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The GC/MS Analysis of 20 Carcinogenic Amines Banned in Textiles and Consumer Products and of Interest in Environmental Monitoring of Colorization Process Wastes

Application Note

Agilent Technologies 6890/5973 GC/MSD System

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Abstract

Some azo dyes, upon application to textiles and other materials, may produce carcinogenic amines. These pose health hazards in two ways: direct contact with consumer products and environmental contamination of water and sludge from wastes generated in colorization processes. Germany has banned the import of certain dyed goods that contain concentrations of these amines above established limits, as determined by analysis using mass spectrometry (MS). By coupling the 5973 mass selective detector (MSD) with the 6890 gas chromatograph (GC), the German regulatory limits with respect to textiles and consumer products were easily met and exceeded. For example, the limit of detection (LOD) with a method using pulsed splitless injection and SIM mode for the MS detection was determined to be 5 ppb. Such a low LOD provides flexibility to the analyst: exploit the low LOD for low level samples or trade-off some of the tedious sample preparation to save on labor and increase sample through-put. Moreover, the achievable LODs and improved data handling tools are well-matched to analysis of environmental samples associated with monitoring contamination from colorization process waste streams. Customized reports of results were produced using the powerful MSD Productivity ChemStation.

Key Words

GC/MS, SIM, amines, azo dyes, Germany, textiles, limit of detection, environmental monitoring, consumer products

Introduction

Due to their versatility, synthetic azo dyes have been used extensively since the mid-nineteenth century. However, it has been found that under reducing conditions, such as during application, these dyes produce amines, of which 20 are known carcinogens. In response, Germany has banned the import, stock and sale of textiles and other materials which have prolonged contact with human skin, and which exceed the regulatory limits of the specified amines.[1] The regulations require identification by at least two different separation techniques and quantitation by either HPLC/DAD (high performance liquid chromatography/diode array detection), GC/FID (gas chromatography/flame ionization detection), or GC/MS (gas chromatography/mass spectrometry). Specific methodology is not prescribed.

Other analytical techniques are possible: HPTLC (high performance thin layer chromatography) and LC/MS (liquid chromatography/mass spectrometry). However, compared to HPTLC, GC/FID, and LC/DAD, GC/MS offers more positive confirmatory information about analyte identity. Compared to LC/MS, GC/MS is more cost-effective.

In the work described in this note, the 6890/5973 GC/MSD system was found to be flexible enough to measure both high concentrations and trace amounts of the 20 banned amines. The trace levels are consistent with sensitivities necessary for environmental monitoring as described in references 2 and 3, which also cite a number of useful references.

Optimized Instrumentation Meets Analytical Requirements for Textile and Consumer Products

There have been specific design enhancements for all three major components of the GC/MSD system to improve overall system performance. For the GC, a pulsed splitless injection transfers all of the sample to the column. With respect to the mass selective detection, the 5973 MSD has a high energy dynode (HED) detector, independently heated ion source and quadrupole regions, and a faster data acquisition rate. The MS Productivity ChemStation not only supports the advances in the GC and MSD instruments but also provides tools for more flexible and productive data analysis for laboratories doing target compound analysis.

For the analysis of amines from a range of consumer products, a GC/MSD system can be configured and operated in a manner that is most suitable to achieve necessary detection levels. World-class performance with respect to reliability continues to lead commercially available GC/MS instrumentation—an important consideration for laboratories doing routine GC/MS work. Data handling tools previously available for very specific environmental analyses have been expanded for more general use, providing greater ease-of-use in data analysis and customized reporting. In particular, users have found that the Q-Edit tool can provide significant improvements in productivity in reviewing data produced by running long sample sequences.

In the work described here, pulsed splitless injection methods (with MS detection in scan and SIM modes) were compared against a split injection method (with MS detection in scan mode). The lowest concentration measured using split injection corresponded to 20 ppb reaching the column (i.e., 1/25 of the 0.5 ppm concentration of a prepared standard or extract). This was close to the limit of detection (LOD) for a method based on split injection/scan mode.

In splitless-injection-based analyses, the lowest concentration measured was a 50 ppb standard. The results showed this to be about 10 times the LOD in SIM. (The GC/MSD LOD for any compound is approximately 5:1 signal-to-noise (S/N, peak-to-peak) for the extracted target ion.)

These LODs are well below the current requirements of a 30 ppm cut-off, enabling an analyst to make the tradeoffs that are most appropriate to a specific laboratory operation:

- Configuration of GC injection port that best accommodates the range in sample types
- Range of amine levels
- Increased laboratory productivity by eliminating some sample preparation steps

While not the primary focus of this study, there are other instrumentation options and tools the user can pursue for improved productivity and/or lower detection limits.

- Fast chromatography[4] can reduce chromatographic separation times by a factor of three to five and has been applied by others to the analysis of the 20 banned amines.[5] This can be achieved by using Method Translation Software[6] along with capillary columns of smaller internal diameter. The faster scan rates of the 5973 MSD support data acquisition across the narrower chromatographic peaks[7] while preserving superior performance in sensitivity and repeatability.
- Large volume injections, LVI[8,9] of sample aliquots of 5 to 250 µL can be done if the injection port is configured with PTV option (programmable temperature vaporizer). Such large injection volumes (compared to typical 1–2 µL injections) allow the analyst to put less effort into some of the sample preparation steps (concentrations) or to push detection limits even lower.[10,11]

Experimental Approach

Table 1 lists the 20 amines and the internal standard (ISTD), 1-napthylamine, with target and qualifier ions, chromatographic retention times, CAS numbers and TexLab numbers.

A custom mixture of the 20 amines was obtained from ChemService, Inc. (West Chester, PA, USA): 200 µg/mL (200 ppm) each component in methanol (Residual Analyzed grade). Since one goal of this work was to explore levels of parts-per-billion, the custom mixture was subjected to dilutions creating lower concentrations of 100 ppm, 20 ppm, 10 ppm, 2 ppm, 1 ppm, 0.2 ppm, 0.1 ppm and 0.05 ppm. The choice of the diluent (or reconstitution) solvent can greatly impact the chromatographic approach with respect to injection port configuration, injection setpoints, and other chromatographic conditions; four solvents were explored.

Table 1. 20 Analytes with Target Ions, Qualifiers, CAS Numbers, Retention Times (RT), and TexLab Numbers

Name	Target Ions Signal	Qualifier Q1 Signal	Qualifier Q2 Signal	CAS	RT	TexLab No.
1-Napthylamine (ISTD)	143	115		134-32-7	8.89	21
o-Toluidine	107	77		95-53-4	3.72	18
p-Chloroaniline	127	129		106-47-8	5.24	7
p-Cresidine	137	122		120-71-8	6.05	14
2,4,5-Trimethylaniline	135	120		137-17-7	6.25	20
4-Chlor-o-toluidine	141	143	106	95-69-2	6.35	3
Toluene-2,4-diamine	121	122		95-80-7	7.27	19
2,4-Diaminoanisole	123	138	95	615-05-4	8.22	8
2-Naphthylamine	143	115		91-59-8	9.04	4
2-Amino-4-Nitrotoluene	152	106		99-55-8	9.52	6
4-Aminobiphenyl	169			92-67-1	10.93	1
4,4'-Oxydianiline	200	171		101-80-4	13.82	16
Benzidine	184			92-87-5	13.90	2
4,4'-Methylene Dianiline	198	182		101-77-9	13.97	9
o-Amino Azotoluene	225	106		97-56-3	15.22	5
4,4'-Methylene-bis-(o-toluidine)	226	211		838-88-0	15.52	13
3,3'-Dimethylbenzidine	212			119-93-7	15.75	12
4,4'-Thiodianiline	216	184		139-65-1	16.31	17
3,3'-Dichlorobenzidine	252	254		91-94-1	17.42	10
4,4'-Methylene-bis-(2-chloraniline)	266	268	231	101-14-4	17.47	15
3,3'-Dimethoxybenzidine	244	201		119-90-4	17.59	11

The Impact of the Reconstitution Solvent upon the GC Injection Process

In general, starting from initial textile samples, the sample preparation protocols usually involve an extraction of the fabric with a specified solvent, concentration of the extract, clean-up with SPE (solid phase extraction), concentration of the SPE fraction containing the amines, and a reconstitution to a known volume. As in any analytical method, it is important to select the reconstitution solvent with respect to both the analytes to be redissolved and the compatibility with the analytical instrument to be used.

In this work, four different solvents were explored for the impact on the GC method: methanol, methylene chloride, ethyl acetate, and 50:50 methanol:methylene chloride. Even though the stock solution (i.e., the custom mixture described above) was based on methanol, the sets of diluted standards were dominated by the diluent solvent (which would correspond to the reconstitution solvent when working with textile samples).

Splitless Injections. In GC analyses based on splitless injections, large expansion volumes of some polar solvents, including methanol, can result in loss of sample out the purge vent. Pulsed splitless injections can help reduce the expansion volume. However, this may cause some sample to reach the column as a liquid instead of a gas and poor chromatographic peak shapes may result, mainly in the form of "fronting." To eliminate fronting in this work, the initial oven temperature was set to 80°C, which is 15°C above the boiling point of methanol. This improved the chromatographic peak shape of the early eluting compounds, though some peak tailing was observed.

Tailing is a result of the polarity of the analytes and the solvent relative to the polarity of the stationary phase of the chromatographic column. HP-5 is a non-polar stationary phase. If splitless injections with methanol become the technique of choice, more polar phases would be appropriate. However, note that polar stationary phases may have lower upper temperature limits so the final GC oven temperature may need modification.

The liner used for the splitless injections was a single taper liner without glass wool. A small amount of glass wool might help reduce the observed peak tailing. It is recommended that the single tapered liner with glass wool, P/N 5062-3587, be investigated.

Split Injections. In this work, split injections did not show chromatographic fronting or tailing for any of the diluent solvents explored. In particular, since the quantity of methanol making it to the column was a factor of 25 less than with splitless mode, experiments done in the split mode showed much better chromatographic profiles. For analyte concentrations above 10 ppm, split injections are recommended.

For the split injection samples, the liner used was SGE's FocusLiner, which is designed for high reproducibility in split injections. The FocusLiner is packed with glass wool at the 20 mm height, then deactivated. The split/splitless liner can be used as a substitute; however, it is quite important to deactivate the liner (e.g., following the silanization instructions discussed by Doherty[12,13]).

GC/MSD System

The 6890 GC coupled with the 5973 MSD was used for all experiments. The system was configured with a turbomolecular pump, a split/splitless injection port, and an ALS autosampler. The system was controlled by the MSD Productivity ChemStation. Tables 2 and 3 outline the instrument method parameters used for the experiments.

Table 2. Instrument Parameters for the 6890 GC

	Pulsed Splitless Method	Split Method
GC Column	HP-5MS: 30 m × 0.25 mm ID × 0.25 µm film; P/N 19091S-433	HP-5MS: 30 m × 0.25 mm ID × 0.25 µm film; P/N 19091S-433
Injection Volume	1 µL	1 µL
Injection Port Liner	Deactivated Taper Liner, • No glass wool • P/N 5181-3316	FocusLiner™ • SGE P/N 092002 Split/Splitless Liner — Alternative[12, 13] • P/N 19251-60540
Split/Splitless Inlet Temperature	250°C	250°C
GC Oven Ramp	Initial: 80°C 1 min hold Ramp 1: 12°C/min to 210°C Ramp 2: 15°C/min to 230°C Ramp 3: 3°C/min to 250°C Ramp 4: 40°C/min to 300°C	Initial: 60°C 1 min hold Ramp 1: 12°C/min to 210°C Ramp 2: 15°C/min to 230°C Ramp 3: 3°C/min to 250°C Ramp 4: 40°C/min to 300°C
Pressure Program	Initial Pulse: 30 psi for 1 min Constant Flow: 1.5 mL/min He	Flow Rate: 1.6 mL/min He
Splitless Time	1.2 min	
Split Ratio		25:1
Autosampler	7673B	7673B

Table 3. Instrument Parameters for the 5973 MSD

Temperatures	Transfer line = 300°C Source = 230°C Quadrupole = 150°C
Tune	Autotune
Scan Mode	45–300 amu 2^3 a/d sampling rate
SIM Mode	EMV = 200 volts above tune voltage
Solvent Delay	• 3.2 min — splitless injections • 3.5 min — split injections
Dwell per Ion	100 msec
Emission Current[14, 15]	35 µamp
ChemStation	G1701 BA

Results and Discussion

Detection Limits

Of the four diluent solvents studied, methanol showed the best detection limits, with ethyl acetate second.

Low-level concentrations of amines were analyzed by pulsed splitless injection (scan and SIM modes), and split injection (scan mode). Pulsed splitless injection, SIM mode, yields the highest sensitivity; ultimate detection limits can be achieved by this method.

As seen in Figure 1, all 20 amines at the 0.1 ppm level are easily detected in scan mode. This shows that the detection limits for these compounds in scan mode are actually less than 0.1 ppm because the extracted ion signal is even stronger, as demonstrated in Figure 2.

Figure 2 is an example of extracted ion signals in both scan and SIM modes for a 0.05 ppm standard. Because the scan sample was run at a threshold of 100, only an approximate S/N (signal-to-noise ratio) could be calculated, and therefore, the S/N results were not printed.

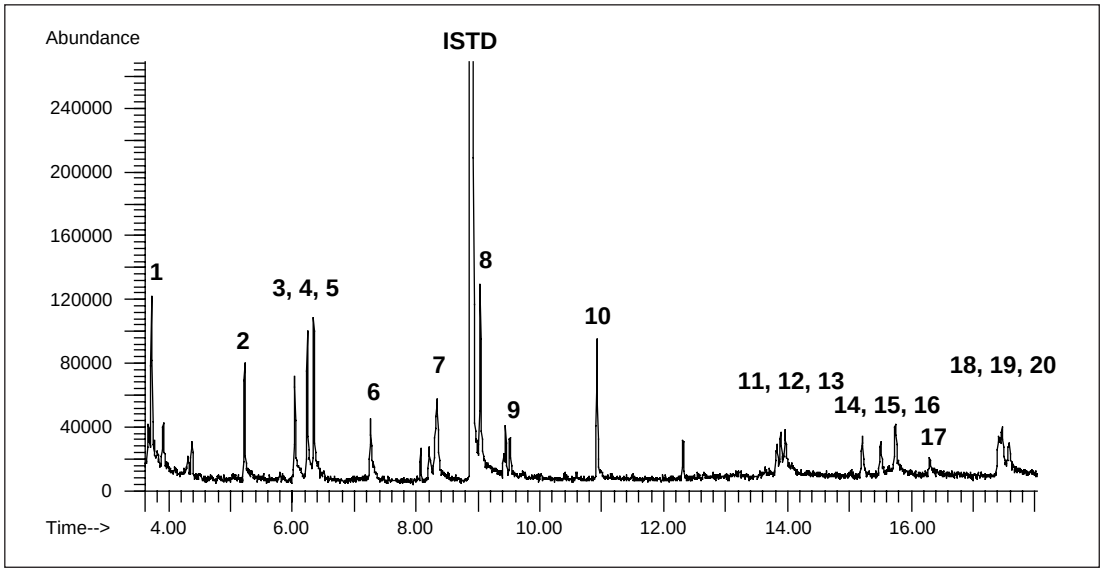

Figure 1. Total Ion Chromatogram of 1 µL of a 0.1 ppm solution, pulsed splitless injection, scan mode.

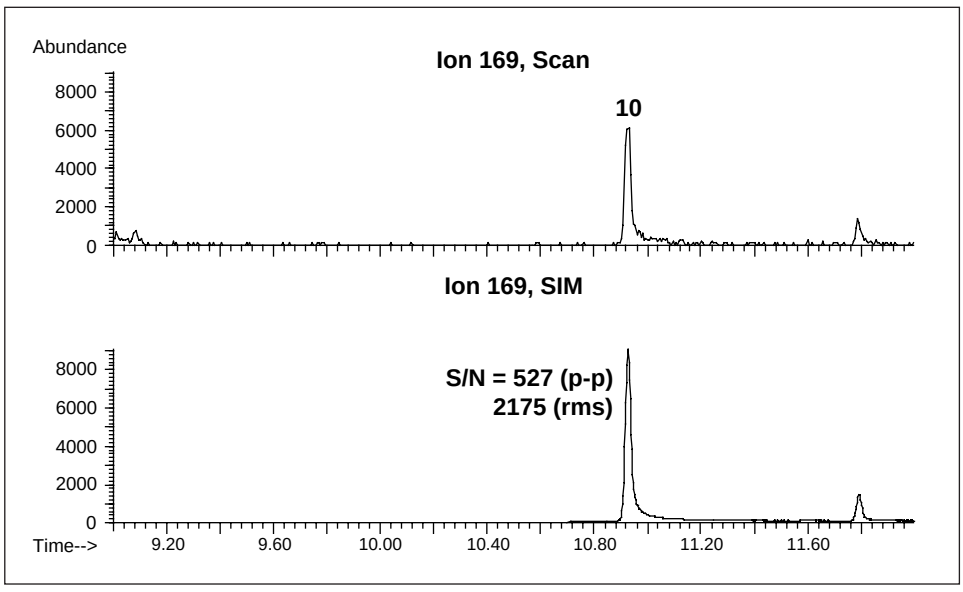

Figure 2. Extracted ion chromatogram at 0.05 ppm, pulsed splitless injection, Scan and SIM Modes.

When the 0.05 ppm standard was re-analyzed in a subsequent run in scan mode at a threshold of zero, the S/N (peak-to-peak) was found to be 50 and the S/N (rms) was 265. For this same standard, in SIM mode, the S/N results were calculated as S/N (p-p) = 527 and S/N (rms) = 2175. The high signal-to-noise ratio indicates that *analysis down to 5 ppb is achievable.*

Even with split injections, where only a fraction of the sample reaches the column (and the MSD), quantitation of low concentration levels is easily achievable. This demonstrated in Figure 3 where all the amines can be seen for a 1-µL injection of the 1-ppm standard (representing 0.04 ppm at the detector).

MSD Productivity ChemStation

Samples may contain unknowns, including compounds with retention times similar to those of the target analytes. The MSD Productivity ChemStation was used to verify structures, identify unknown compounds, and to enhance the presentation of results (Figure 4).

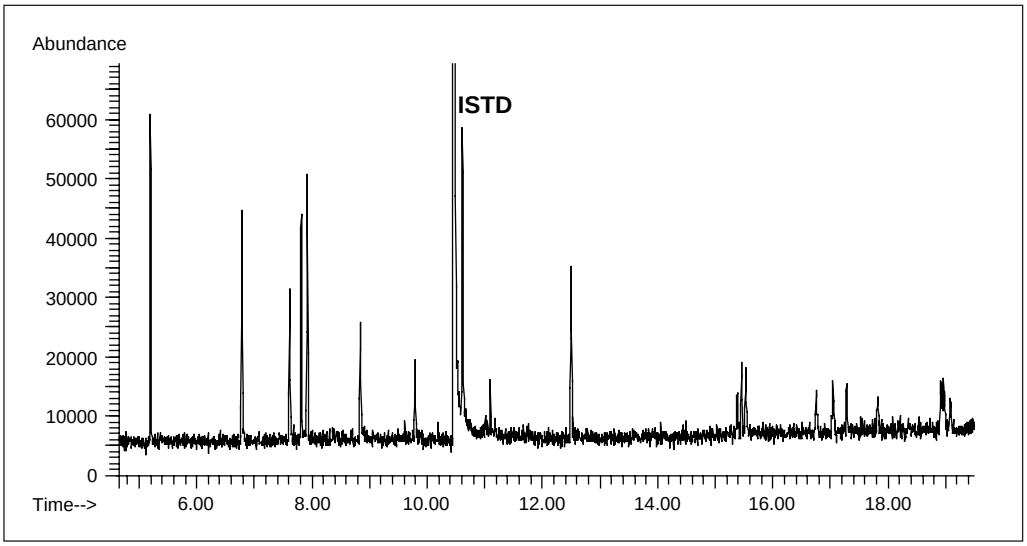

Figure 3. Total Ion Chromatogram resulting from 1 µL of a 1-ppm standard with split injection (25:1 split ratio yielding 0.04 ppm on column) in scan mode.

20 Banned Amines – Customized Report

Data File Name	2NG01.D
Operator	Doherty
Date Acquired	01/12/97 16:58
Method File	AMINESS
Sample Name	2 ppm amines with 5 ppm ISTD, 1uL
Misc Info	1.5 mL/min He, pulsed splitless, MeOH solvent
Vial Number	33

#	NAME	CAS	RT	AMOUNT	TARGET RESPONSE	TEXLAB #
1)	o-Toluidine	95-53-4	3.72	2.31 ppm	1120833	18
2)	p-Chloroaniline	106-47-8	5.24	2.18 ppm	1300106	7
3)	p-Cresidine	120-71-8	6.05	2.07 ppm	751972	14
4)	2,4,5-Trimethylaniline	137-17-7	6.25	2.17 ppm	1209182	20
5)	4-Chlor-o-toluidine	95-69-2	6.35	2.23 ppm	1072988	3
6)	Toluene 2,4 diamine	95-80-7	7.27	2.00 ppm	887877	19
7)	2,4-Diaminoanisole	615-05-4	8.22	1.85 ppm	625096	8
	1-Naphthylamine (ISTD)	134-32-7	8.89	5.00 ppm	4318258	21
8)	2-Naphthylamine	91-59-8	9.04	2.41 ppm	2246939	4
9)	2-Amino-4-Nitrotoluene	99-55-8	9.52	1.78 ppm	330646	6
10)	4-Aminobiphenyl	92-67-1	10.93	2.37 ppm	2508188	1
11)	4,4'-Oxydianiline	101-80-4	13.82	1.77 ppm	709530	16
12)	Benzidine	92-87-5	13.90	2.30 ppm	2053643	2
13)	4,4'-Methylene Dianiline	101-77-9	13.97	2.06 ppm	1021093	9
14)	o-Amino Azotoluene	97-56-3	15.22	2.09 ppm	696944	5
15)	3,3'Dimethyl 4,4'-Diamino	838-88-0	15.52	2.01 ppm	922710	13
16)	3,3'-Dimethylbenzidine	119-93-7	15.75	2.26 ppm	2406667	12
17)	4,4'-Thiodianiline	139-65-1	16.31	1.71 ppm	548381	17
18)	3,3'-Dichlorobenzidine	91-94-1	17.42	2.18 ppm	1311473	10
19)	4,4'-Methylene-bis	101-14-4	17.47	2.13 ppm	430749	15
20)	3,3'-Dimethoxybenzidine	119-90-4	17.59	1.87 ppm	712072	11

Figure 4. Design a report with Custom Reports.

Figure 4 is a custom report produced with the MSD Productivity ChemStation. This report can be printed with every sample analyzed. For this application, the textile (TexLab) number is an important parameter. This was easily set in the user definable fields for each compound in the compound database.

Summary

The performance of the 6890/5973 GC/MSD system made it easy to exceed German regulatory requirements with respect to textiles and consumer products. Because the concentration of the amines in products may vary, scan or SIM mode methods have been developed. With split injection of 1-µL aliquots, MS detection in scan mode allows easy detection at 0.1 ppm and ultimate detection down to 0.02-0.04 ppm. The highest sensitivity is obtained by pulsed splitless injection combined with SIM mode, yielding a limit of detection of 5 ppb for injection volumes of 1 µL. For samples with concentrations greater than 10 ppm, split injections are recommended.

Methanol is a good solvent for split injections, giving excellent chromatographic peak shapes and low detection limits. Other solvents such as ethyl acetate should be investigated thoroughly. Ethyl acetate is a better solvent choice for the pulsed splitless technique because of its lower expansion volume.

The MSD Productivity ChemStation proved to be a valuable tool for data collection, analysis, and presentation. Besides the efficient verification of structures and identification of unknowns using the Q-Edit tool, the ChemStation was easily used to create the necessary custom results reports.

For those analysts who need to routinely determine the levels of amines in textiles, other consumer products, or environmental samples, the 6890/5973 GC/MSD system with the MSD Productivity ChemStation provides positive analyte identification, accurate and precise quantitation from low ppb through ppm levels, reports formatted according to the laboratory's needs, and numerous approaches to increasing laboratory productivity.

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