

# Industrial Chemicals

# Applications note

Lit. cat.# 59552

## GC/MS Analysis of Azo Dye Compounds

Some consumer goods that come into contact with the human body, such as clothing, jewelry, and toys, may contain azo dyes that metabolize into carcinogenic aryl amines. Consumer safety regulations in Germany prohibit the distribution of all consumer goods containing azo dyes,<sup>1</sup> and other European countries may follow suit in the near future. In 1996, the German government institute BgVV published four methods for azo dye analysis, including a gas chromatography (GC) method.<sup>2</sup>

Analysts must overcome several obstacles when performing a GC analysis of aryl amines. First, response factors rise for the first several injections before reaching an equilibrium state, especially when using standard sleeve, wool, and column deactivation. Restek's proprietary amine deactivation for inlet liners and wool, when used in combination with the Rtx®-5 Amine column, will eliminate the adsorption that causes this "priming effect." As a result, the reproducibility of the analysis also will improve. Second, the inability to obtain pure, stable calibration standards is a problem. Raw material characterization and purification is essential for the preparation of suitable calibration standards. Restek's azo dye standards offer the most

pure and stable calibration available. The following describes an optimized gas chromatography/mass spectrometry (GC/MS) method of azo dye analysis, based on the German method (Figure 1).

### Sample Preparation

1g of material (e.g. leather or cotton) is solvated in 17mL of 60mM, pH 6 sodium citrate buffer at 70°C for 30 minutes. Add 3mL of 20% sodium dithionite to the sample and heat at 70°C for 30 minutes to chemically reduce the azo dyes into aryl amines.

### Sample Extraction

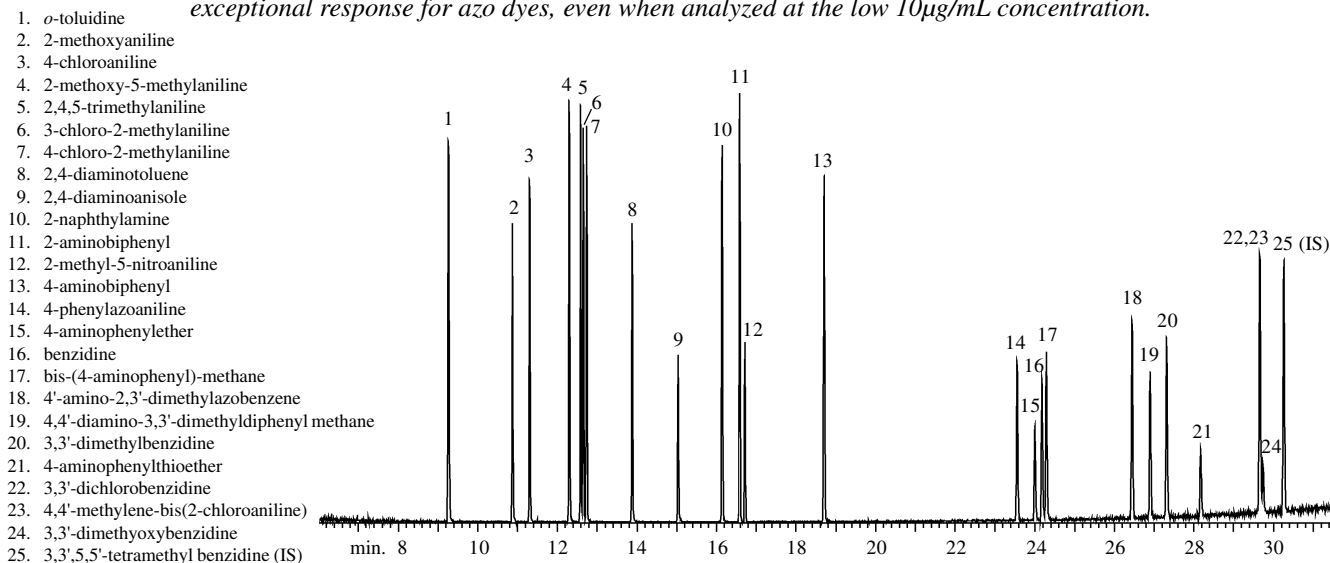
Samples may be extracted and concentrated for analysis using solid phase extraction (SPE) or liquid/liquid extraction.

### Procedure #1, Solid Phase Extraction

This procedure uses E. Merck Extrelut® 20 cartridges or equivalent. The aqueous, chemically-reduced samples are loaded onto the activated cartridges and allowed to stand for 5 minutes.

Figure 1

Restek's proprietary amine deactivated sleeves, fused silica wool, and Rtx®-5 Amine columns combine to give exceptional response for azo dyes, even when analyzed at the low 10µg/mL concentration.



30m, 0.25mm ID, 0.25µm Rtx®-5 Amine (cat.# 12323). 1µL splitless injection using a 4mm gooseneck sleeve with base deactivated wool for HP GCs (cat.# 20798-211.1).

Concentration: 10µg/mL in ethylacetate. Oven temp.: 50°C to 200°C @ 10°C/min. to 270°C @ 5°C/min.; Inj./det. temp.: 250°/300°;

Carrier gas: helium @ 1mL/min.; Detector type: MSD; Splitless hold time: 1 min.

The target analytes are eluted from the cartridge with two additions of 40mL methyl-*tert*-butyl ether. The two fractions are combined and evaporated to about 1mL on a rotary evaporator under vacuum at 50°C. The extracts are then redissolved in 1mL of methanol for GC analysis.

### Procedure #2, Liquid/Liquid Extraction

The chemically-reduced sample also may be concentrated by liquid/liquid extraction. The aqueous sample first is adjusted with sodium hydroxide to a pH greater than 10. Sodium chloride is added until saturation. The sample then is extracted twice with 25mL of methyl-*tert*-butyl-ether. The ether fractions are combined and evaporated to dryness. Finally, the extract is reconstituted in 2mL of methanol for GC/MS analysis.

## Your Chromatographic Problems Solved

### Problem: Priming Effects

Analysts commonly see priming effects in this analysis. While standard sleeve, wool, and column deactivations work well to decrease this in most situations, they do not perform well with amines in splitless injections at low concentrations.

### Solution: Amine Deactivation

Restek has developed the ideal GC products to eliminate the resulting priming effects in the analysis of azo dye. The use of Restek's proprietary amine-deactivated sleeves, amine-deactivated wool, and an Rtx®-5 Amine column will ensure the highest level of system inertness and excellent reproducibility. The priming effect when using standard deactivated products was evaluated by comparing the response factors during repetitive runs. The results indicated that the response factors for the first injection were up to 10% lower than the tenth injection. However, the comparison analysis that used amine deactivation showed no priming effect. The amine deactivations also improved repeatability and lowered standard deviations to < 5%.

**Restek Trademarks:** Rtx.

**Other Trademarks:** Extrelut (E. Merck).

### Problem: Calibration Standards

A lack of accurate, stable calibration standards presents another obstacle to the analysis of azo dyes. Many of the target compounds are not commercially available in a pure, stable form. Some are only available as the hydrochloric, carbonate, or sulfate salts of the free base. While these salts have better stability as a neat material, they do not make good raw materials for GC calibration standards. Standards prepared from the salt forms of azo dyes will yield poor response and peak shape when analyzed using GC. The salt forms also will cause difficulty in assigning the actual weight/weight purity. The best standards for GC analysis are prepared from the free base forms of the azo dye target analytes.

### Solution: Calibration Standard Purity

To ensure accurate preparation of calibration standards, these target compounds must be tested and re-purified. One of the best methods for final purification of the free base is sublimation. When performed under an inert atmosphere, this produces a very pure material for final preparation of a calibration standard. Using multiple purity assays is required to detect impurities. While GC will detect only organic impurities, differential scanning calorimetry (DSC) will detect both organic and inorganic impurities. All of the raw materials for Restek's azo dye mixes are carefully tested and purified to provide the most accurate and stable calibration standards available.

### Conclusion

When using the German azo dye method and Restek's amine-deactivated column, sleeve, and wool, analysts can achieve reproducible and accurate results.

### References

1. German Consumers Goods Act of July 15, 1994, Lebensmittel- und Bedarfsgegenständegesetz, §35 LMBG
2. BgVV, Bundesgesundhbl. 2/96

## Product Listing

### Rtx®-5 Amine Column

30m, 0.25mm ID, 0.25µm, cat.# 12323

### Base-Deactivated Fused Silica Guard Columns

5m, 0.25mm ID    cat.# 10000, ea.    cat.# 10000-600, 6-pk.

### 4mm Gooseneck Base-Deactivated Inlet Sleeves Packed with Base-Deactivated Wool

GC	each	5-pk.	25-pk.
HP	20798-211.1	20799-211.5	20800-211.25
Varian	20904-211.1	20905-211.5	20906-211.25
CE*	20945-211.1	20946-211.5	20947-211.25

\*5mm Gooseneck base-deactivated inlet sleeve.

### Azo Dye Mix #1

Compounds 1-24 from Figure 1 chromatogram  
10µg/mL ea. in ethyl acetate, 1mL/ampul

	Each	5-pk.	10-pk.
	31466	31466-510	
w/data pack	31466-500	31466-520	31566

### Internal Standard for Azo Dye Method

3,3',5,5'-tetramethylbenzidine mix  
1000µg/mL in ethyl acetate, 1mL/ampul

	Each	5-pk.	10-pk.
	31467	31467-510	
w/data pack	31467-500	31467-520	31567

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