

Application of chromatographic techniques for determination of azo dyes and their degradation products in consumer goods

Małgorzata MOŚCIPAN*, Magdalena ZARĘBSKA, Renata KULESZA – Institute of Heavy Organic Synthesis „Blachownia”, Kędzierzyn-Koźle, Poland

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Textiles

Information on the earliest textiles date back to Neolithic period (about 5000 BC) and their examples, mainly in the form of ancient people relicts have been found in Africa and Peru. However, the form of textiles at that time differed from that contemporary one. At the beginning grasses, creepers, branches and straps of animal skins have been braided creating not large plaits. The first evidences for thin linen, cotton, wool and silk fabric existence have been found in Ancient Egypt (about XXVII-XXII BC) mostly in painting form and mentions about flax utilization by Ancient Egyptians [1].

Ancient people used chemicals to enhance textiles colours, moreover, they used dyes for decorating purpose which has significant role in society. Animal skins protected from heat, cold and rain were worn directly or processed with natural chemicals of both vegetable (e.g. indigo, curcuma, *Rubia tinctorum*) and animal origin (e.g. carminic acid, Tyrian red). Natural dyes characterize very beautiful colours and most of them show high durability of light exposition which is best proven by e.g. old carpets and tapestry which colour survived many centuries without being damaged [1].

The second half of the XIXth century supported by industrial revolution in Europe and outside it, become the breakthrough in synthetic dyes commercialization area. First synthetic dyes fuchsine (synthesized in 1855 by J. Natanson) and mauveine (obtained in 1856 by W. Perkin during work on quinine) have caused the development of the most numerous synthetic dyes group which three years later were named azo dyes due to P. J. Greiss diazo compounds discovery in 1858 [2].

Nowadays, the cheaper and easier to use synthetic dyes are used. More than ¾ of dyes production consume the textile industry, additionally, leather, paper, plastics, printing, cosmetics and construction. Dyes are also used in analytical chemistry as indicators.

Azo dyes

Azo dyes constitute a numerous group of synthetic organic dyes which are characterized by presence of azo chromophore group ($-N=N-$). In the molecule structure is one or more (generally 1-4) azo groups attached from the one side to aromatic ring (benzene or naphthalene) and often modified by functional groups such as: amine ($-NH_2$), hydroxyl ($-OH$), methyl ($-CH_3$), sulfo ($-SO_3H$), and from the second side to aromatic, cyclic, heterocyclic or aliphatic fragment. Advantages such as: simplicity of achieving, chemical stability and wide range of available colours, decide about versatility of azo dyes use. They are used in textile and leather industry as pigments to varnishes, paints, cosmetics, plastics, foodstuffs, oils and papers production. Currently, more than 3000 different azo dyes which constitute about 65% dyes and pigments market are applied. World azo dyes consumption reach 700 thousands tons per year and just in textiles industry 2/3 of world production is used [2-4].

Azo dyes are created in coupling reaction diazonium compounds with phenols or aromatic amines (Fig. 1).

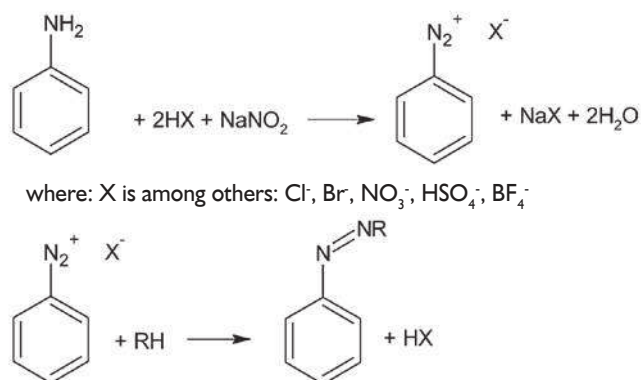


Fig. 1. Azo compounds synthesis diagram

Available on market azo dyes are sold in the form of polishes, powders and as a liquid where their concentration varies from 6 to 100% [2].

Classification of azo dyes includes among others the following classes:

- acid: anionic dyes water-soluble, include one or more sulfo or carboxyl groups, they are effective in dyeing the synthetic fiber nylon, silk, modified acryl fibers, wool, paper, food and cosmetics;
- basic: water-soluble dyes that are mainly applied to colouring modified polyesters, acryl fibers, nylon and paper;
- direct: anionic compounds, water-soluble in the presence of electrolytes, that are used to dyeing cellulose and polyamide fibers, nylon, leather and paper;
- reactive: that is capable of directly reacting with the fibre substrate due to presence of active groups in molecules which can react with wool, silk, polyamide fibers amino groups or cellulose hydroxyl groups.

Azo dyes are relatively resistant on degradation process in oxygen conditions, whereas in anaerobic they can decompose to aromatic amines as a result of reductive cleavage of azo group (Fig. 2).

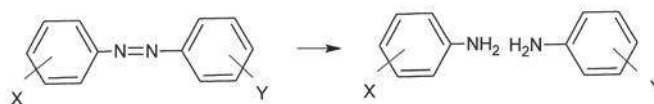


Fig. 2. Decomposition of azo dyes to aromatic amines diagram

According to REACH regulation annexe no VII [5] replacing 2002/61/EC directive [6], in European Union countries list of 22 aromatic amines (as specified in Tab. 1) is in force. These amines derive from azo dyes decomposition and their presence should be permanently monitored due to carcinogenic properties. Detectable quantity of these analytes in complete products or their dyeing fragments which have direct contact with the skin or mouth should not exceed 30 ppm.

Corresponding author:

Małgorzata MOŚCIPAN – Ph.D., e-mail: moscipan.m@icso.com.pl

Table I
List of the 22 aromatic amines formulated in REACH regulation
annexe VII [5]

No.	Aromatic amine	CAS	Structure
1	4-aminodiphenyl	92-67-1	
2	Benzidine	92-87-5	
3	4-chloro-o-toluidine	95-69-2	
4	2-naphtylamine	91-59-8	
5	4-amino-2',3-dimethylazobenzene	97-56-3	
6	2-amino-4nitrotoluene	99-55-8	
7	4-chloroaniline	106-47-8	
8	2,4-diaminoanisole	615-05-4	
9	4,4'-diaminodiphenylmethane	101-77-9	
10	3,3'-dichlorobenzidine	91-94-1	
11	3,3'dimethoxybenzidine	119-90-4	
12	3,3'-dimethylbenzidine	119-93-7	
13	3,3'-dimethyl-4,4'-diaminodiphenylmethane	838-88-0	
14	4-cresidine	120-71-8	
15	4,4'-methylene-bis-(2-chloroaniline)	101-14-4	
16	4,4'-oxydianiline	101-80-4	
17	4,4'-thiodianiline	139-65-1	
18	2-aminotoluene	95-53-4	
19	2,4-diaminotoluene	95-80-7	
20	2,4,5-trimethylaniline	137-17-7	
21	2-methoxyaniline	90-04-0	
22	2-methoxyaniline 4-aminoazobenzene	60-09-3	

Carcinogenic properties of aromatic amines for the first time have been described by German doctor Ludwig Ren who has observed increased incidence on tumour diseases (bladder cancer) in men employed in factories producing dyes who were long-term exposed to big amount of aniline [3].

Some of azo dyes after azo group reduction, so after transformation to appropriate aromatic amines, show biological activity and mutagenic properties. These amines generally are more toxic than initial compounds what is due to physicochemical properties. Hence of these amines influence on living organisms is strictly connected with the character and position of substituent attached to aromatic molecule fragment. According to European Union regulations [6] azo dyes are characterized by low acute toxicity-LD₅₀ in the range of 250-2000 mg/kg of body weight. Some

of them which belong to acid, basic or direct azo dyes can be very toxic in relation to aquatic organisms, whereas reactive azo dyes show toxicity just in concentrations above 100 mg/dm³ [4].

Sample preparation

Determination of azo dyes in consumer goods samples can require several consecutive steps which include: reduction and extraction process, extract treatment and then quality and quantity determination with instrumental analysis techniques.

For azo compounds reduction mostly sodium dithionite is used [7-10]. M. C. Garrigós et al. made a research on influence a number of parameters on reduction process (quantity of reducing reagent, reduction temperature and time). Afterwards, developed method was applied for reduction of 9 azo dyes used for toys production [8]. Another reductors in use are tin oxide (II) [11], zink powder in the presence of ammonium formate or formic acid [12].

The next step, which included sample preparation before analytes determination is extraction from the matrix. For extraction of aromatic amines resulting from dyes reduction the following methods are applied: solvent extraction [7], microwave assisted extraction (MAE) [9, 13], supercritical fluid extraction (SFE) [9, 13], Soxhlet extraction [13], accelerated solvent extraction (ASE) [14, 15] (mainly to solid and semisolid samples) and solid phase extraction (SPE) (for liquid samples) [16, 17].

For extraction of amines, which were created after commercially available dyes reduction, methyl acetate was used [7], whereas to separate analytes from hair dyes dichloromethane was used [18]. Sparr Eskillson et al. have compared efficiency of two aromatic amines extraction methods: methanol microwave assisted extraction and carbon dioxide supercritical extraction. For both methods following analytes recovery values: 62 % and 60% respectively, were obtained for leather samples [9]. M. C. Garrigós et al. have also influence of above described extraction methods investigated as well as Soxhlet extraction on efficiency of three dyes separation (Solvent Yellow 14, Solvent Red 24 i Disperse Red 1) from polyvinyl chloride samples enriched of this dyes. They have optimized procedures to select many parameters and they have compared it for reproducibility, selectivity and recovery. The highest recovery values were achieved for microwave assisted extraction. Authors have also shown optimization of carbon dioxide supercritical extraction and application of this method for selected amines separation (4-chloro-o-toluidyne, 2-naphtylamine, 4-aminodiphenyl, benzidine) from finger paints for children [19, 20].

Dionex has shown application note concerning accelerated solvent extraction which enable dyes extraction with simultaneous sodium dithionite reduction to appropriate aromatic amines. This method was used to separate analytes from textiles. The procedure concludes detailed description of parameters applied to extraction and chromatographic separation [14]. This application was used by J. Paterson et al. for 22 aromatic amines from textiles determination using HPLC with UV and MSQ Plus detector [15].

Azo dyes and aromatic amines determination

Determination of selected aromatic amines derived from azo dyes decomposition constitutes an important element for security control in industries which use this dyes. Many European and international regulations (REACH, GB i KC MARK) [21] strictly limit its presence and often demand aromatic amines analyzing using at least two independent chromatographic methods in order to avoid misidentification due to the presence of matrix interferences or structural isomers. For determination of trace quantity azo dyes and derive from them aromatic amines in different matrixes, a few analytical techniques are used: thin layer chromatography (TLC) [22, 23], gas chromatography (GC) [7, 16, 17, 22, 24-30] and high performance liquid chromatography (HPLC) [8, 9, 18, 22, 31-35]

with different detectors types. While TLC and GC are generally used to quality analyze, HPLC can be applied in quantitative analyte determination.

In the literature examples of aromatic amines determination are accessible. The matrix can be: environmental samples (water, sewages, soils, sediments) [16, 17, 27–29], biological [36] and in food [35, 37], whereas just not numerous examples refer to identification of discussed substances group in consumer goods samples [13, 20, 22, 26, 28].

Gas chromatography

In determination of both free amines and this obtained after azo compounds degradation, gas chromatography coupled to mass spectrometry (GC-MS) was used. This technique enable to testing compounds determination, however, the method is often insufficiently selective in case of simultaneous determination of wide range analytes. Besides, it is needed to sample derivatization to obtain both lower values of detection limits and better chromatographic separation. As an derivatizing agent heptafluorobutyric acid anhydride (HFBA) [24] and pentafluoropropionic acid anhydride (PFPA) [25] are used. Procedure of 73 primary and secondary aromatic amines determination which constitute alkyl-, chloro- and nitro- derivatives of aniline, benzidine, aminonaphthalene and aminobiphenyl M. Lango i A. Cavallaro have shown. Amines were analyzed by gas chromatography with ion trap coupled to mass spectrometer using HFBA for derivatization. Low values of detection limits were contained in the range of 0.01–66.3 pg and prepared method has found application to analyte coming from polluted underground water determination [24]. M. S. Narveker i A. K. Srivastava for derivatization of samples containing 20 aromatic amines benzene sulfonyl chloride, acetyl chloride, dansyl chloride have used, however, just PFPA application enabled to obtain analytes derivatives with high efficiency. Prepared method was used to chloroaniline isomers determination in samples of commercially available dyes [25].

S. W. Oh et al. have showed analyses method of aromatic amines which come after azo dyes sodium dithionite reduction [7]. To post reduction analytes separation the thin-layer chromatography was used and the GC/MS for extract achieving. 2-naphthylamine, 2,4-toluenediamine, 4,4'-diaminesiphenylemethane, 4-aminephenol and benzidine were detected in dyes in the quantity above 50 mg per kg of dye and above 5 mg per kg of dyed products.

A. Plum et al. have described fast and simple method of direct analyse of 21 aromatic amines using pyrolysis gas chromatography coupled to mass spectrometry. The described method didn't require any initial stage of sample preparation. In testing textiles samples (cotton, wool, synthetic leather, polyamide) 2-nahtylamine, benzidine, 3,3-dimethylbenzidine and 4-aminebiphenyl in the range of 5–700 ng/kg of sample were detected [26].

High performance liquid chromatography

High performance liquid chromatography is definitely the most frequent technique applied for determination of discussed chemical compounds group. HPLC enable quantity and quality analysis with different chromatographic column and gradient programs based on water/buffer and methanol/acetonitrile as a mobile phase.

M. C. Garrigós et al. have shown the method of 22 aromatic amines determination using high performance liquid chromatography with spectrophotometric detector and methanol-phosphate buffer as a mobile phase in gradient program. This method was used for amines from toys determination. Selection of following process parameters was also made: reaction temperature, time of duration, quantity of reductive reagent (sodium dithionite) [8].

The mixture of methanol and phosphate buffer as a mobile phase was also used for o-toluidyne, 4-chloroaniline, benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine and 3,3'-dichlorobenzidine derived from following azo dyes: acid red 035,

acid orange 031, acid black 077, acid black 209, direct blue 015 and direct red 061. Analytes extraction from leather samples was carried out by two methods: microwave assisted extraction (MAE) and supercritical fluid extraction (SFE). The second method proved more efficient and gave higher recovery values of tested compounds [9].

A high performance liquid chromatography with electrochemical detection was shown by T. M. Lizier i M. V. Boldrin Zanoni. For 16 aromatic amines separation a mixture of methanol and ionic liquid BMIm[NTf₂] was used as a mobile phase and recovery values in the range of 95–103% were achieved. Optimized method was used for amines from hair dyes determination with dichloromethane previous extraction [18].

The table mentioned below (Tab. 2) shows collective specification of tested aromatic amines occurring in textiles and determined by high performance liquid chromatography and gas chromatography with previous stage of sample preparation (derivatization, extraction, reduction).

Summary

The most appropriate analytical technique, which enables for aromatic amines and azo dyes determination in consumer goods, is high performance liquid chromatography. It characterizes both quick sample preparation stage (ASE) without previous derivatization and quick analysis (several minutes) with simultaneous high recovery and low detection limits of tested azo dyes and aromatic amines.

Table 2
Determination methods of aromatic amines derived from azo dyes in consumer goods

Analyte (No. from Tab. 1)	Matrix	Sample preparation	Detection	Literature item
(1-22)	Textiles	chlorobenzene extraction and/or sodium dithionite reduction	TLC, HPLC-DAD, HPLC-MS, GC-FID, GC-MS, CE	[22]
2,5-dimethylaniline, (1-20)	Textiles (cotton, wool, synthetic leather, polyamide)	Lack of preparation step, direct analyse	Py-GC-MS	[26]
(3,4,6,7,18,19,21) and its derivatives	Dyes	Test no. 35LMBG, method no. B82.02-2 (1996) pentafluoropropionic acid anhydride derivatization	GC-MS	[25]
(2,7,10-12,18)	Leathers	MAE, SFE	HPLC-DAD	[16]
(1,2,4,6,7,9-12,14-16,18,21)	Hair dyes	Solvent extraction	HPLC-ED	[28]
(1-20)	Dyes, dyeing products (textiles, leather)	Sodium dithionite reduction	GC-MS	[7]
1,4-phenylenediamine, aniline, 2,4-dimethylaniline, 2,6-dimethylaniline, (1-22)	Textiles, leather	Procedure ISO 14362-1:2012 Procedure ISO 17234-1:2010 and 17234-2:2010	HPLC-MS (QQQ)	[31]
Solvent Yellow 14, Solvent Red 24, Disperse Red 1	Polyvinyl chloride	Solvent extraction in Soxhlet, MAE, SFE	HPLC-UV	[13]
(1-4)	Finger paints	SFE	GC	[20]
(1-22)	Textiles	ASE with direct sodium dithionite reduction	HPLC-UV HPLC-MS	[14]
(1-22)	Textiles	ASE with direct sodium dithionite reduction	HPLC-UV/ MSQ Plus Detector	[15]

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*Małgorzata MOŚCIPAN - Ph.D., graduated from the Faculty of Chemistry, Opole University (2009). Her doctoral thesis (Environmental Ph.D. Studies in Opole and Wrocław University of Technology) was graduated in 2013. Scientific interests: environment analytics, agrobiotechnology, compounds synthesis. Nowadays she is an assistant in the liquid chromatography laboratory of Analytical Department in the Institute of Heavy Organic Synthesis "Błachownia", Kędzierzyn-Koźle
e-mail: moscipan.m@icso.com.pl, phone: +48 77 487 3492

Magdalena ZARĘBSKA - M.Sc., (Eng.), graduated from the Faculty of Chemistry, Silesian University of Technology (2004). Scientific interests: analytics in quality control and environment protection, biodegradation, liquid chromatography, metrology. Nowadays she is an assistant in the liquid chromatography laboratory of Analytical Department in the Institute of Heavy Organic Synthesis "Błachownia", Kędzierzyn-Koźle
e-mail: zarebska.m@icso.com.pl, phone: +48 77 487 3492

Renata KULESZA - Ph.D., (Eng.), graduated from the Faculty of Engineering and Chemical Technology, Cracow University of Technology (1992). Her doctoral thesis (Environment Engineering Department, Cracow University of Technology) was awarded in 2003. Nowadays she is a Manager of the Analytical Department in the Institute of Heavy Organic Synthesis "Błachownia", Kędzierzyn-Koźle. Scientific interests: analytics, engineering and environment protection, chemical technology, products safety, management of innovative projects. She is the author of 6 chapters in monographs, 11 articles in scientific journals and co-author of 42 communications and posters at national and international conferences. She is the co-author of 3 patent application.
e-mail: kulesza.r@icso.com.pl, phone: +48 77 487 3568