

Detection, Identification, and Quantitation of Azo Dyes in Leather and Textiles by GC/MS

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Keywords: azo dyes, cancerogenic amines, textiles, leather, EN ISO standard method, ISQ Series GC/MS, fast full scan detection, quantitation, confirmation, library search.

Introduction

Azo dyes are compounds characterized by their vivid colors and provide excellent coloring properties. They are important and widely used as coloring agents in the textile and leather industries. The risk in the use of azo dyes arises mainly from the breakdown products that can be created *in vivo* by reductive cleavage of the azo group into aromatic amines. Due to the toxicity, carcinogenicity and potential mutagenicity of thus formed aromatic amines, the use of certain azo dyes as textile and leather colorants, and the exposure of consumers using the textile and leather colored with azo compounds causes a serious health concern^[1]. The two main routes of consumer exposure are the skin absorption of the azo compounds from the dyed clothes worn, and potential oral ingestion, mainly referring to the sucking of textiles by babies and young children. The manufacturing workers can also be exposed via the inhalation route.

The EU Commission classified 22 amines as proven or suspected human carcinogens. “Azo dyes which, by reductive cleavage of one or more azo groups, may release one or more of these aromatic amines in detectable concentrations, i.e. above 30 ppm in the finished articles or in the dyed parts thereof ... may not be used in textile and leather articles which may come into direct and prolonged contact with the human skin or oral cavity”^[2]. The EU Directive 2002/61/EC has banned the use of dangerous azo colorants, placing textiles and leather articles colored with such substances on the market, and requested the development of a validated analytical methodology for control. Since the azo dyes are one of the longest known synthetic dyes, simple and inexpensive in preparation, available easily in bulk and in great variety, and rarely cause acute symptoms, the textile manufactures can be persuaded to use them despite the regulations — if the strict and reliable analytical control is not imposed.



Experimental Conditions

Sample Preparation

The sample preparation for the analysis of textile samples depends on the nature of the textile. The textiles made of cellulose and protein fibers, for example cotton, viscose, wool, or silk^[3] make the azo dyes accessible to a reducing agent without prior extraction. The EN ISO 17234-1 standard method for the analysis of such textiles is based on the chemical reduction of azo dyes followed by solid phase extraction (SPE) with ethyl acetate providing a ready-to-inject extract after solvent concentration.

The analysis of synthetic fibers like polyester, polyamide, polypropylene, acrylic or polyurethane materials requires prior extraction of the azo dyes and is described in the EN 14362-2 standard method. The analysis of leather samples follows the EN ISO 17234 standard method.

The azo group of most azo dyes can be reduced in the presence of sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) under mild conditions ($\text{pH} = 6$, $T = 70^\circ\text{C}$), resulting in the cleavage of the diazo group and formation of two aromatic amines as the reaction products. The amines are extracted by liquid-liquid extraction with t-butyl methyl ether (MTBE), concentrated, adjusted to a certain volume with MTBE, then analyzed by GC/MS. The quantitation is performed

with the internal standards Naphthalene-d8 and 2,4,5-trichloroaniline.

In the EN ISO 17234-1 standard method, the directly reduced amines are isolated by SPE and analyzed by GC/MS. Some aromatic compounds with nitro groups can also be reduced to amines under the conditions of this standard method, resulting in the false positive detection. The Thermo Scientific™ TraceFinder™ data processing method takes care of false positive peaks by checking confirming ions at the expected retention time of each compound, see the list of target and confirming ions in Table 1.

GC/MS Analysis

The GC/MS analysis has been carried out using a Thermo Scientific™ ISQ™ Series single quadrupole GC/MS system. The analytical conditions for GC and MS are given in the Tables 2 and 3, the GC oven temperature program is shown in Figure 1.

Table 2: Thermo Scientific™ TRACETM 1310 GC method

Injector:	split/splitless
Injection mode:	splitless, 1 min splitless time
Injection volume:	1 µL
Injector temp.:	200 °C
Analytical column:	TG-35MS or equivalent polarity column
Column dimensions:	30 m length×0.25 mm ID×0.25 µm film thickness (Cat. No. 26094-1420)
Carrier gas:	Helium, 1.0 mL/min, constant flow
Oven program:	60 °C, 1 min 15 °C/min to 200 °C 25 °C/min to 310 °C 310 °C, 5 min
Transfer line temp.:	295 °C

The ISQ mass spectrometer was set to run in full scan mode, providing the complete mass spectra of the detected compounds for identification, confirmation and quantitation. The fully removable electron impact (EI) ion source of the ISQ LT GC-MS has been setup by using the standard autotune program delivered with the instrument.

Table 3: ISQ MS method

Ionization:	EI, 70 eV
Ion source temp.:	220 °C
Scan mode:	Full scan
Mass range:	50 – 350 Da
Scan time:	75 ms

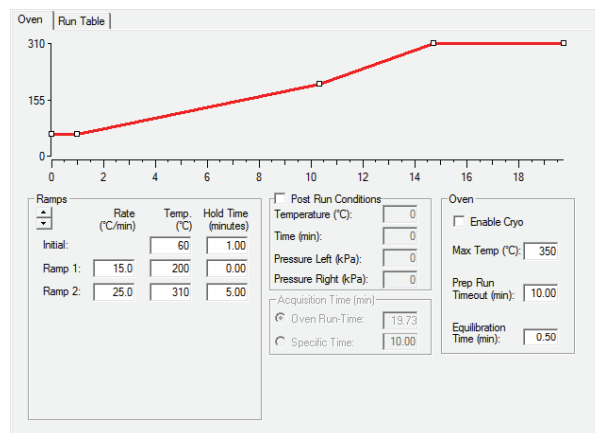


Figure 1. TRACE 1310 GC oven program

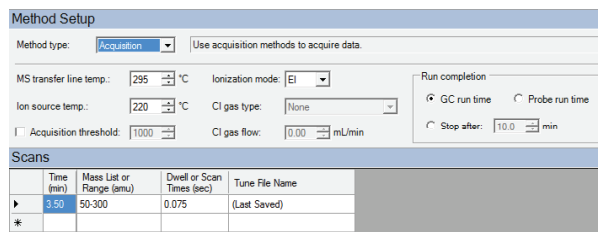


Figure 2. ISQ MS acquisition method setup

Table 4. Amine compounds included in the method*

Amine Compound	CAS#	RT	Quan [min]	comment [m/z]
Aniline	62-53-3	5.42	93	2
o-Toluidine	95-53-4	6.43	106	
2,4-Xylidine	95-68-1	7.35	121	2
2,6-Xylidine	87-62-7	7.39	121	2
Naphthalene-d8	1146-65-2	7.50	136	ISTD
2-Methoxyaniline	90-04-0	7.61	108	
p-Chloroaniline	106-47-8	7.97	127	
m-Anisidine	536-90-3	8.40	123	2
p-Cresidine	120-71-8	8.50	122	
2,4,5-Trimethylaniline	137-17-7	8.57	120	
4-Chloro-o-toluidine	95-69-2	8.90	106	
1,4-Phenylenediamine (1,4-Benzendiamine)	106-50-3	8.91	108	1,2
2,4-Toluenediamine	95-80-7	10.09	122	1
2,4-Diaminoanisole	615-05-4	10.91	123	1
2,4,5-Trichloroaniline	636-30-6	11.08	195	ISTD
2-Naphthylamine	91-59-8	11.44	143	
5-Nitro-o-toluidine	99-55-8	11.85	152	
4-Aminodiphenyl	92-67-1	12.66	169	
p-Aminoazobenzene	60-09-3	14.36	92	
4,4-Oxydianiline	101-80-4	14.62	200	
4,4-Diaminodiphenylmethane	101-77-9	14.66	198	
Benzidine	92-87-5	14.71	184	
o-Aminoazobenzene	2835-58-7	15.01	106	2
3,3-Dimethyl-4,4-diaminodiphenylmethane	838-88-0	15.31	226	
3,3'-Dimethylbenzidine	119-93-7	15.47	212	
4,4'-Thiodianiline	139-65-1	16.04	216	
4,4-Methylene-bis-2-chloroaniline	101-14-4	16.23	231	
3,3'-Dimethoxybenzidine	119-90-4	16.24	244	
3,3-Dichlorobenzidine	91-94-1	16.26	252	

*CAS # is CAS Registry Number, RT is the expected retention time, Quan is the quantitation ion.

Comments:

1. Compounds are unstable and tend to deteriorate at temperatures above 20°C
2. Additional user specific compounds included in the assay, not part of EN ISO 17234-1

Results

The scan rate of the ISQ LT GC-MS in the full scan mode has been set to a very fast scan speed of only 75 ms/scan. This allows a very high chromatographic resolution especially of unresolved chromatographic peaks in the injected complex mixture of amines. Short analysis cycle times can be achieved for an increased sample throughput.

The GC oven program and flow conditions have been optimized accordingly for short run times of ca. 20 minutes and the particular requirements for the target compound separation criteria.

Isomeric amine compounds need to be separated on the chromatographic time scale for individual component quantitation. The mass spectra of isomers are very similar and typically do not offer unique quantitation ions for the independent quantitation. This is the case for the isomeric xylidine compounds 2,4-xylidine and 2,6-xylidine. The applied method separated both compounds very well with 7.33 and 7.38 minutes retention time, see Figure 3.

All other target amine analytes produce distinct mass spectra with unique ions available for selective quantitation, even if the chromatographic peaks in the total ion chromatogram are not resolved from each other. In these situations, when several analytes coelute, individual mass spectra can still be isolated and identified by a library search. The intensities of the unique ions allow the interference-free quantitation based on the separated mass traces. In the case of the coelution of 4,4-methylene-bis-2-chloroaniline (RT 16.23 min), 3,3'-dimethoxybenzidine (RT 16.24 min), and 3,3'-dichlorobenzidine (RT 16.24 min) shown in Fig. 4, only a partial chromatographic resolution with the given conditions of the method is achieved, but a safe peak integration and quantitation have been accomplished.

For all co-eluting target compounds, the quantitation has been performed on the corresponding unique fragment ions. The fast scanning mode of ISQ LT mass spectrometer allows the detection of small retention time differences so that even the clean spectra of each of the compounds can be isolated for library search, see Figure 5 a-c. All three compounds are safely identified by searching the NIST library. The spectra have been taken from a spiked calibration file at the 3 ppm level. The high sensitivity of the ISQ LT mass spectrometer provides complete mass spectra for a reliable compound confirmation, as well as quantitation, even at the required low detection level and fast scanning speed.

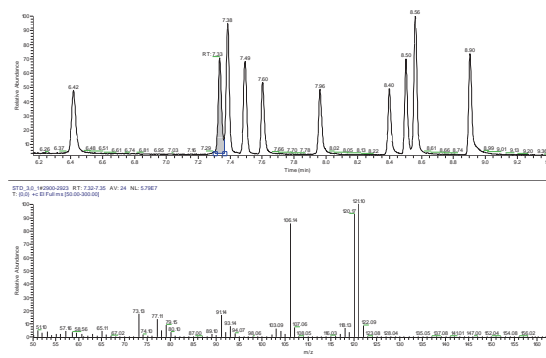


Figure 3. Chromatographic separation of 2,4- and 2,6-xylidine at retention times 7.33 and 7.38 min, with mass spectrum of the integrated 2,4-Xylidine peak below.

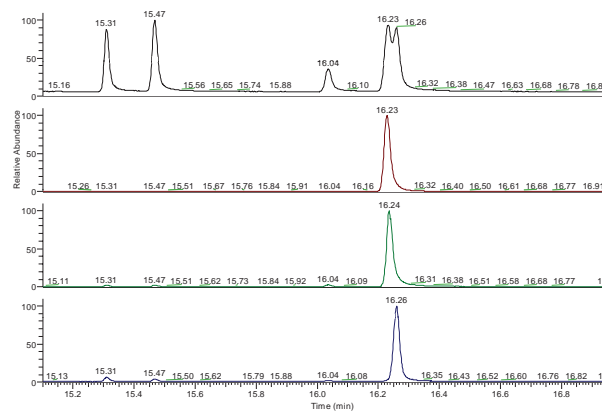


Figure 4. Coelution of three different amines, separated by individual mass traces.

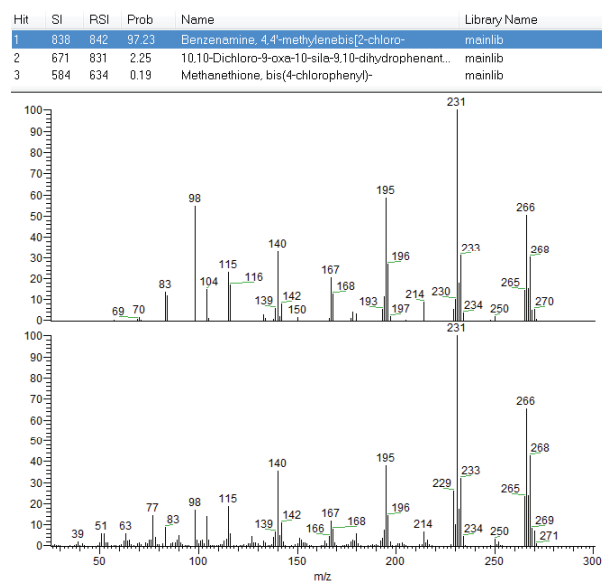


Figure 5a.

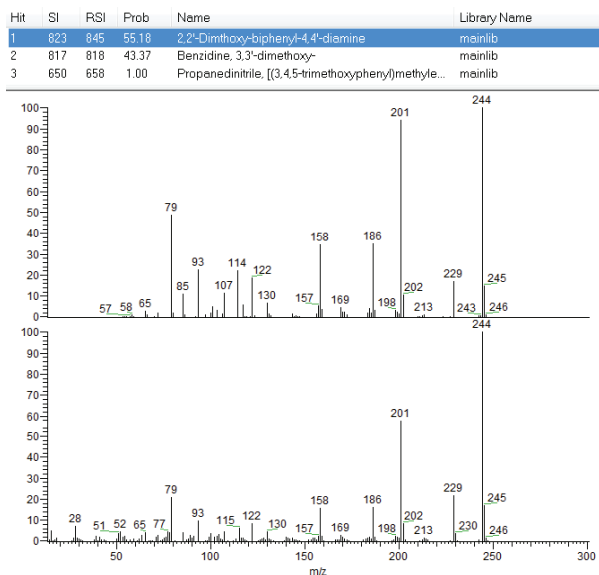


Figure 5b.

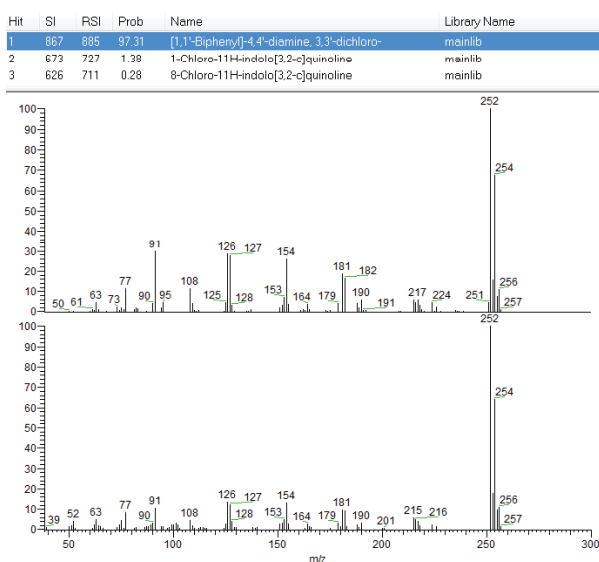


Figure 5c.

Figure 5. Acquired mass spectra and library search results for the coeluting compounds of Figure 4. (top acquired spectrum from data file in Figure 4, bottom spectrum from NIST library)

- 4,4-Methylene-bis-2-chloroaniline (CAS 101-14-4)
- Dimethoxybenzidine (CAS 119-90-4)
- 3,3-Dichlorobenzidine (CAS 91-94-1)

The quantitation was done by using the two internal standards Naphthalene-d8 RT 7:50 minutes and 2,4,5-trichloroaniline RT 11:08 minutes. A linear calibration with $R^2 > 0.9998$ was created using standards over three levels at 0.5, 3.0, and 10.0 ppm, representing 5, 30, and 100 ppm in the textile. An example of a routine QC report for the reporting level of 30 ppm is shown in Figure 6. The list of compounds and the chromatogram of the QC sample is shown. All compounds were detected, integrated and passed the confirmation check based on the compound mass spectrum.

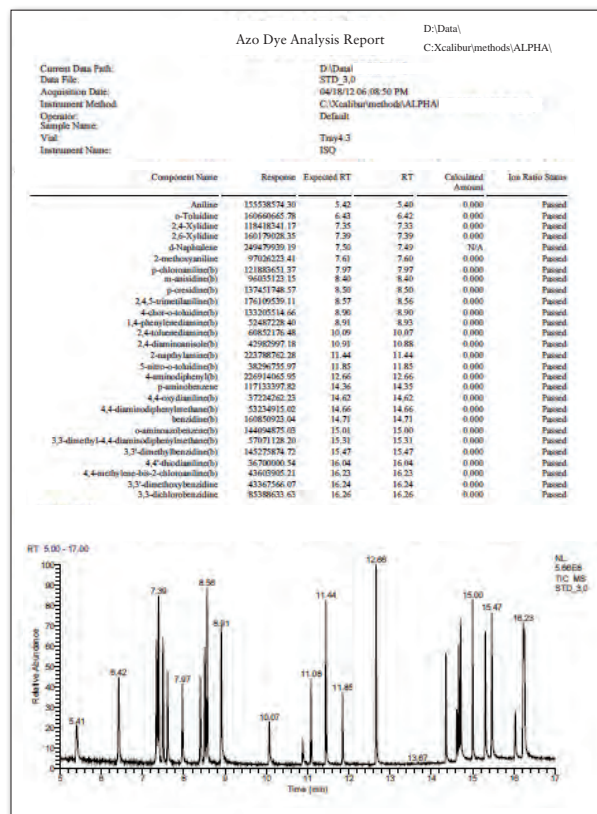


Figure 6. Report of a routine QC sample at the reporting level of 30 ppm.

Conclusions

It has been demonstrated that the Thermo Scientific ISQ Series GC/MS system achieved high sensitivity in full scan mode together with high productivity in the analysis of azo dyes in textiles. The described method has the advantage of the complete mass spectra for identification by library search, quantitation on the compound specific fragment ions and the confirmation of positive findings by comparing the spectral information with the detected peak.

The high acquisition rate allows excellent chromatographic resolution for difficult to separate compounds, while maintaining short cycle times for high productivity with high number of samples to run.

Together with the data processing method and QA/QC checks, this application is compliant with the requirements of the EN ISO 14362-1 standard procedure for the analysis of certain azo dyes in cotton and silk textiles. Following appropriate sample preparation methods, the described analytical setup can be used for the analysis of azo dyes in leather and synthetic fabric as well.

References

- [1] European Commission - Health and Consumers, Scientific Committees Opinion, see http://ec.europa.eu/health/scientific_committees/environmental_risks/opinions/sctee/sct_out27_en.htm, Brussels, 18 January 1999.
- [2] Directive 2002/61/EC of the European Parliament and of the Council of 19 July 2002 amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azocolourants). of 19 July 2002 amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azocolourants).
- [3] EN ISO 14362-1, Textiles - Methods for the determination of certain aromatic amines derived from azo colorants - Part 1: Detection of the use of certain azo colorants accessible without extraction.

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