Application Note

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Determination of Aromatic Amines Derived from Azo Colorants by GC/MS Using Supported Liquid Extraction Chem Elut S Cartridges

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Abstract

Agilent Chem Elut S is a sample preparation product that uses synthetic media for supported liquid extraction (SLE). This study uses the Chem Elut S cartridges for the quantitative analysis of aromatic amines derived from azo dyes according to European method ISO 14362-1. A sodium citrate/sodium hydroxide solution containing aromatic amines was loaded onto the cartridge and held for 15 minutes, then eluted with methyl *tert*-butyl ether (MTBE) to give an extract for GC/MS analysis. The method measured amines at 30 µg/mL for three different SLE cartridge formats, all delivering high recovery (87 to 119%) and excellent reproducibility (<9% RSD). Results demonstrate that Chem Elut S is a high-performance SLE product for the determination of aromatic amines.

Introduction

Azo dyes are the mostly widely used dyes in industry, accounting for two-thirds of all synthetic dyes.[1] Many common products, including textile fibers, foods, drugs, and cosmetics, use them as coloring additives. Certain azo dyes are banned in textile manufacturing due to environmental hazards caused by reductive degradation into carcinogenic aromatic amines.[2]

The quantification of aromatic amines from their corresponding azo dyes in textiles is outlined in European method ISO 14362-1.[3] The azo dye is extracted from the material using refluxing chlorobenzene, which is then dried down. The extraction residue is treated with a sodium citrate/sodium hydroxide buffer and sodium dithionate to reduce azo dyes to aromatic amines. This work evaluated the Chem Elut S extraction recovery and reproducibility by spiking the appropriate aromatic amines into a blank sodium citrate/sodium hydroxide solution.

In traditional SLE, an aqueous sample is loaded onto the sorbent bed containing diatomaceous earth (DE) where the aqueous sample coats as a thin film on the material. A water-immiscible solvent is then passed through the SLE bed, which extracts target analytes from the sample with high efficiency, and elutes them into a collection tube for analysis with or without post-treatment. This feature provides significant time and labor savings over conventional LLE, and user-to-user reproducibility is improved.

The Chem Elut S products use a synthetic medium optimized to overcome the issues of traditional DE sorbent. Unlike irregularly shaped DE particles that have considerable variability and fines, Chem Elut S particles have a narrow particle size distribution and are free of fines. These features promote ideal flow characteristics and higher reproducibility. Also, Chem Elut S has higher sample holding capacity than DE sorbents, delivering efficient sample adsorption and reducing the chance of sample breakthrough. The three large format Chem Elut S cartridges, 5 mL, 20 cc; 10 mL, 60 cc; and 20 mL, 60 cc, operate with gravity loading and elution, simplifying the workflow.

This work demonstrates high recovery and reproducibility of aromatic amines using Chem Elut S cartridges and GC/MS (SIM) detection.

Experimental

All reagents and solvents were HPLC or analytical grade. MTBE was from VWR-BDH Chemicals (Radnor, PA, USA). Sodium citrate dihydrate (Sigma-Aldrich, St. Louis, MO, USA) was used to prepare citrate buffer at 0.06 M in water. Sodium dithionite was from Sigma-Aldrich and prepared as a 200 mg/mL solution daily. Sodium hydroxide was from Sigma-Aldrich as a 50% solution in water. The aromatic amines and internal standards were purchased from Sigma-Aldrich and AccuStandard (New Haven, CT, USA) as neat solids and liquids for preparation of stock standards. Table 1 lists retention times, CAS numbers, and GC/MS ions for target amines.

Standards and solutions

Individual stock solutions were prepared for each amine at 10 mg/mL in water or DMSO, as required. These standards were then combined into working standards for calibrants and sample spiking.

Sample preparation equipment and supplies

- Agilent Chem Elut S 5 mL, 20 cc (p/n 5610-2009);
- Agilent Chem Elut S 10 mL, 60 cc (p/n 5610-2010)
- Agilent Chem Elut S 20 mL, 60 cc (p/n 5610-2011)
- 40 and 150 mL glass collection bottles
- Eppendorf pipettes

Instrument conditions

- Agilent 7890 GC
- Agilent 5977 GC/MSD

Agilent 5977 GC/MSD Parameters	
GC Column	Agilent J&W DB-35ms, 30 m × 250 µm × 0.250 µm (p/n 122-3832)
Liner	Ultra Inert single taper, splitless, with wool (p/n 5190-2293)
Injection Volume	1 µL
Inlet Temperature	280 °C
Flow Rate	2 mL/min, constant flow
Oven Temperature	100 °C then 10 °C/min to 320 °C
Aux Temperature	320 °C
MS Source	250 °C
Quad Temperature	180 °C
MS Mode	SIM (see Table 1 for ions)

Table 1. Target amines and internal standards, retention time, SIM parameters, and recovery specification.[3]

Analyte	CAS No.	Retention Time (min)	Quantifier Ion (*m/z*)	Qualifier Ion 1 (*m/z*)	Qualifier Ion 2 (*m/z*)	%Rec Spec.[3]
o-Toluidine	95-53-4	2.783	106	107	89	>50
4-Chloroaniline	106-47-8	4.511	127	129	100	>70
2,4,5-Trimethylaniline	137-17-7	4.872	120	135	134	>70
p-Cresidine	120-71-8	5.224	122	137	94	No spec
3-Chloro-*o*-toluidine	87-60-5	5.585	141	106	140	>70
4-Chloro-*o*-toluidine	95-69-2	5.700	141	106	140	>70
2,4-Diaminotoluene	95-80-7	7.271	121	122	94	>50
3-Nitro-*p*-toluidine	119-32-4	9.268	152	107	135	>70
2-Naphthylamine	91-59-8	9.326	143	115	116	>70
2-Aminobiphenyl	90-41-5	9.427	169	168	167	>70
4-Aminobiphenyl	92-67-1	11.591	169	168	170	>70
Anthracene-d_{10}	1719-06-8	11.784	188	184	189	I.S.
p-Aminoazobenzene	60-09-3	15.200	197	92	120	>70
4,4′-Oxydianiline	101-80-4	15.836	200	171	108	No spec
4,4′-Diaminophenylmethane	101-77-9	15.937	198	197	106	>70
Benzidine	92-87-5	16.012	184	185	92	>70
3,3′-Dimethyl-4,4′-diaminodiphenylmethane	838-88-0	17.363	226	211	120	No spec
3,3′-Dimethylbenzidine	119-93-7	17.623	212	213	106	No spec
4,4′-Thiodianiline	139-65-1	18.520	216	184	215	>70
4,4′-Methylenebis(2-chloroaniline)	101-14-4	18.856	231	266	195	No spec
3,3′-Dimethoxybenzidine	119-90-4	18.973	244	201	229	No spec

Sample preparation

Sample pretreatment procedure: The E.4.6. check protocol was followed in ISO 14362-1 to determine the SLE extraction efficiency of aromatic amines.[3] A solution was prepared with 0.06 M sodium citrate adjusted to pH 6 with sodium hydroxide. The sample was then spiked at 30 µg/mL with aromatic amines and mixed well prior to SLE extraction. After the addition of the amines, the solution was bright orange/yellow.

SLE procedure

1. Set up Chem Elut S cartridges with collection tubes/bottles.
2. Transfer the sample onto a cartridge and load under gravity (see Table 2 for volumes).
3. Hold the sample in media for 15 minutes.
4. Gravity elute with methyl *tert*-butyl ether (see Table 2 for volumes).
5. Mix eluent and aliquot into autosampler vials for GC/MS SIM analysis.

Recovery and reproducibility evaluation

The Chem Elut S protocol for aromatic amines was tested for recovery and reproducibility. Each cartridge was run in three replicates at 30 µg/mL, as indicated in the EN method.[3] Isotopically labeled internal standard (IS) anthracene-d_{10} was spiked at 30 µg/mL to ensure accurate volume correction. Calibrants were prepared at 7.5 µg/mL in MTBE to account for a 4x dilution, as indicated in the SLE procedure.

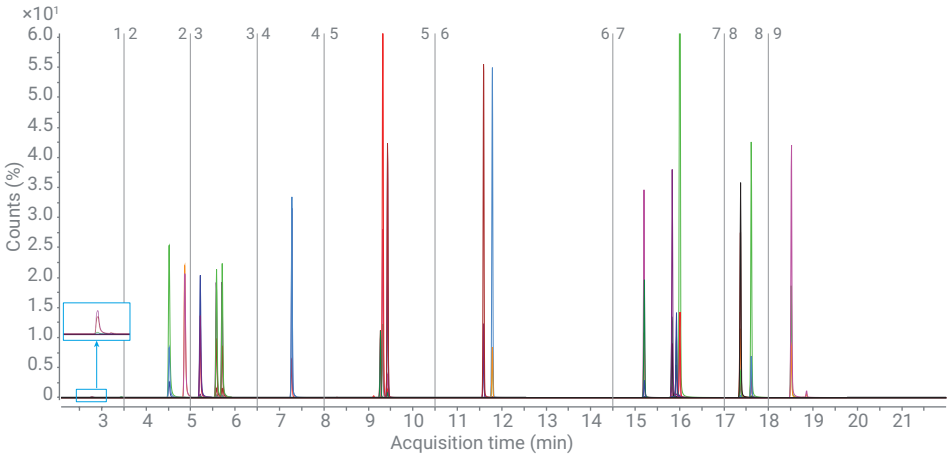

Figure 1. GC/MS SIM chromatogram overlay of 20 aromatic amines and IS at 7.5 µg/mL.

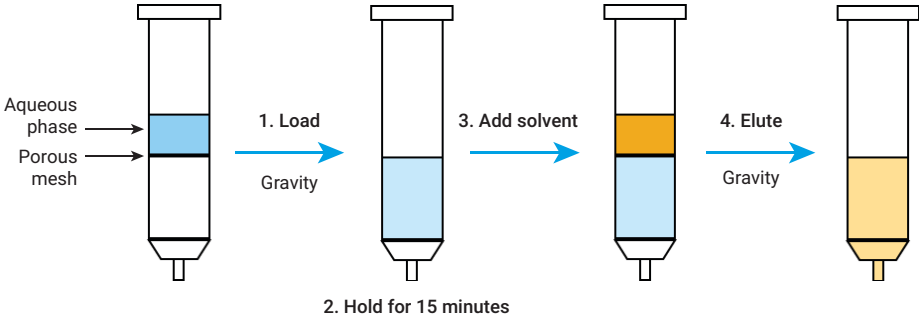

Figure 2. Diagram of the general Agilent Chem Elut S workflow. The steps include: 1) loading sample with gravity; 2) holding the sample in the SLE media for 15 minutes; 3) adding a water-immiscible solvent to extract the analytes; and 4) eluting the organic solvent with gravity.

Table 2. Cartridge load and elution volumes for aromatic amine SLE extraction.

Cartridge	Part Number	Load Volume (mL)	Elution Volume (mL)
5 mL, 20 cc	5610-2009	5	20
10 mL, 60 cc	5610-2010	10	40
20 mL, 60 cc	5610-2011	20	80

Results and discussion

Recovery and reproducibility results

The study produced excellent results, as shown in Table 3. Recovery was between 87 and 119% for all three Chem Elut S formats tested, with %RSD <9. Extending the SLE hold time to 15 minutes increased recoveries for *p*-aminoazobenzene, 4,4'-methylenebis(2-chloroaniline), and 3,3'-dimethoxybenzidine by 29 to 38% (Figure 3). These recovery improvements were demonstrated on both synthetic and DE-based SLE tubes. All large-format cartridges performed exceptionally well, particularly the 10 mL, 60 cc tube, which demonstrated excellent reproducibility.

Sample preparation with Chem Elut S

The Chem Elut S tubes were simple to use, fast, and gave high analyte recovery and excellent precision for aromatic amines. The synthetic medium is carefully manufactured to provide high sample holding capacity, uniform packing, consistent batch-to-batch reproducibility, and optimal flow characteristics. Chem Elut S 5 mL, 20 cc, 10 mL, 60 cc, and 20 mL, 60 cc cartridges are designed to allow gravity sample loading and elution for simple operation. This application benefits from a 15-minute hold time to ensure exceptional recovery for all analytes. Performance characteristics were consistent with DE-based sorbents in direct comparisons. These features provide excellent data quality, ease-of-use, and matrix removal (that is, salts).

Table 3. Recovery and reproducibility of carcinogenic aromatic amines by Agilent Chem Elut S.

Analyte	Chem Elut S Format					
	5 mL, 20 cc		10 mL, 60 cc		20 mL, 60 cc	
	%Rec.	%RSD	%Rec	%RSD	%Rec	%RSD
o-Toluidine	96.8	5.7	91.9	2.4	91.3	4.5
4-Chloroaniline	98.6	3.7	91.3	0.7	92.2	4.7
2,4,5-Trimethylaniline	98.8	3.9	92.2	0.8	93.8	4.3
p-Cresidine	96.9	4.3	90.6	0.8	91.3	3.9
3-Chloro-*o*-toluidine	97.8	3.7	91.0	1.0	92.3	4.4
4-Chloro-*o*-toluidine	96.4	3.9	89.8	0.6	90.7	4.7
2,4-Diaminotoluene	96.7	4.4	90.7	0.7	90.6	4.2
3-Nitro-*p*-toluidine	99.8	4.7	95.0	0.8	94.9	5.0
2-Naphthylamine	97.8	4.3	91.7	0.8	93.0	5.1
2-Aminobiphenyl	96.4	3.4	90.7	0.6	92.8	4.4
4-Aminobiphenyl	97.9	4.3	93.5	0.6	94.1	4.5
p-Aminoazobenzene	98.9	6.2	96.2	0.5	95.3	4.1
4,4'-Oxydianiline	112.9	6.5	109.5	0.8	107.7	5.8
4,4'-Diaminophenylmethane	99.5	6.1	95.3	0.6	95.0	5.5
Benzidine	116.4	5.6	110.4	1.3	111.7	6.1
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	119.6	2.4	114.0	0.5	113.1	5.3
3,3'-Dimethylbenzidine	103.4	5.4	96.8	0.4	96.8	5.7
4,4'-Thiodianiline	107.4	5.9	101.9	1.0	99.7	5.9
4,4'-Methylenebis(2-chloroaniline)	107.2	5.8	101.6	1.0	99.6	5.7
3,3'-Dimethoxybenzidine	91.6	6.1	91.5	8.2	87.1	5.8

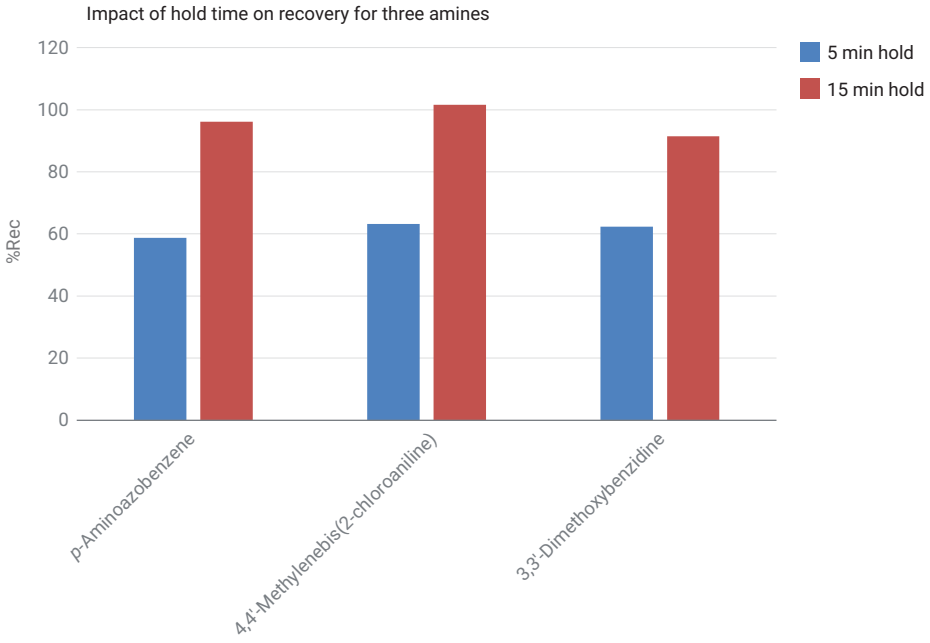

Figure 3. Experiment demonstrating the impact of holding time on recovery of three aromatic amines. Samples containing 30 µg/mL amines were extracted with Agilent Chem Elut S 10 mL, 60 cc tubes (n = 3).

Conclusion

Agilent Chem Elut S uses a synthetic medium designed for consistency and high aqueous sample holding capacity. In this study, Chem Elut S delivered high recovery and reproducibility for aromatic amines derived from azo dyes as outlined in ISO 14362-1. Excellent performance was demonstrated for all three large Chem Elut S cartridges tested, giving 87 to 119% recovery and <9% RSD for all 20 aromatic amines. Many other sample and analyte types benefit from Chem Elut S sample preparation and are featured in other Agilent applications.

References

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Printed in the USA, June 24, 2019
5994-0951EN

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