White Paper 035
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Rapid quantitation of azo dyes in water and textiles by GC–TOF MS

This study describes the use of fast GC–TOF MS for high-throughput analysis of azo dye aromatic amines in textiles and water.

Introduction

Azo dyes are a class of compounds containing the azo (R–N=N–R') functional group, and are widely used colouring agents in consumer goods, especially in the leather and textile industries.

However, the use of azo dyes has sparked serious health concerns in recent years – mostly attributed to reductive cleavage of the azo bond, which can lead to harmful breakdown products, including a range of aromatic amines.[1] The toxicity, carcinogenicity and potential mutagenicity of these aromatic amines to humans, as well as their potential toxicity to aquatic organisms, has accelerated the need for rigid control measures.

The risk presented by azo dyes has resulted in numerous countries – including India, USA and Europe – banning their use. For example, EU Commission Regulation (EC) No 1907/2006 (later amended to 552/2009 – Annex XVII) restricts the use of azo dyes that can form any of the 22 named aromatic amines that are classified as proven or suspected human carcinogens. The regulations state that azo dyes, which release one or more of the classified compounds in concentrations greater than 30 mg/kg, must not be used in leather and textile consumer products that may come into direct and prolonged contact with human skin or the oral cavity.[2]

Nevertheless, azo dyes are still appealing to the textile industry since they offer a wide spectrum of colours, are inexpensive to prepare and simple to use – meaning that robust standard methods are required to ensure compliance with regulations.

Multiple standard methods have been developed, including ISO EN 14362-1[3] and Indian Standard 15570.[4] However, the analysis remains a challenge due to the complex matrices involved, the low detection limits imposed and the high turnover required to test all clothing prior to retail.

In this study, we develop a rapid method for the analysis of 26 aromatic amines using gas chromatography and time-of-flight mass spectrometry (GC–TOF MS), resulting in a total run time of just seven minutes and detection limits <1 ng/mL.

Experimental

Samples: Calibration series prepared for 26 target amines at concentrations of 1, 2, 10, 20, 100, 200 and 1000 ng/mL.

Autosampler: 1 µL injection; 5:1 split.

GC: Seven-minute injection-to-injection cycle time.

MS: BenchTOF-Evolve™ (SepSolve Analytical); Mass range: m/z 45–600; Acquisition rate: 10 Hz.

Software: Instrument control and data processing by TOF-DS™.

Please contact SepSolve for full analytical parameters.

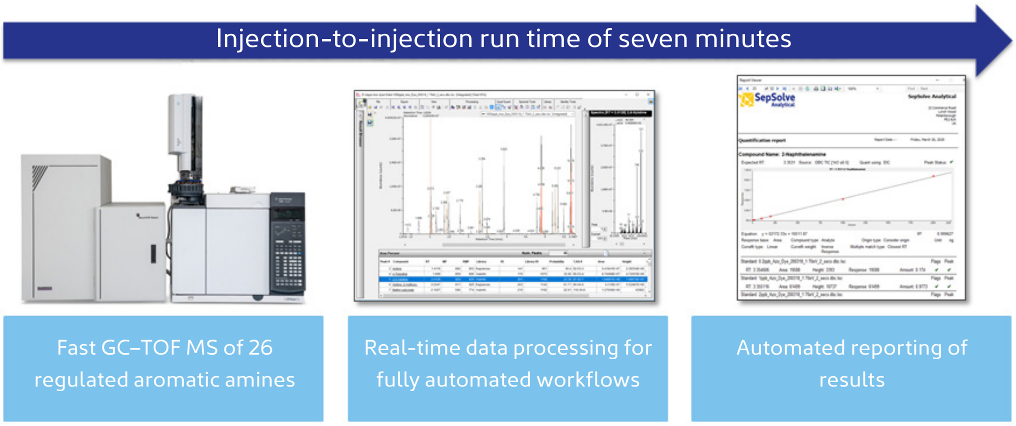

Figure 1

Schematic of the workflow for analysis of aromatic amines.

Results

Development of a fast GC–TOF MS method

A rapid GC–TOF MS method, with an injection-to-injection run time of seven minutes, was developed (Figure 2). All 26 aromatic amines were confidently identified despite co-elutions. Peak identifications are listed in Table 1.

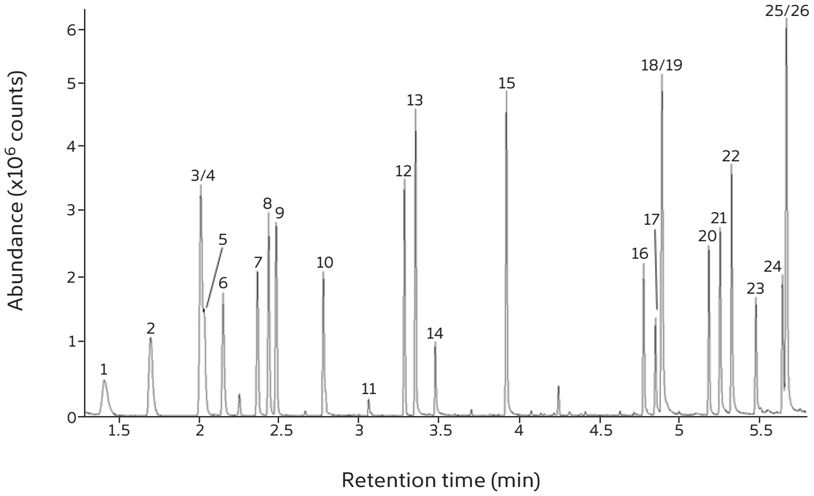

Figure 2

GC–TOF MS chromatogram for the analysis of a 26-component aromatic amines standard.

The BenchTOF™ time-of-flight mass spectrometer used in this study offers fast acquisition speeds and high sensitivity, at the same time as generating reference-quality library-searchable spectra. Unlike quadrupole MS, TOF detectors do not suffer from spectral skew, enabling more robust deconvolution of co-eluting analytes (Figure 3).

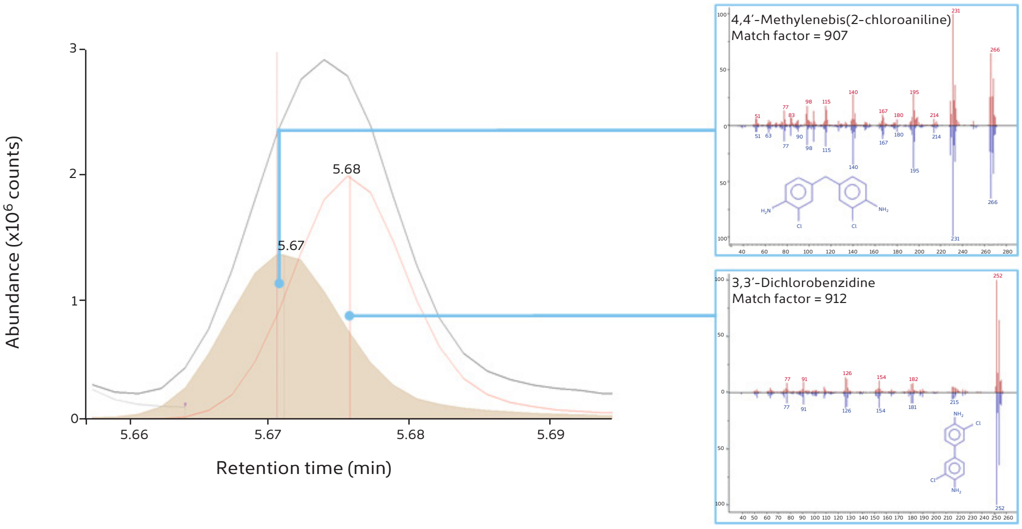

Figure 3

Confident identification of two co-eluting analytes using TOF-DS deconvolution and comparison of BenchTOF spectra (red) to the NIST database (blue).

Peak no.	Compound	RT (min)	R^2	%RSD (n = 8)	IDL (ng/mL)	% Recovery
1	Aniline	1.397	0.9999	1.87	0.56	107.7%
2	*o*-Toluidine	1.685	0.9999	1.35	0.40	108.5%
3	2,4-Xylidine	2.005	0.9989	1.24	0.37	109.3%
4	2,6-Xylidine					
5	2-Methoxybenzenamine	2.026	0.9995	2.15	0.64	103.6%
6	*m*-Chloroaniline	2.145	0.9997	2.04	0.61	103.5%
7	*p*-Cresidine	2.362	0.9999	1.89	0.57	107.6%
8	2,4,6-Trimethylaniline	2.432	0.9990	1.26	0.38	104.7%
9	4-Chloro-*o*-toluidine	2.479	0.9995	2.06	0.62	103.7%
10	Toluene-2,4-diamine	2.776	1.0000	2.47	0.74	91.7%
11	2,4-Diaminoanisole	3.061	0.9997	2.74	0.82	90.1%
12	2,4,5-Trichloroaniline	3.285	0.9996	1.45	0.43	98.2%
13	2-Naphthalenamine	3.353	0.9999	1.74	0.52	96.4%
14	2-Nitro-*p*-toluidine	3.477	0.9997	2.83	0.85	95.0%
15	4-Biphenylamine	3.923	1.0000	2.60	0.78	93.8%
16	*p*-Aminoazobenzene	4.783	0.9994	2.80	0.84	107.9%
17	4,4'-Oxydianiline	4.856	0.9994	2.46	0.74	90.2%
18	Benzidine	4.895	0.9984	2.35	0.71	90.1%
19	4,4'-Methylenedianiline	4.899	0.9990	1.91	0.57	91.0%
20	*o*-Aminoazotoluene	5.191	0.9992	2.88	0.86	102.4%
21	4,4'-Methylene bis(2-methylaniline)	5.260	0.9995	2.23	0.67	91.8%
22	*o,o*'-Tolidine	5.333	0.9996	2.93	0.88	91.0%
23	*p,p*-Thiodianiline	5.484	0.9988	2.27	0.68	86.7%
24	3,3'-Dimethoxybenzidine	5.651	0.9987	1.95	0.59	88.9%
25	4,4'-Methylenebis(2-chloroaniline)	5.673	0.9999	2.12	0.64	87.7%
26	3,3'-Dichlorobenzidine	5.678	0.9994	2.70	0.81	90.2%

Table 1

Overview of validation results for the fast GC–TOF MS method.

Repeatability

Repeatability of the method was measured by eight replicate injections of the 10 ng/mL standard. Relative standard deviations (RSD) were calculated for the peak areas – with all values <3%, indicating excellent repeatability. All values are displayed in Table 1.

Linearity

Linearity of the method was then evaluated using a calibration series spanning 1–1000 ng/mL. Unique quantifier ions were selected for all aromatic amines (except 2,4- and 2,6-xylidine). Figure 4 shows the calibration curves generated for all analytes, while R^2 results are summarised in Table 1. The R^2 values were found to be >0.995 for all components, with an average of 0.9995, indicating strong linearity.

Typically, much of the time associated with quantitative analyses is devoted to data processing and review of results. To reduce this time burden, the TOF-DS software suite enables real-time data processing to be employed during analysis. This allowed the chromatograms to be background-subtracted, integrated and identified while the samples were still acquiring – meaning that all results were ready to view immediately.

Reports can also be automatically generated during acquisition for further time savings. These easy-to-use reports are created from pre-defined templates and can be saved in Excel, pdf or Word formats.

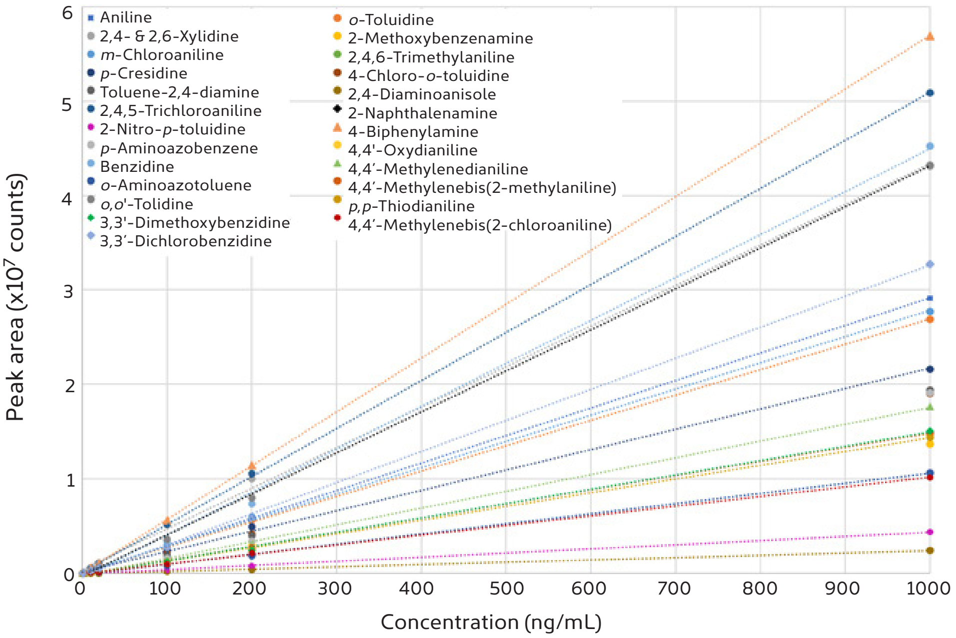

Figure 4

Calibration curves generated for the 26 aromatic amines (1–1000 ng/mL).

Instrument detection limits

The sensitivity of the method was also evaluated by calculating instrument detection limits (IDLs). The IDLs were calculated using the following equation:

IDL = t × RSD × concentration

where t = 2.998 for eight replicate injections of the 10 ng/mL standard.

All IDLs were found to be <1 ng/mL (Table 1) for trace-level detection of aromatic amines.

Recovery assessment

Finally, the percent recovery was assessed by spiking a relevant matrix (in this case, a section of a nitrile glove) with 10 ppb of the aromatic amine standard. The GC–TOF MS chromatogram for the spiked sample is provided in Figure 5. It can be seen that the intensity of the matrix is over two orders of magnitude greater than that of the target analytes. Nevertheless, all target components were detected and their calculated concentrations were within ±15% of the known spiked amount, as provided in Table 1.

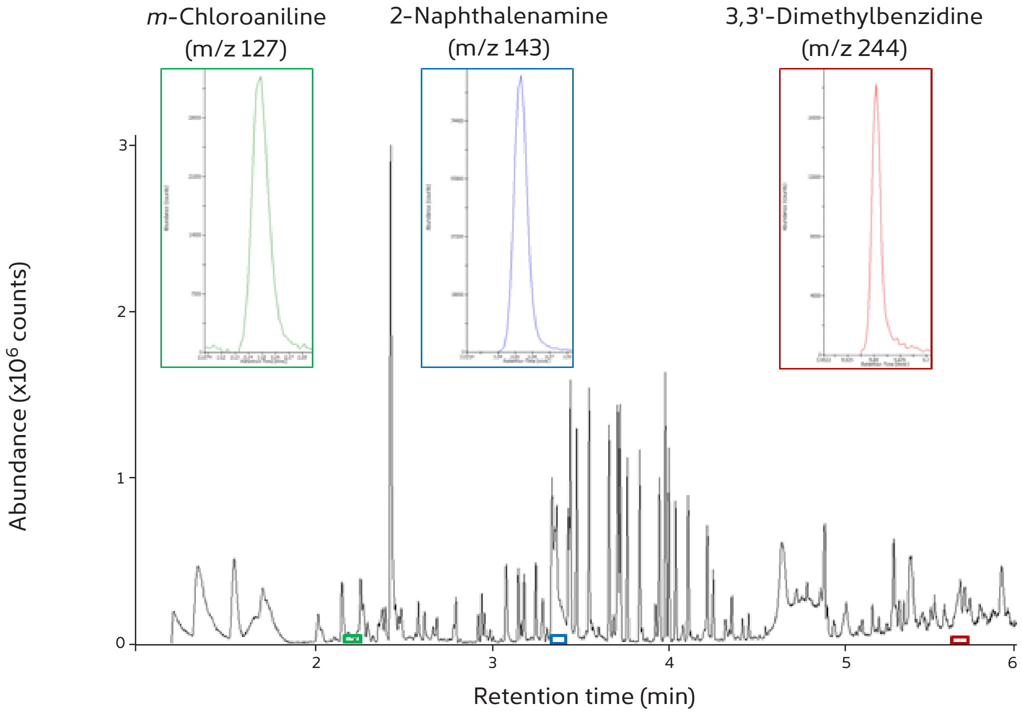

Figure 5

GC–TOF MS chromatogram of a nitrile glove extract spiked with 10 ppb of the aromatic amines standard. Insets show EICs for a selection of the target compounds, which are hidden by the matrix in the TIC chromatogram.

Summary

A rapid GC–TOF MS method was developed and validated for the analysis of azo dye aromatic amines.

- High sample throughput with an injection-to-injection run time of seven minutes.
- Confident identification against commercial libraries using high-quality BenchTOF spectra.
- Excellent linearity (all R^2 values >0.995) across a wide concentration range from 1–1000 ng/mL.
- Method repeatability (n = 8) gave %RSD values of <3% for all target compounds.
- Recoveries from a suitable matrix were all within +15% of the spiked concentration.
- TOF-DS software enables real-time data processing for immediate results and streamlined reporting.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

References

[1] H.S. Freeman, Aromatic amines: use in azo dye chemistry, *Frontiers in Bioscience*, Landmark Edition, January 1, 2013, 18: 145–164.

[2] Commission Regulation (EC) No 552/2009 of 22 June 2009 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XVII.

[3] ISO EN 14362-1: Textiles — Methods for determination of certain aromatic amines derived from azo colorants — Part 1: Detection of the use of certain azo colorants accessible with and without extracting the fibers.

[4] Indian Standard 15570: Textiles – Method of test – Detection of banned azo colourants in coloured textiles.

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