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Quantitative Determination of Aromatic Amines formed from Azo Dyes in Textile using GCMS/MS

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Introduction

Azo dyes are extensively used for colouring a variety of consumer goods, such as leather, clothes and toys[1]. Under certain conditions they can be reduced to form potential carcinogens. Reductive cleavage of azo radicals (-N=N-) from azo dyes and azo pigments can form aromatic amines (Figure 1) that are suspected carcinogens.

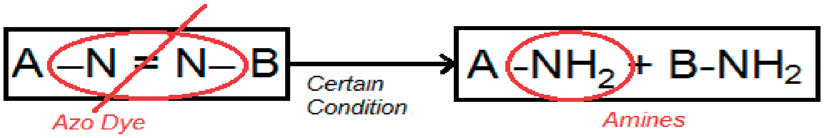


Figure 1. Reductive cleavage of azo radical to amines

The various regulations stipulate different groups of aromatic amines with varying concentration limits. Among these, the Chinese regulation GB 18401:2010 is most stringent. It allows individual limit of 20 mg/kg for 24 aromatic amines.
The EU Commission has classified 22 amines as either proven or suspected human carcinogens. Azo dyes, by reductive cleavage of 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]. Hence, it is necessary to quantify these banned amines in such products.
GCMS-TQ8040 triple quadrupole system (Figure 2) from Shimadzu Corporation Japan with Smart MRM Optimization tool was used to create easy, selective and reproducible analytical method.

Figure 2. GCMS-TQ8040 Triple quadrupole system by Shimadzu

Key Features of GCMS-TQ8040

1. Smart Productivity : Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.
2. Smart Operation : Smart MRM technology creates optimal MRM methods automatically. The “MRM Optimization Tool” automates best MRM transitions for new compounds.
3. Smart Performance : ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest MRM 800 trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.

Method of Analysis

Extraction of Banned Amines from Textile sample

The sample preparation depends on the nature of the textile. Synthetic fibers like polyester, polyamide, polypropylene, acrylic or polyurethane materials require prior extraction of the azo dyes and is described in the standard EN 14362-2 method. The analysis of leather samples follow the standard EN ISO 17234-2 method. The EN ISO 17234-1 method for the analysis of textiles like cotton, viscose, wool, or silk utilizes chemical reduction of azo dyes followed by solid phase extraction (SPE) with ethyl acetate (EA) providing a ready-to-inject extract after solvent concentration.

The azo group present in dyes was reduced by using sodium dithionite ($Na_2S_2O_4$) under mild conditions of pH 6 and 70 °C temperature. This resulted in cleavage of the diazo group and formation of two aromatic amines as the reaction products. These amines were extracted by liquid-liquid extraction with EA, concentrated, adjusted to a certain volume with EA. Injected 1 μL of extract into Shimadzu GCMS-TQ8040 system.

Standard Preparation

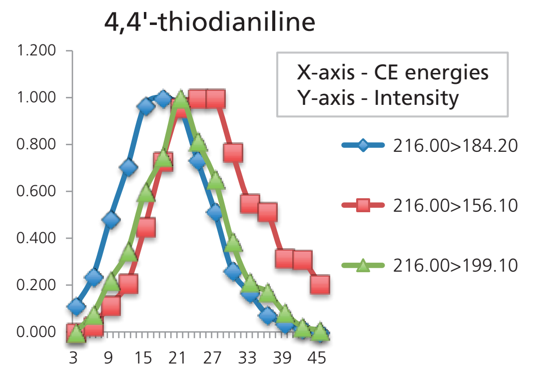
About 100 ppm Banned Amines standard mixture procured by Sigma Aldrich® was prepared in methanol. This stock solution was diluted to prepare linearity solutions of 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm along with 1-Naphthylamine as an internal standard.

MRM method development

For MRM optimization, about 10 ppm standard mixtures was analyzed in scan mode. Retention times of individual components were identified and their precursor ions were selected. Using selected precursor ion, product ion scan was performed with different Collision Energies (CE). MRM transitions for each component were studied to plot optimization graph as shown in Figure 3. From this, MRM transition with appropriate CE were determined. All the above steps were simplified with the help of Smart MRM optimization tool as mentioned in Table 1. These optimized MRM transitions were registered to Smart Database. Using Smart Database, MRM method with overlapping segments was created. Overlapping segments enabled monitoring of MRM transition with higher dwell times leading to better sensitivity for close eluting peaks as shown in Figure 4.

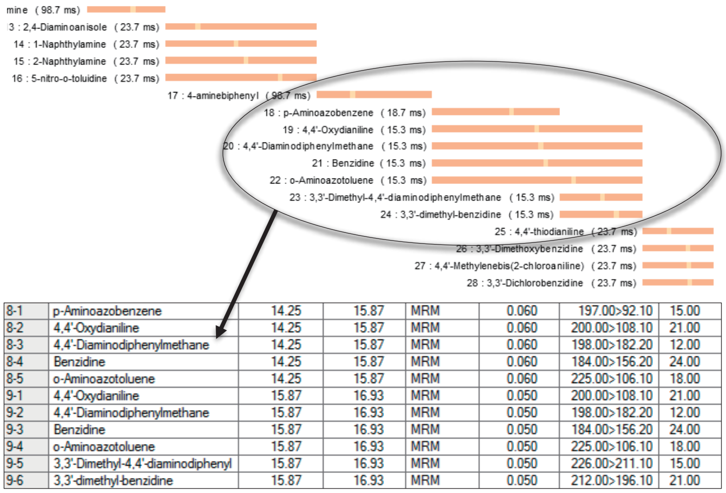
Table 1. Method creation using Smart MRM feature of GCMS-TQ8040

Type of compounds	Step.1	Step.2	Step.3	Step.4
Banned Amines	Measure in Scan mode and Determine Pre-cursor ion	**MRM Optimization Tool** Create Batch sequence and Method file of several Collision Energy automatically.	**MRM Optimization Tool** Analyze acquired data files, and select the best transitions and collision energy automatically. And the result can be exported to Smart MRM database on a mouse click.	Method creation using Smart Database



Product m/z	216.00>184.20	216.00>156.10	216.00>199.10
Max Int.	15095	2967	2253
CE	18	24	21

Figure 2. CE Optimization graph for 4, 4’-trichloroaniline



8-1	p-Aminoazobenzene	14.25	15.87	MRM	0.060	197.00>92.10	15.00
8-2	4,4'-Oxydianiline	14.25	15.87	MRM	0.060	200.00>108.10	21.00
8-3	4,4'-Diaminodiphenylmethane	14.25	15.87	MRM	0.060	198.00>182.20	12.00
8-4	Benzidine	14.25	15.87	MRM	0.060	184.00>156.20	24.00
8-5	o-Aminoazotoluene	14.25	15.87	MRM	0.060	225.00>106.10	18.00
9-1	4,4'-Oxydianiline	15.87	16.93	MRM	0.050	200.00>108.10	21.00
9-2	4,4'-Diaminodiphenylmethane	15.87	16.93	MRM	0.050	198.00>182.20	12.00
9-3	Benzidine	15.87	16.93	MRM	0.050	184.00>156.20	24.00
9-4	o-Aminoazotoluene	15.87	16.93	MRM	0.050	225.00>106.10	18.00
9-5	3,3'-Dimethyl-4,4'-diaminodiphenyl	15.87	16.93	MRM	0.050	226.00>211.10	15.00
9-6	3,3'-dimethyl-benzidine	15.87	16.93	MRM	0.050	212.00>196.10	21.00

Figure 3. MRM method with overlapping segments

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8040 as per the conditions given in Table 2.

Table 2. Analytical conditions

Chromatographic parameters

Column	: SH-Rxi™-35Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Split
Split Ratio	: 10.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 36.5 cm/sec
Column Flow	: 1.00 mL/min
Purge Flow	: 3.0 mL/min
Injection Volume	: 1.0 µL
Total Program Time	: 20.83 min
Column Temp. Program	:

Rate (°C /min)	Temperature (°C)	Hold time (min)
	60.0	1.00
15.00	200.0	0.00
20.00	310.0	5.00

Mass Spectrometry parameters

Ion Source Temp.	: 230.0 °C
Interface Temp.	: 280.0 °C
Ionization Mode	: EI (Electron Ionization)
Acquisition Mode	: MRM

Results

All the linearity solutions and extracted samples were analyzed using the analytical method parameters shown in Table 2.
Linear response with $r^2 \geq 0.995$ was obtained. %RSD achieved for 1ppm standard mixture was less than 3 for six repetitions as shown in Table 3.

Banned Amines in the sample were quantified against linearity curve and results are shown in Table 4. Figure 4 depicts detailed data for one of the Banned Amines, benzenamine, N,N-dimethyl- (1 ppm) (ID. No. 2).
Acquired data was processed using Shimadzu LabSolutions Insight software as shown in Figure 5 and 6.

Table 3. Quantitation results

ID	Banned Amines	Retention time (min)	Target MRM (m/z)	r^2	% RSD at 1 ppm (n=6)
1	Aniline	5.635	93.00>66.10	0.9980	0.72
2	Benzenamine, N,N-dimethyl-	6.665	120.00>77.10	0.9999	0.50
3	o-Toluidine	6.705	106.00>77.10	0.9982	1.18
4	2,6-Xylidine	7.655	121.00>106.10	0.9992	0.33
5	2,4-Xylidine	7.715	121.00>106.10	0.9979	0.36
6	o-Anisidine	7.930	123.00>108.10	0.9984	0.31
7	p-Chloroaniline	8.300	127.00>65.10	0.9982	0.48
8	p-Cresidine	8.855	137.00>122.10	0.9985	0.31
9	2,4,5-trimethyl-aniline	8.920	135.00>120.10	0.9996	1.36
10	1,4 phenylenediamine	9.225	108.00>80.10	0.9940	3.00
11	4 Chloro-o-toluidine	9.265	141.00>106.10	0.9998	0.18
12	2,4-Toluenediamine	10.445	122.00>105.10	0.9938	1.34
13	2,4-Diaminoanisole	11.270	138.00>123.10	0.9992	0.95
14	1-Naphthylamine*	11.755	143.00>115.10	ISTD	ISTD
15	2-Naphthylamine	11.885	143.00>115.10	0.9981	0.58
16	5-nitro-o-toluidine	12.320	152.00>106.10	0.9929	2.37
17	4-aminebiphenyl	13.240	169.00>167.10	0.9975	0.80
18	p-Aminoazobenzene	15.260	197.00>92.10	0.9958	1.22
19	4,4'-Oxydianiline	15.585	200.00>108.10	0.9974	1.20
20	4,4'-Diaminodiphenylmethane	15.635	198.00>182.20	0.9978	0.77
21	Benzidine	15.695	184.00>156.20	0.9978	1.27
22	o-Aminoazotoluene	16.050	225.00>106.10	0.9960	1.36
23	3,3'-Dimethyl-4,4'-diaminodiphenylmethane	16.415	226.00>211.10	0.9984	1.21
24	3,3'-dimethyl-benzidine	16.595	212.00>196.10	0.9995	1.39
25	4,4'-thiodianiline	17.265	216.00>184.20	0.9968	1.84
26	3,3'-Dimethoxybenzidine	17.520	231.00>195.10	0.9971	1.54
27	4,4'-Methylenebis(2-chloroaniline)	17.511	244.00>201.10	0.9972	1.60
28	3,3'-Dichlorobenzidine	17.529	252.00>154.10	0.9977	0.94

Note * : Internal Standard (ISTD)

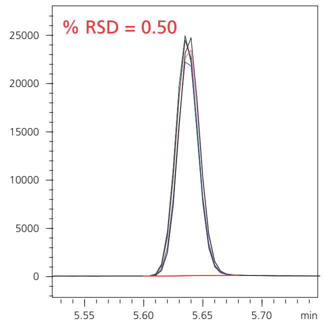
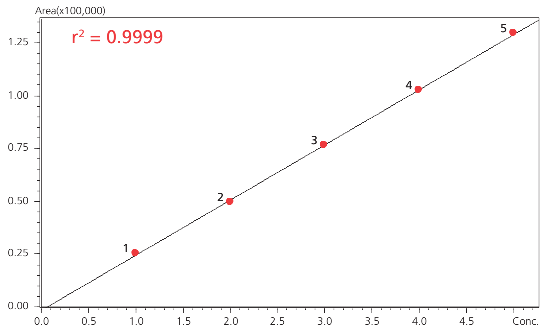
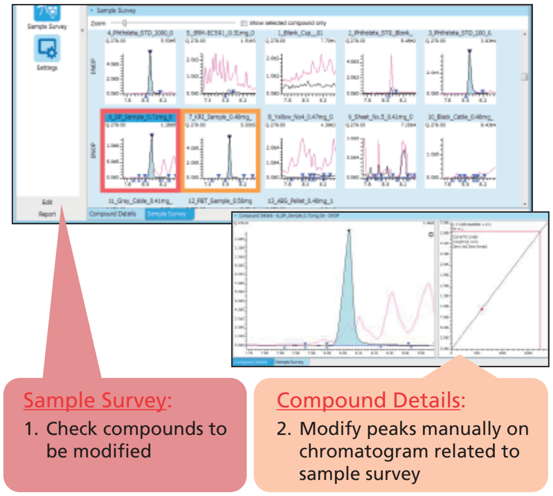
Overlay of precision data	Calibration curve
	

Figure 4. Detailed data for Benzenamine, N,N-dimethyl- (1 ppm)

Features of LabSolutions Insight

- Quantitative result for a complete series of data files can be displayed side by side for comparison and QC review.
- All the chromatograms for a selected target compound can be displayed simultaneously, making it easy to review the detected peaks and confirm the quantitative results.
- Color-coded QA/QC flags quickly identify any outliers that require further examination.
- Results can be displayed in a variety of ways, allowing users to select a view that is best suited for their workflow, and when necessary, peaks can be reintegrated and re-quantified directly from LabSolutions Insight.



Sample Survey:
1. Check compounds to be modified

Compound Details:
2. Modify peaks manually on chromatogram related to sample survey

Figure 5. Sample Survey Function

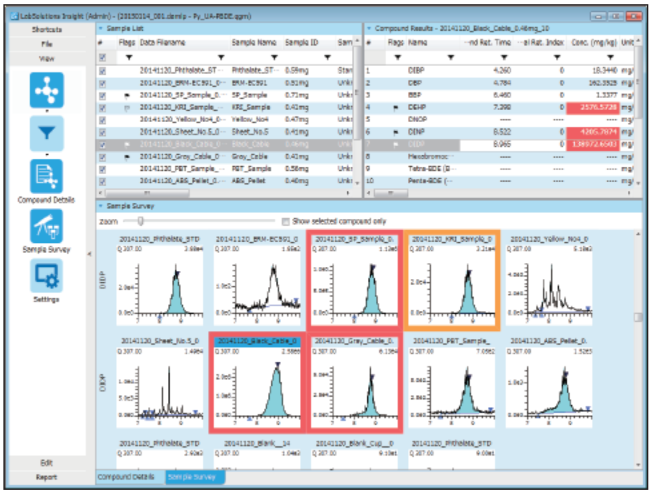


Figure 6. Flagging Function

Table 4. Sample results

ID	Banned Amines	Retention time (min)	Sample A	Sample B
1	Aniline	5.635	ND	ND
2	Benzenamine, N,N-dimethyl-	6.665	ND	ND
3	o-Toluidine	6.705	ND	ND
4	2,6-Xylidine	7.655	ND	ND
5	2,4-Xylidine	7.715	ND	ND
6	o-Anisidine	7.930	ND	ND
7	p-Chloroaniline	8.300	ND	ND
8	p-Cresidine	8.855	ND	ND
9	2,4,5-trimethyl-aniline	8.920	ND	ND
10	1,4 phenylenediamine	9.225	ND	ND
11	4 Chloro-o-toluidine	9.265	ND	ND
12	2,4-Toluenediamine	10.445	ND	ND
13	2,4-Diaminoanisole	11.270	41.14	ND
14	1-Naphthylamine*	11.755	ISTD	
15	2-Naphthylamine	11.885	ND	ND
16	5-nitro-o-toluidine	12.320	ND	ND
17	4-aminebiphenyl	13.240	ND	ND
18	p-Aminoazobenzene	15.260	ND	ND
19	4,4'-Oxydianiline	15.585	ND	ND
20	4,4'-Diaminodiphenylmethane	15.635	ND	ND
21	Benzidine	15.695	5.23	ND
22	o-Aminoazotoluene	16.050	ND	ND
23	3,3'-Dimethyl-4,4'-diaminodiphenylmethane	16.415	ND	ND
24	3,3'-dimethyl-benzidine	16.595	ND	ND
25	4,4'-thiodianiline	17.265	ND	ND
26	3,3'-Dimethoxybenzidine	17.520	ND	ND
27	4,4'-Methylenebis(2-chloroaniline)	17.511	ND	ND
28	3,3'-Dichlorobenzidine	17.529	ND	ND

Note * : Internal Standard (ISTD)

🟥 : Above Detectable concentration 🟨 : Below Detectable concentration

Conclusion

- Smart Database feature in Shimadzu GCMS-TQ8040 enabled automatic creation of MRM method with overlapping segments and optimum dwell times for achieving high sensitivity.
- LabSolutions Insight features eased multi-analyte data review, QA/QC flagging, re-integration, re-quantitation and reporting.

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).

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