amines [11].

As it can be observed in figures 5–8 and table 3, all amines were evaluated at the wavelength corresponding to their maximum absorption; compounds are well separated at their baseline, with no fronting, tailing or overlapping peaks.

Table 3

Wavelength corresponding to maximum absorption	Amine	HPLC Results		
		t_R (average)	R_S (average)	RSD%
240 nm	Amine 8	3.955	3.19	0.59
	Amine 19	4.890	29.93	0.50
	Amine 16	12.284	5.58	0.86
	Amine 21	13.624	4.46	0.77
	Amine 18	14.901	9.90	0.73
	Amine 9	17.617	3.65	0.46
	Amine 7	18.618	1.06	1.43
	Amine 6	18.922	3.57	0.85
	Amine 14	20.090	1.73	0.60
	Amine 23	20.837	1.36	0.54
	Amine 24	21.209	5.43	0.61
	Amine 4	22.553	6.02	0.97
	Amine 3	24.207	4.11	0.96
	Amine 13	25.272	1.42	0.82
	Amine 20	25.603	16.81	0.69
	Amine 15	28.670	16.81	0.07
280 nm	Amine 2	11.601	37.75	0.85
	Amine 12	19.680	47.12	0.66
	Amine 1	27.727	5.43	0.51
	Amine 10	28.306	5.43	0.23
305 nm	Amine 11	19.362	1.53	0.64
380 nm	Amine 5	28.605	12.25	0.13
	Amine 22	29.173	12.25	0.09

Precision

Measurement precision express the closeness of the results obtained from a series of multiple measurements of aliquots from the same homogeneous sample in specific conditions.

Reproducibility, component of precision, was addressed in the present study by participating in the

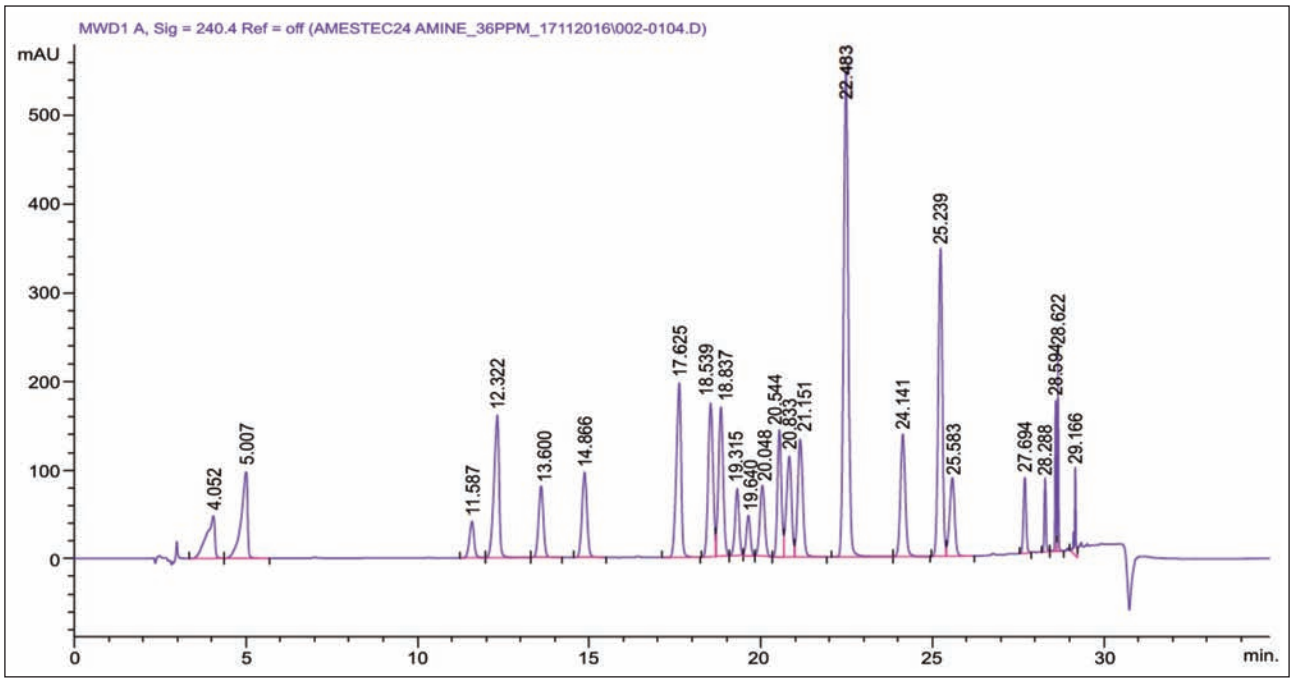

Fig. 5. Chromatogram of 24 amines mixture, concentration 36 ppm, detection at 240 nm

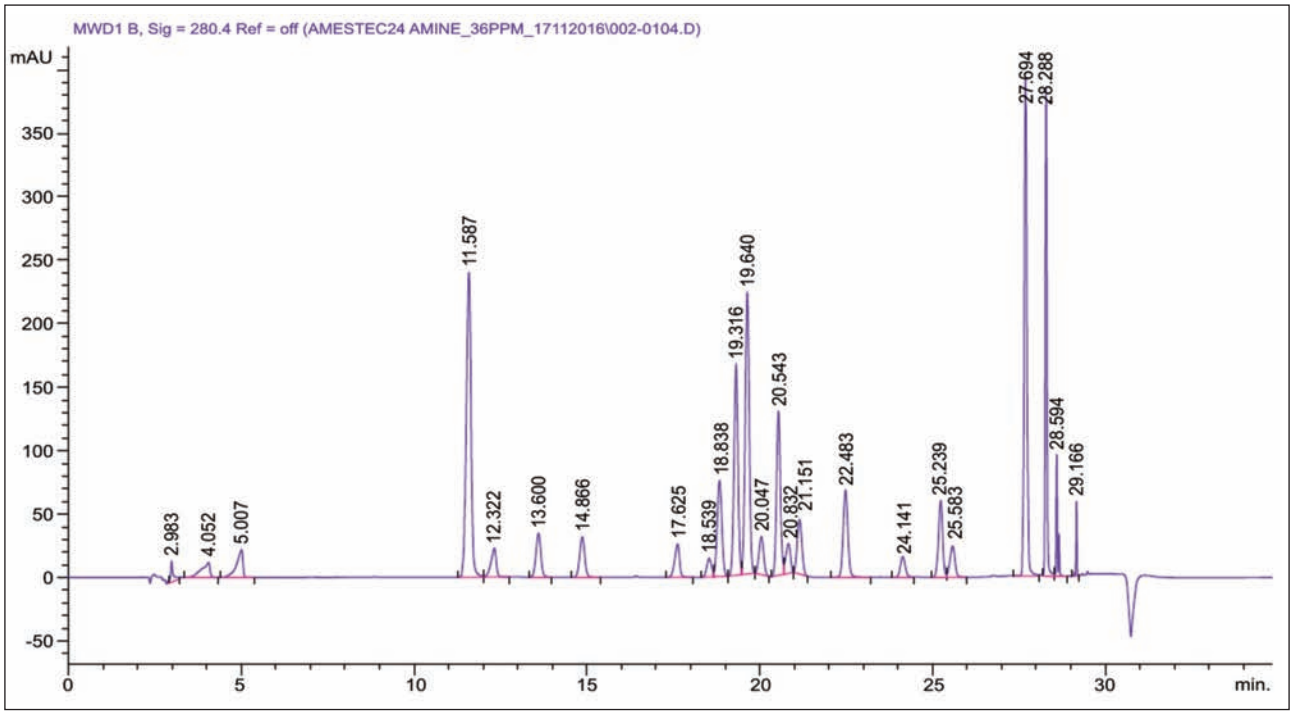

Fig. 6. Chromatogram of 24 amines mixture, concentration 36 ppm, detection at 280 nm

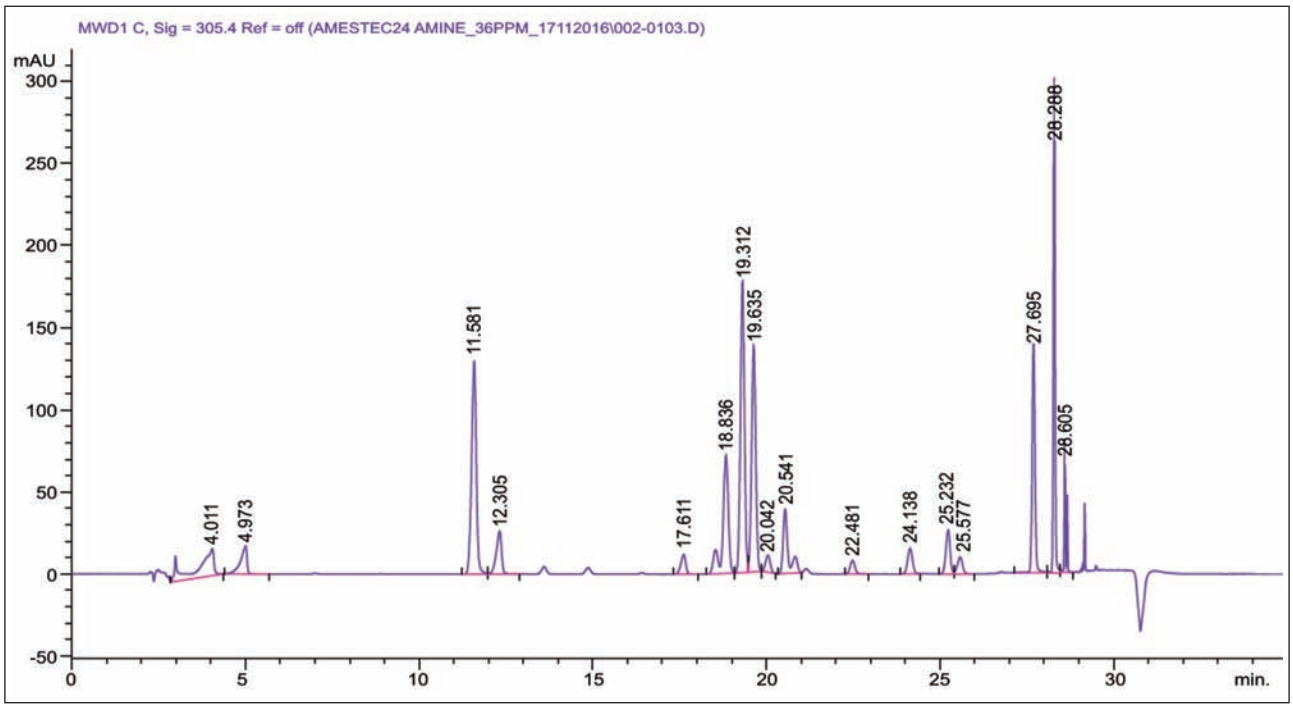

Fig. 7. Chromatogram of 24 amines mixture, concentration 36 ppm, detection at 305 nm

interlaboratory comparison with the Institute for Interlaboratory Studies in Spijkenisse, the Netherlands. In this study, 170 laboratories from 34 different countries participated. Two different samples of fabric were made available – a sample of cotton and a sample of polyamide, each dyed with azo dyes. In the cotton sample, 3,3'-dimethoxybenzidine aromatic amine was identified by the laboratory that organized the study, and in the polyamide sample the aromatic amines 3,3'-dimethylbenzidine and 2,4-xylidine were found.

The z score is calculated to evaluate the performance of the participating laboratories. For this determination, the calculated z score was our laboratory was –1.92, which is satisfactory according to literature [3] (figure 9).

For this determination, the calculated z score was –0.01 (good), indicating a good reproducibility of the method for determining the aromatics of textile materials (figure 10).

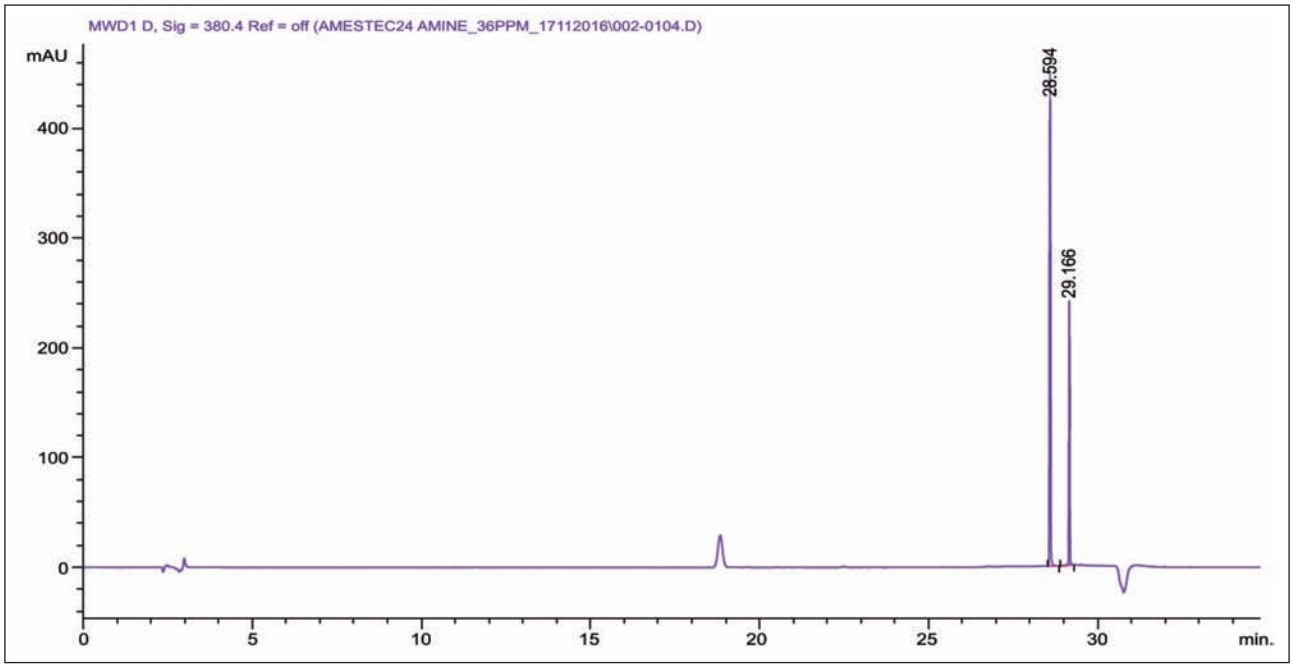

Fig. 8. Chromatogram of 24 amines mixture, concentration 36 ppm, detection at 380 nm

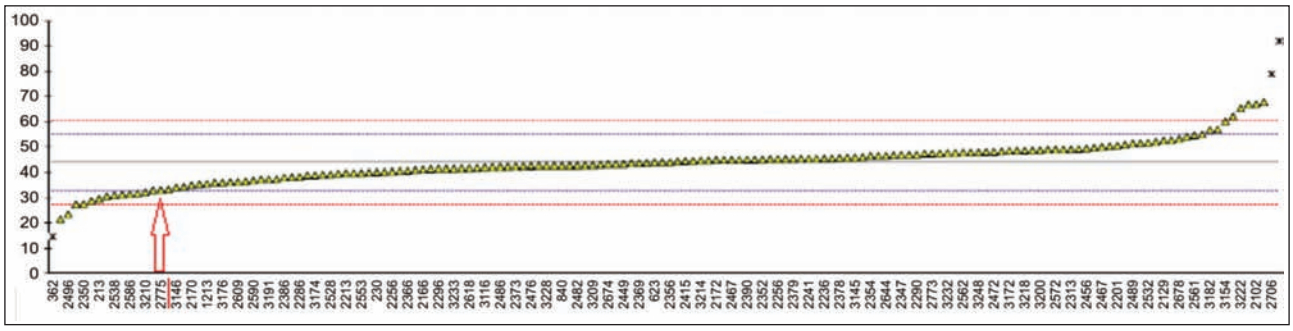

Fig. 9. Concentrations determined by the participating laboratories for 3,3'-dimethoxybenzidine in the cotton sample and positioning of our laboratory (code 2775) in the graph

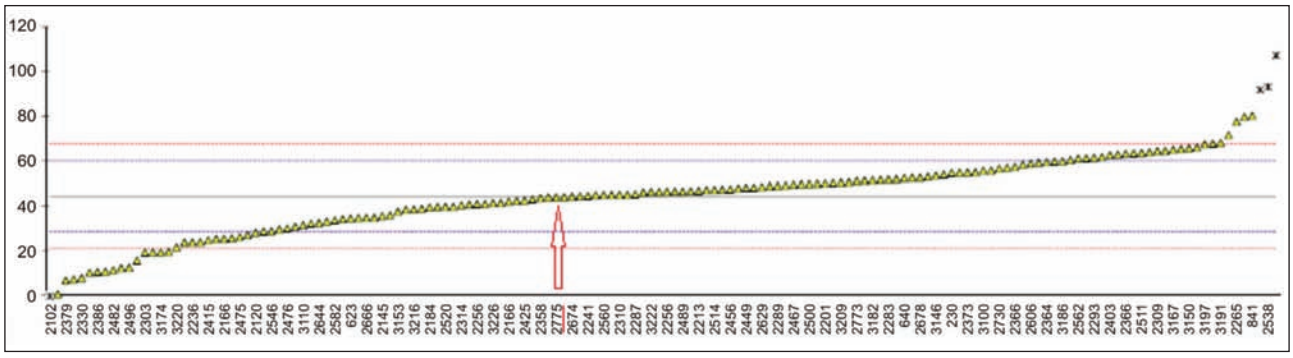

Fig. 10. Concentrations determined by the participating laboratories for 3,3'-dimethylbenzidine in the poliamide sample and positioning of our laboratory (code 2775) in the graph

The limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection is the lowest value of the analyte concentration providing a signal (a chromatographic peak) at least equal to 3σ (σ – the standard deviation of the noise in the chromatogram baseline) [3].

The limit of quantification is the value of the concentration determined in the sample under well-specified measurement conditions with acceptable repeatability and accuracy.

To determine the detection limit, an injection of the 24 amine mixture was performed by both gas and liquid chromatography, of 6 independent blank samples fortified at the lowest acceptable concentration (3 ppm), measured once each. The values obtained for the detection limits of the 24 individual amines for the two

Table 4

Amine	Average conc. – HPLC [ppm]	s_{HPLC}	LOD_{HPLC}	Average conc. – GC [ppm]	s_{GC}	LOD_{GC}
Amine 8	2.9533	0.0404	**0.12**	3.7920	0.1809	**0.54**
Amine 19	2.1981	0.0316	**0.09**	4.0280	0.1859	**0.55**
Amine 16	2.9898	0.0298	**0.08**	4.0480	0.1571	**0.47**
Amine 21	3.0051	0.0137	**0.04**	3.3360	0.2140	**0.64**
Amine 18	3.0085	0.0143	**0.04**	3.4840	0.1534	**0.46**
Amine 9	2.9956	0.0281	**0.08**	3.9360	0.2459	**0.73**
Amine 7	2.9743	0.0176	**0.05**	3.5020	0.1480	**0.44**
Amine 6	2.9752	0.0178	**0.05**	3.8700	0.1339	**0.40**
Amine 14	2.9676	0.0169	**0.05**	3.4780	0.1770	**0.53**
Amine 17	2.9412	0.0239	**0.07**	4.1000	0.0780	**0.23**
Amine 23	3.0019	0.0188	**0.05**	4.4200	0.6325	**1.89**
Amine 24	2.9923	0.0198	**0.05**	4.3600	0.3145	**0.94**
Amine 3	3.0118	0.0364	**0.10**	3.4000	0.1476	**0.44**
Amine 13	2.9890	0.0191	**0.05**	4.3820	0.0538	**0.16**
Amine 20	3.0447	0.0217	**0.06**	3.7820	0.1541	**0.46**
Amine 15	2.9335	0.0150	**0.04**	4.3480	0.0567	**0.17**
Amine 2	2.9857	0.0212	**0.06**	3.9800	0.0738	**0.22**
Amine 12	2.9313	0.0479	**0.14**	4.2060	0.0550	**0.16**
Amine 1	2.9785	0.0265	**0.07**	4.2440	0.1183	**0.35**
Amine 10	2.9795	0.0202	**0.06**	4.2420	0.0412	**0.12**
Amine 11	2.9900	0.0226	**0.06**	3.8860	0.0898	**0.26**
Amine 5	2.9773	0.0169	**0.05**	3.9180	0.0979	**0.29**
Amine 22	3.0718	0.0213	**0.06**	3.9360	0.1179	**0.35**

Table 5

Amina	Average conc. – HPLC [ppm]	s_{HPLC}	LOQ_{HPLC}	Average conc. – GC [ppm]	s_{GC}	LOQ_{GC}
Amina 8	2.9533	0.0404	**0.40**	3.7920	0.1809	**1.80**
Amina 19	2.1981	0.0316	**0.31**	4.0280	0.1859	**1.85**
Amina 16	2.9898	0.0298	**0.29**	4.0480	0.1571	**1.57**
Amina 21	3.0051	0.0137	**0.13**	3.3360	0.2140	**2.13**
Amina 18	3.0085	0.0143	**0.14**	3.4840	0.1534	**1.53**
Amina 9	2.9956	0.0281	**0.28**	3.9360	0.2459	**2.45**
Amina 7	2.9743	0.0176	**0.17**	3.5020	0.1480	**1.47**
Amina 6	2.9752	0.0178	**0.17**	3.8700	0.1339	**1.33**
Amina 14	2.9676	0.0169	**0.16**	3.4780	0.1770	**1.77**
Amina 17	2.9412	0.0239	**0.23**	4.1000	0.0780	**0.77**
Amina 23	3.0019	0.0188	**0.18**	4.4200	0.6325	**6.32**
Amina 24	2.9923	0.0198	**0.19**	4.3600	0.3145	**3.14**
Amina 3	3.0118	0.0364	**0.36**	3.4000	0.1476	**1.47**
Amina 13	2.9890	0.0191	**0.19**	4.3820	0.0538	**0.53**
Amina 20	3.0447	0.0217	**0.21**	3.7820	0.1541	**1.54**
Amina 15	2.9335	0.0150	**0.14**	4.3480	0.0567	**0.56**
Amina 2	2.9857	0.0212	**0.21**	3.9800	0.0738	**0.73**
Amina 12	2.9313	0.0479	**0.47**	4.2060	0.0550	**0.54**
Amina 1	2.9785	0.0265	**0.26**	4.2440	0.1183	**1.18**
Amina 10	2.9795	0.0202	**0.20**	4.2420	0.0412	**0.41**
Amina 11	2.9900	0.0226	**0.22**	3.8860	0.0898	**0.89**
Amina 5	2.9773	0.0169	**0.16**	3.9180	0.0979	**0.97**
Amina 22	3.0718	0.0213	**0.21**	3.9360	0.1179	**1.17**

chromatographic methods are shown in table 4. We determined the values for limits of detection of aromatic amines according to the standard SR EN ISO 14362-1:2017 (table 4); by liquid chromatographic method with spectrophotometric detection HPLC-MWD, values are in the range of 0.04–0.14 mg/L and by the gas chromatographic method with mass selective detector GC-MS are in the range of 0.2–1.9 mg/L, which allows a precise detection of amines in the range of 2–50 mg/L (specified in standard method). The values of the limits of quantification for aromatic amines by liquid chromatographic method HPLC-MWD according to SR EN ISO 14362-1: 2017 are in the range of 0.1–0.5 mg/L, and by gas chromatography method are in the range of 0.4 to 2.5 mg/L, which allows quantification of amines at low levels of concentration (table 5).

CONCLUSION

In this study, some aspects relating to validation of a precise and reliable method for determining aromatic amines derived from banned azo dyes specific to the textile industry are presented; specific UV absorption wavelength for each compound has been identified and the performance characteristics for both HPLC-MWD and GC-MS methods specified in standard SR EN ISO 14362-1:2017 [10] have been determined for an accurate and reliable detection and quantification of multicomponent aromatic amines.

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[9] Postnote "*The Environmental, Health and Economic Impacts of Textile Azo Dyes*", Houses of Parliament, Parliamentary Office of Science and Technology.

[10] SR EN ISO 14362:1/2017 Textile: Metoda de determinare a anumitor amine aromatice derivate din coloranti azoici – Partea 1: Detectia utilizarii anumitor coloranti azoici accesibili cu sau fara extractie.

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