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Parts per trillion level detection of 13 Primary Aromatic Amines (PAAs)

Released by:

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Introduction

Primary Aromatic Amines (PAAs) are a class of organic compounds with a basic chemical structure of an amine functional group bonded to an aromatic ring. PAAs are commonly used in the rubber and plastics industries as antioxidants and as a building block in organic synthesis to produce azo dyes. PAAs have various pathologic effects on humans with some being carcinogenic or suspected to be carcinogenic. Toxic effects of many PAAs are linked to bladder cancer, adverse effects on red cells, and skin sensitization. For these reasons, European legislation has been passed such as EU Cosmetic Regulations (EC) No 1223/2009 on the marketing and use of azo colorants in consumer products. Limits for total PAAs migration of 10 ppb or lower have been set or are being proposed in flexible packaging. To comply with the strict regulations and to minimize the risks of PAAs to consumers, detection and measurement of PAAs with extremely high sensitivity is needed. Current methods available in the analysis of PAAs include HPLC-UV, GC/MS, UHPLC-MS with calibration levels in the ppm or ppb range. In this case study, a quantitation method of 13 PAAs (shown in **Table 1**) with ppt level detection was developed using a state of art UHPLC-Triple Quad MS system.
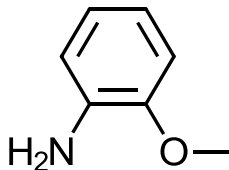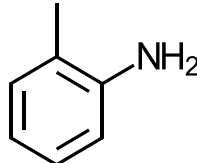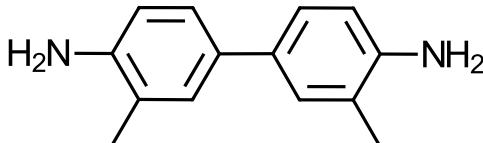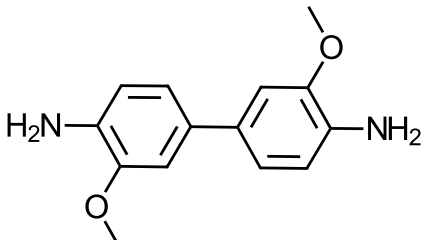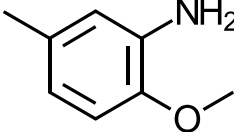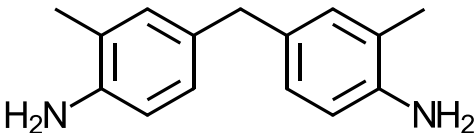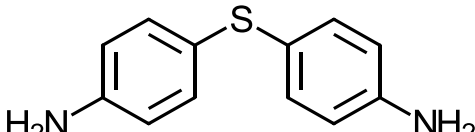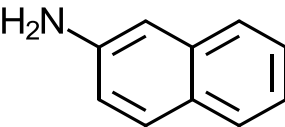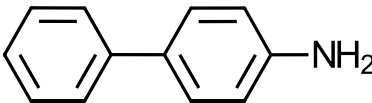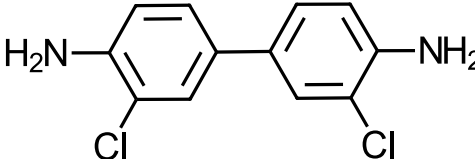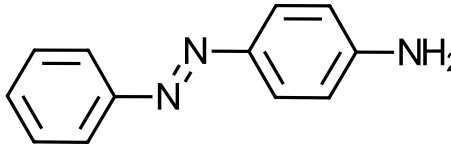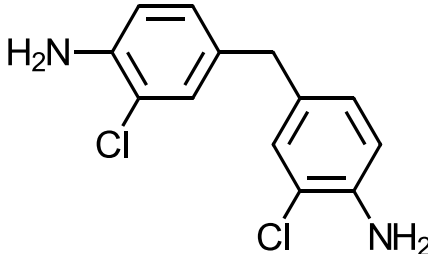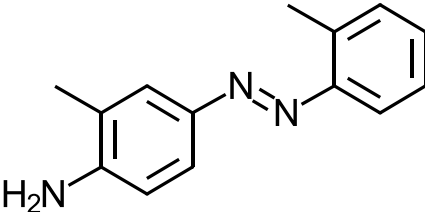
Table 1		
Aromatic Amine	*CAS*	*Structure*
o-Anisidine	90-04-0	
o-Toluidine	95-53-4	
o-Tolidine	119-93-7	
3,3'-Dimethoxybenzidine	119-90-4	

Table 1		
Aromatic Amine	*CAS*	*Structure*
2-Methoxy-5-methylaniline	120-71-8	
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	838-88-0	
4-4'-Thiodianiline	139-65-1	
2-Naphthylamine	91-59-8	
4-Aminobiphenyl	92-67-1	
3-3'-Dichlorobenzidine	91-94-1	
4-Aminoazobenzene	60-09-3	
4-4'-Methylene-bis-2-chloroaniline	101-14-4	
o-Amino-azo-toluene	97-56-3	

September 14, 2016

Dear Customer,

Please find enclosed the test results for your samples described as:

1. PAA Standards Mix

The following test was performed:

1. Ultra High Performance Liquid Chromatography - Triple Quadrupole Mass Spectrometry (UHPLC-QqQ-MS)

Objective

The goal of this analysis was to develop a UHPLC-triple quadrupole MS method to determine poly aromatic amines (PAAs) relevant to their use as azo colorants in textiles.

Summary of Results

A UHPLC-QqQ-MS method was developed for the determination of 13 primary aromatic amines (PAAs). The method was very sensitive with a limit of quantitation (LOQ) of 100 pg/mL (100 ppt). The determination of multiple PAAs derived from banned azo dyes can be performed with the developed method to meet stringent international regulations.

Individual Test Results

A summary of the individual test results is provided below. All accompanying data, including spectra, has been included in the data section of this report.

UHPLC-QqQ-MS

Background: UHPLC-QqQ-MS combines three quadrupole mass filters with the power of an Ultrahigh pressure liquid chromatography separation system to provide highly accurate quantitation of previously-identified compounds.

The presence of an additional quadrupole mass spectrometer (Q) provides the added capability to perform Multiple Reaction Monitoring (MRM) fragmentation experiments. The first quadrupole serves as a pre-filter, allowing only the desired ions to enter the mass spectrometer. These ions are then fragmented in a collision cell yielding product ions. These product ions are then filtered with the third quadrupole before passing to the ion detector. This increases the confidence in identifying the desired analytes and yields highly-accurate quantitation results. Due to differences in ionization efficiency between compounds it is preferable that an internal standard of very similar chemistry to the target analyte be analyzed at the same time as the compound of interest. The ideal internal standard is an isotopically labeled analogue of the analyte of interest when available.

Mass Spectrometry requires that the molecule of interest is ionizable. Ionization can be either positive or negative. Some functional groups or high molecular weight components are less able to ionize and are consequently less suitable for mass spectrometry. Each ionization and MRM fragmentation that is observed in both the analyte and internal standard can be tuned and optimized to achieve the best quality signal. The nature of the mobile phase and the ionization conditions determine the ions formed. For example, in negative ion mode the loss of hydrogen is generally observed which results in the loss of one mass unit (1.0078 amu). Other transformations are also possible including dehydration, dimer formation, etc.

Method

A method development for the determination of PAAs was performed with an Agilent 1290 UHPLC coupled to an Agilent 6470 QqQ Mass Spectrometer. A UHPLC-QqQ-MS chromatogram of a 1000 pg/mL calibration standard is presented in **Figure 1**. **Table 2** lists some of the QqQ MS parameters.
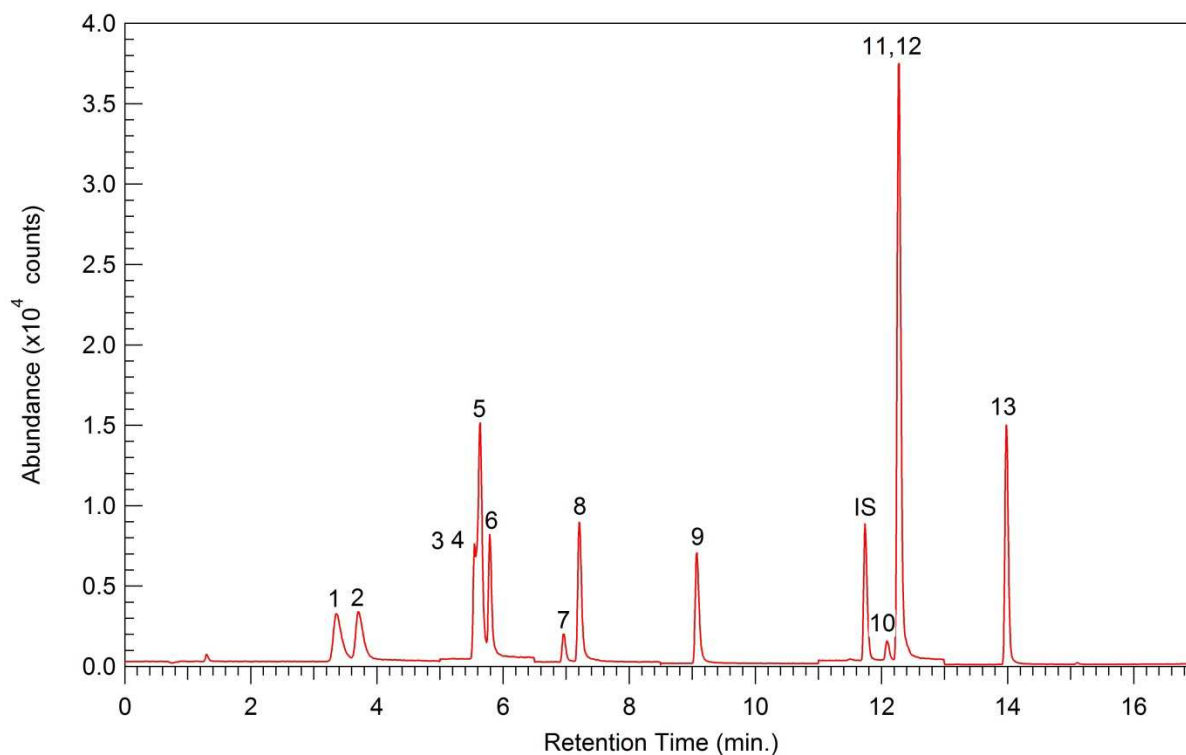
Figure 1: An LC-QQQ-MS chromatogram of a 1000 pg/mL calibration standard. Peak identifications are listed in **Table 2**.

Peak ID	Amine	CAS	RT	Segment Starting Time (min)	Scan Segment	Precursor Ion (m/z)	Product Ion 1 (m/z)
1	*o*-Anisidine	90-04-0	3.340	2.0	1	124.1	109.1
2	*o*-Toluidine	95-53-4	3.690		1	108.1	91.0
3	*o*-Tolidine	119-93-7	5.537		2	213.1	180.0
4	3,3'-Dimethoxybenzidine	119-90-4	5.582		2	245.3	187.0
5	2-Methoxy-5-methylaniline	120-71-8	5.629	5.0	2	138.1	123.4
6	3,3'-Dimethyl-4,4'-diaminodiphenylmethane	838-88-0	5.777		2	227.2	120.0
7	4-4'-Thiodianiline	139-65-1	6.941	6.5	3	217.1	123.9
8	2-Naphthylamine	91-59-8	7.189		3	144.1	127.0
9	4-Aminobiphenyl	92-67-1	9.053	8.5	4	170.1	152.0
IS	Rhodamine B	81-88-9	11.712		5	443.2	399.1
10	3-3'-Dichlorobenzidine	91-94-1	12.075	11.0	5	254.0	154.0
11	4-Aminoazobenzene	60-09-3	12.257		5	198.1	77.0
12	4-4'-Methylene-bis-2-chloroaniline	101-14-4	12.387		5	268.1	232.0
13	*o*-Amino-azo-toluene	97-56-3	13.955	13.0	6	226.3	91.0

Table 2

PAA Standard Preparation

A 1 mg/mL stock of the indicated PAAs was prepared in an 80/20 mixture of isopropyl alcohol (IPA) and methanol. This stock was diluted to 10 ng/mL using the same 80/20 mixture of IPA and methanol before being further diluted using water to known concentrations. Rhodamine B was added to each working standard at a concentration of 500 pg/mL as an internal standard.

Calibration curves were prepared with PAA working standards at 100, 200, 400, 600, and 1000 pg/mL. Each standard was injected in four replicates. All four injections per concentration level were used in constructing the calibration curves. The resulting equations for the linear regression lines and the R^2 values for the lines are included in **Figure 2** to **Figure 14** and are summarized in **Table 3**.

Table 3				
Analyte	*Calibration Curve Equation*	*R^2*	*Regression*	*Weighing Factor*
o-Anisidine	y = 0.0008968x + 0.004436	0.986	Linear	1/x
o-Toluidine	y = 0.0005341x + 0.002985	0.984	Linear	1/x
o-Tolidine	y = 0.0005739x + 0.004297	0.989	Linear	1/x
3,3'-Dimethoxybenzidine	y = 0.0002882x - 0.007131	0.989	Linear	1/x
2-Methoxy-5-methylaniline	y = 0.001558x + 0.011263	0.983	Linear	1/x
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	y = 0.0008111x - 0.0005958	0.983	Linear	1/x
4-4'-Thiodianiline	y = 0.0001677x - 0.0005903	0.985	Linear	1/x
2-Naphthylamine	y = 0.0009175x - 0.002331	0.985	Linear	1/x
4-Aminobiphenyl	y = 0.0009034x + 0.004673	0.983	Linear	1/x
3-3'-Dichlorobenzidine	y = 0.0001148x + 0.001623	0.983	Linear	1/x
4-Aminoazobenzene	y = 0.004296x + 0.02478	0.979	Linear	1/x
4-4'-Methylene-bis-2-chloroaniline	y = 0.00001506 + 0.00007050	0.954	Linear	1/x
o-Amino-azo-toluene	y = 0.001381 + 0.01108	0.982	Linear	1/x

The relative response indicated on the y-axis represents the peak area of the quantifier for the analytes of interest divided by the peak area of the quantifier for the internal standard. Likewise, the relative concentration indicated on the x-axis represents the concentration of the analyte of interest divided by the concentration of the internal standard.
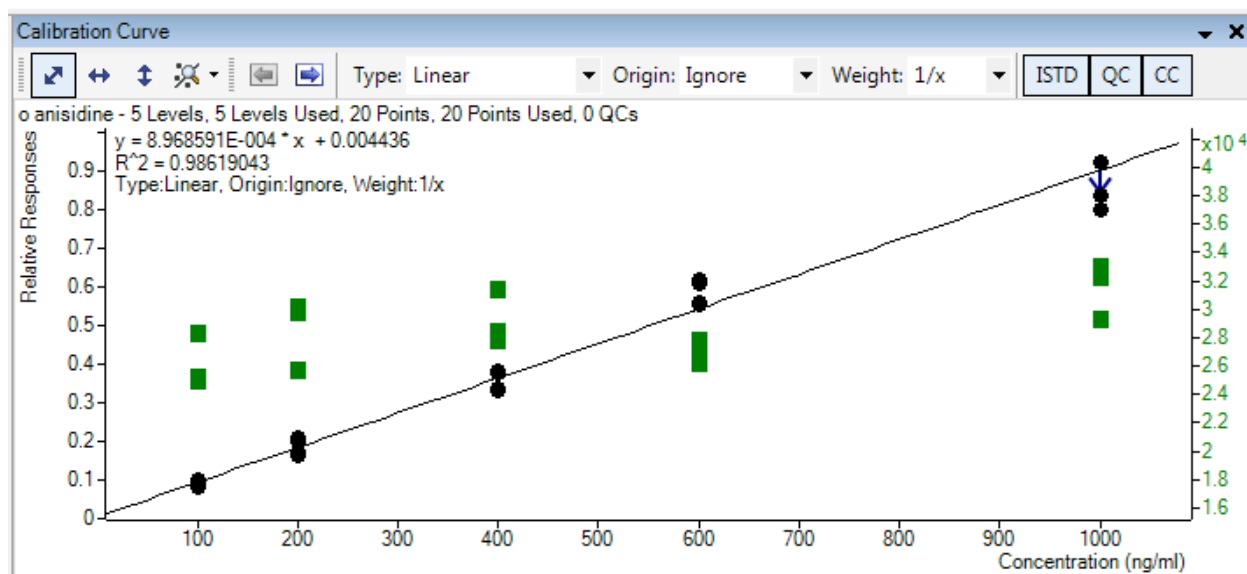

Figure 2: Calibration curve prepared for *o-Anisidine*. The green points represent the relative internal standard response.
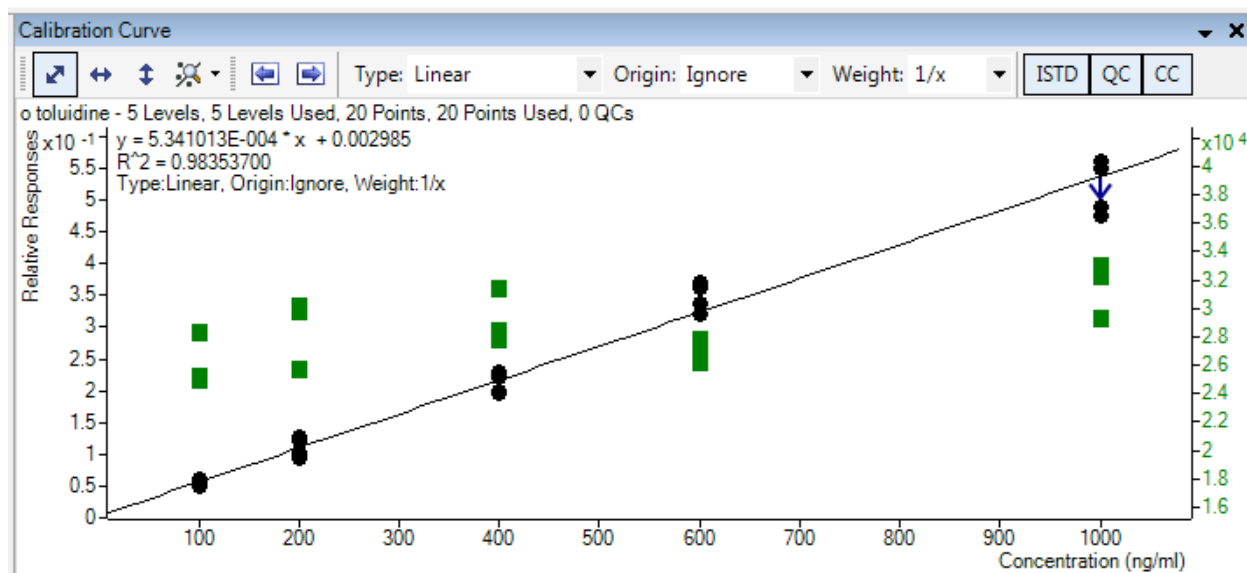
Figure 3: Calibration curve prepared for *o-Toluidine*. The green points represent the relative internal standard response.
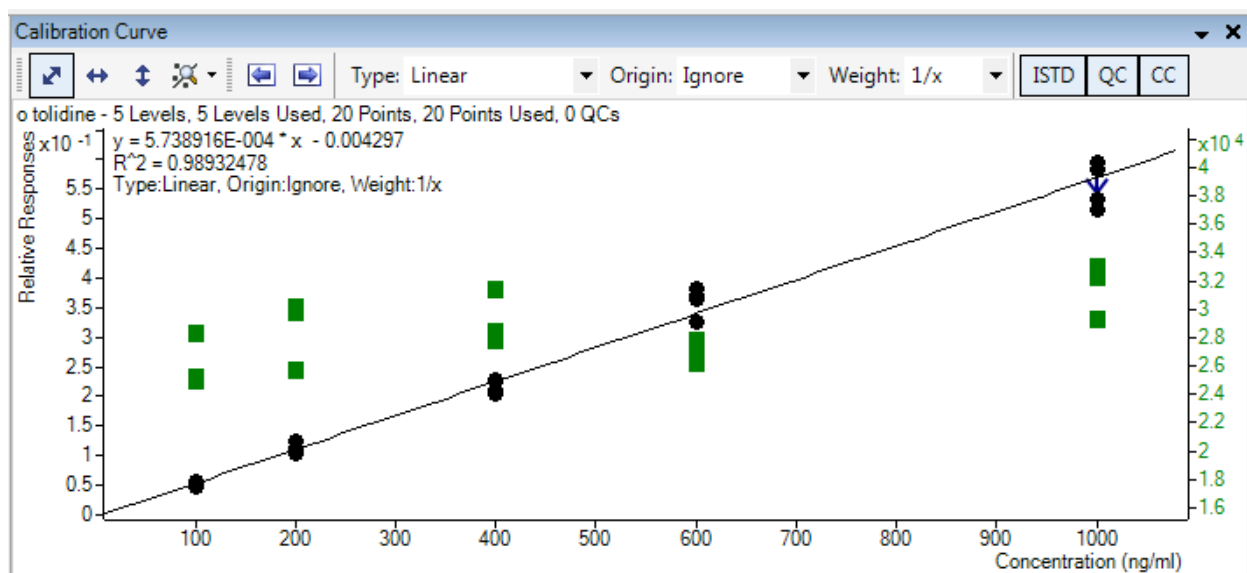

Figure 4: Calibration curve prepared for *o-Tolidine*. The green points represent the relative internal standard response.
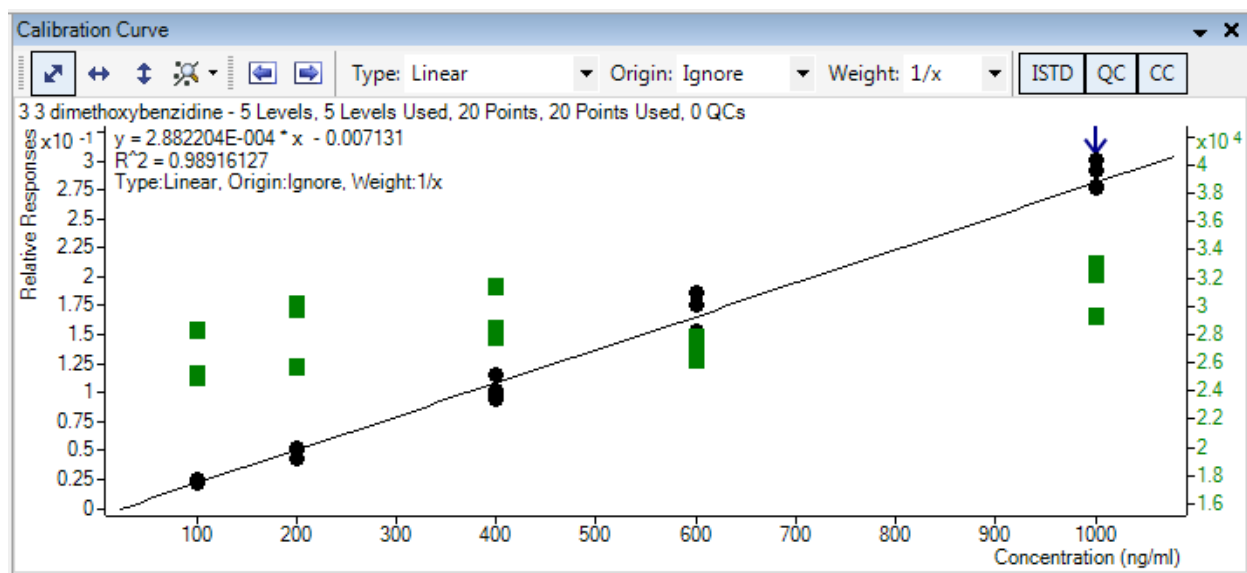
Figure 5: Calibration curve prepared for *3,3'-Dimethoxybenzidine*. The green points represent the relative internal standard response.
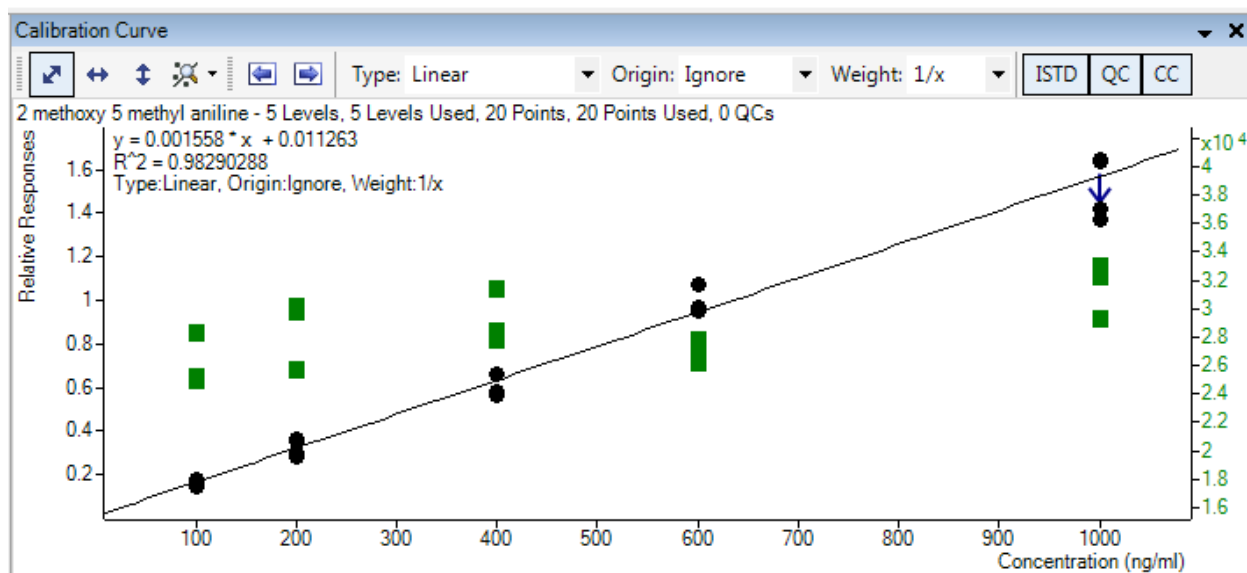

Figure 6: Calibration curve prepared for *2-Methoxy-5-methylaniline*. The green points represent the relative internal standard response.
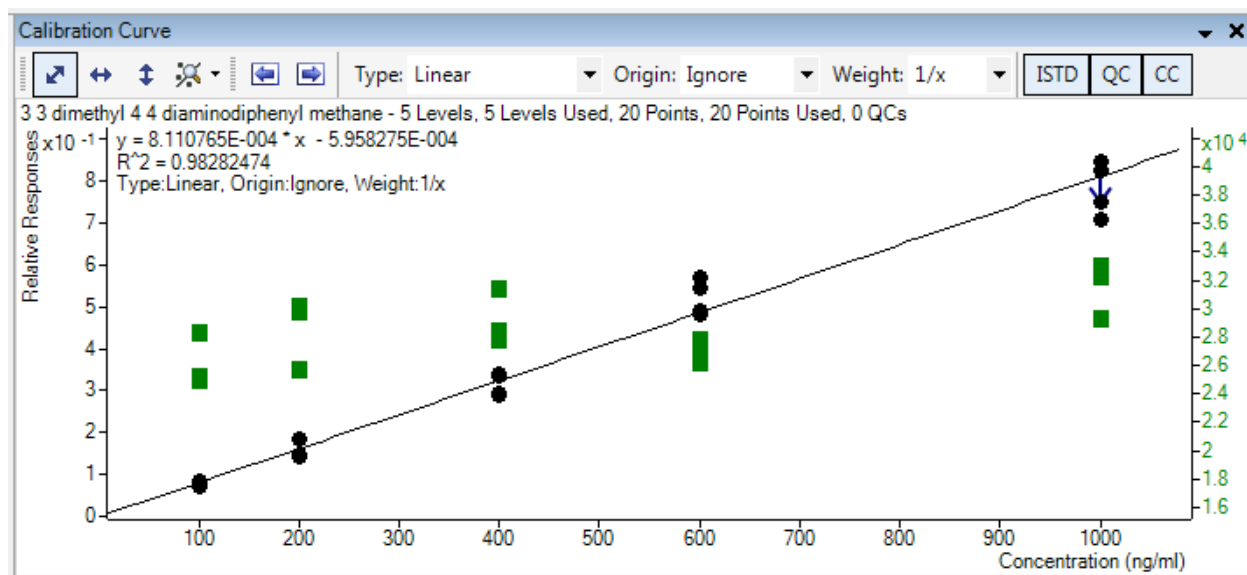
Figure 7: Calibration curve prepared for *3,3'-Dimethyl-4,4'-diaminodiphenylmethane*. The green points represent the relative internal standard response.
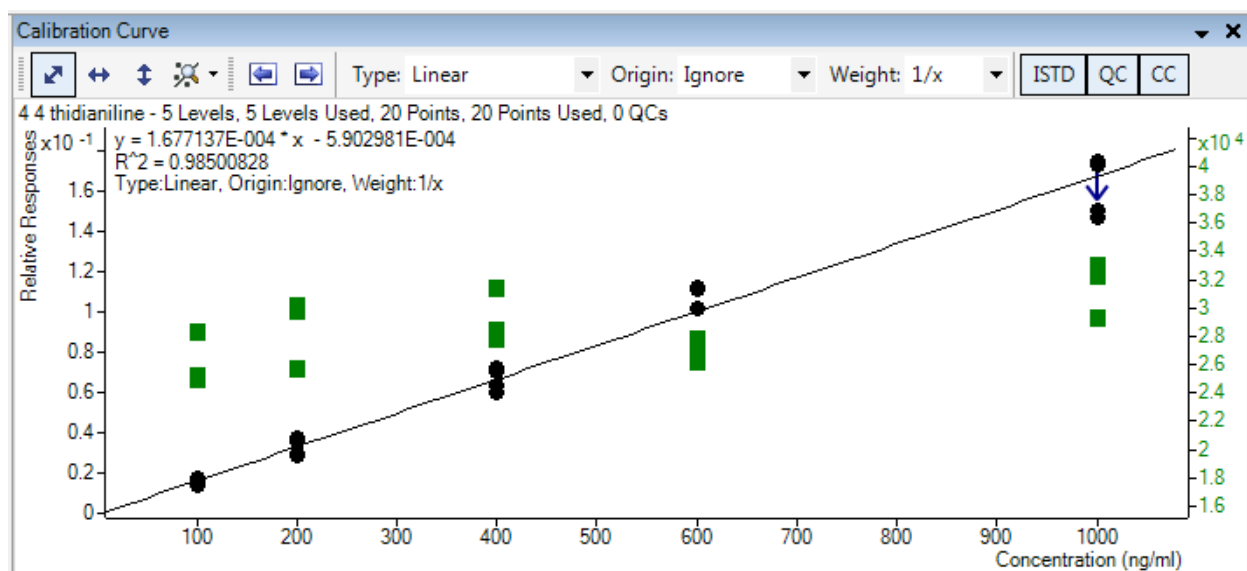

Figure 8: Calibration curve prepared for *4-4'-Thiodianiline*. The green points represent the relative internal standard response.
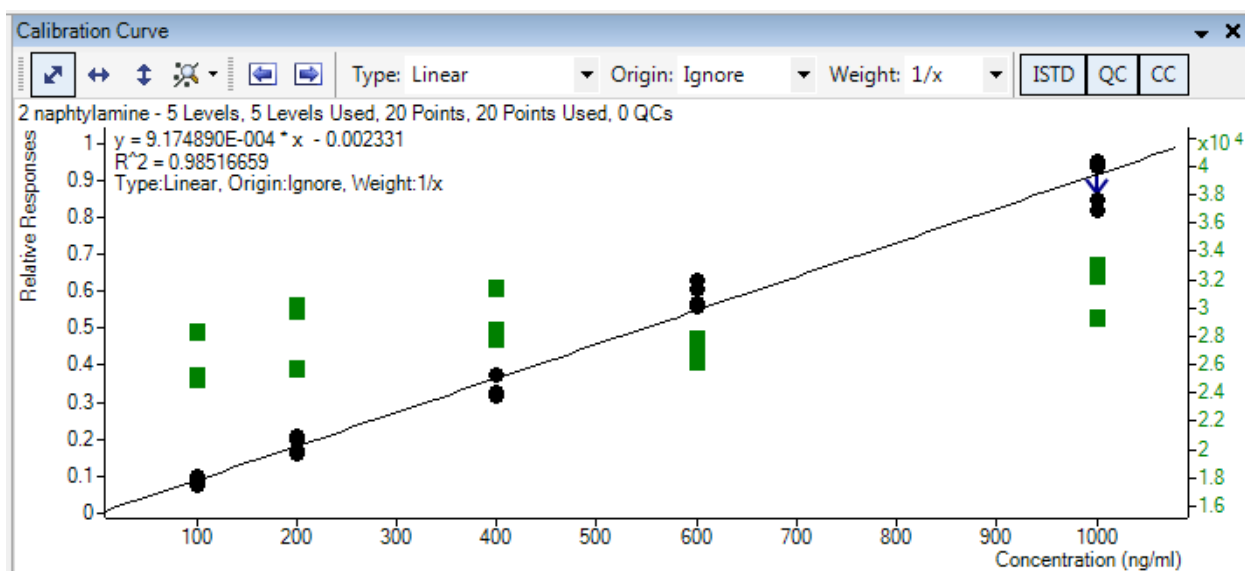
Figure 9: Calibration curve prepared for *2-Naphthylamine*. The green points represent the relative internal standard response.
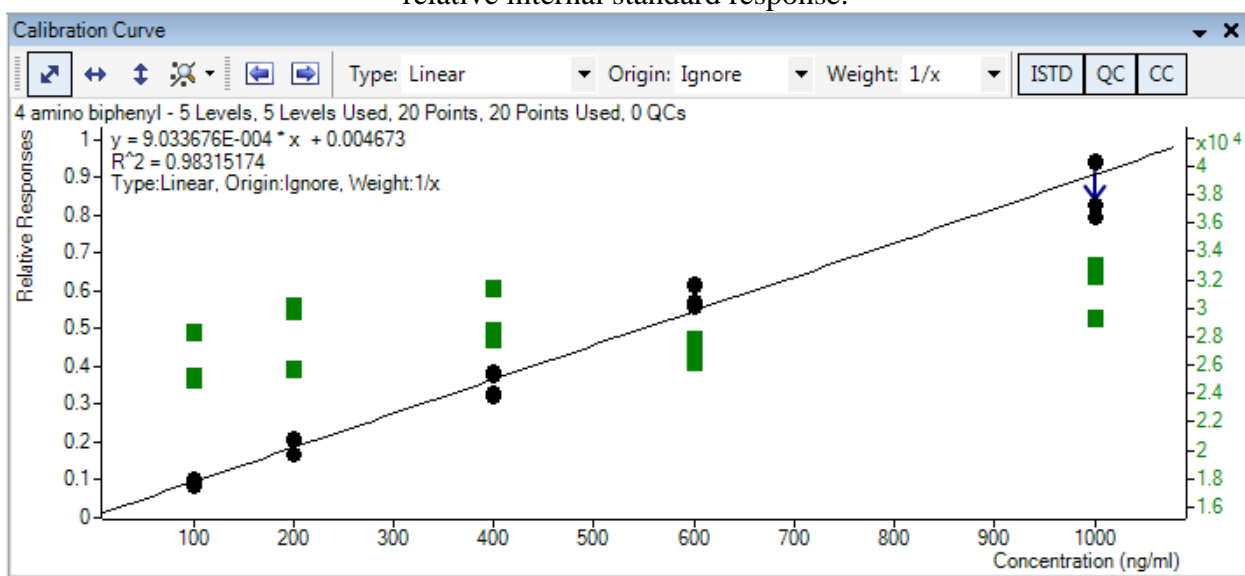

Figure 10: Calibration curve prepared for *4-Aminobiphenyl*. The green points represent the relative internal standard response.
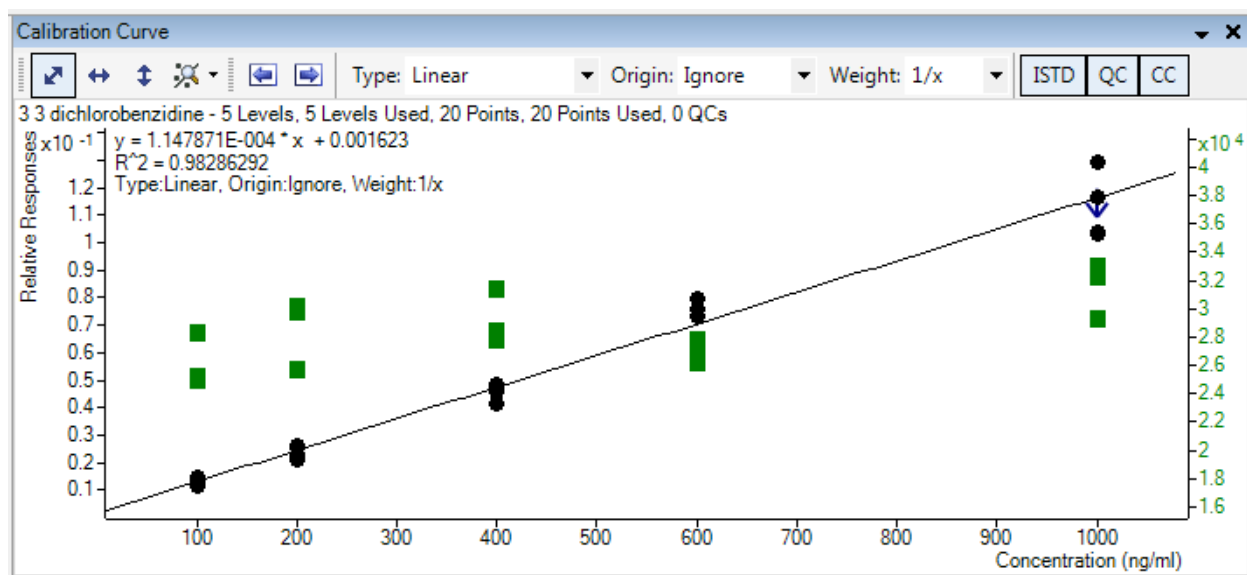
Figure 11: Calibration curve prepared for *3-3'-Dichlorobenzidine*. The green points represent the relative internal standard response.
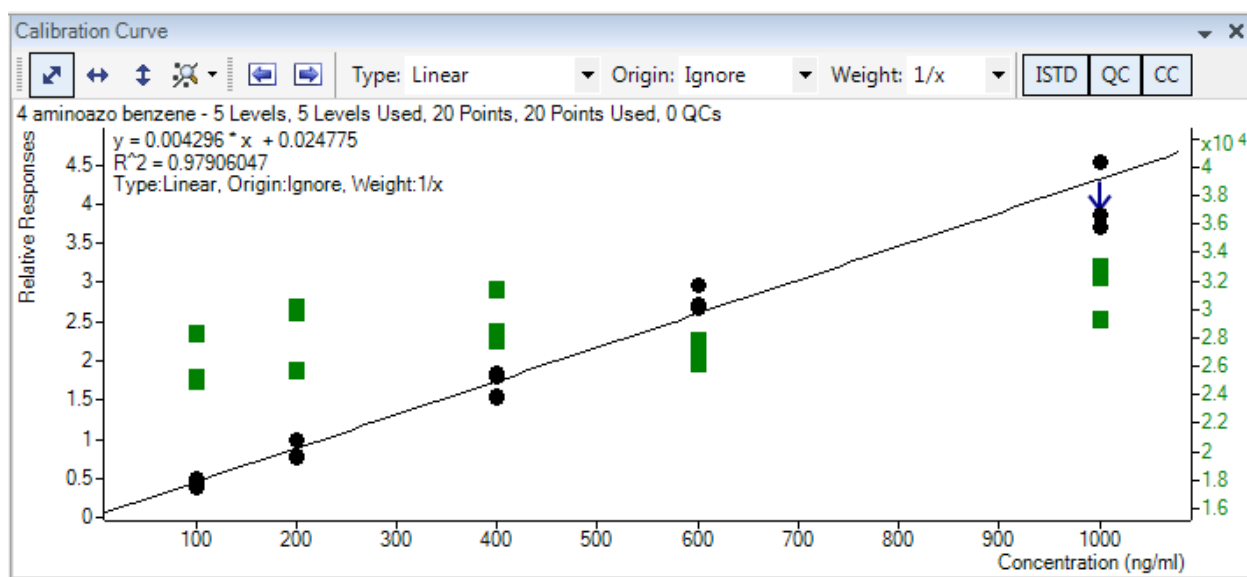

Figure 12: Calibration curve prepared for *4-Aminoazobenzene*. The green points represent the relative internal standard response.
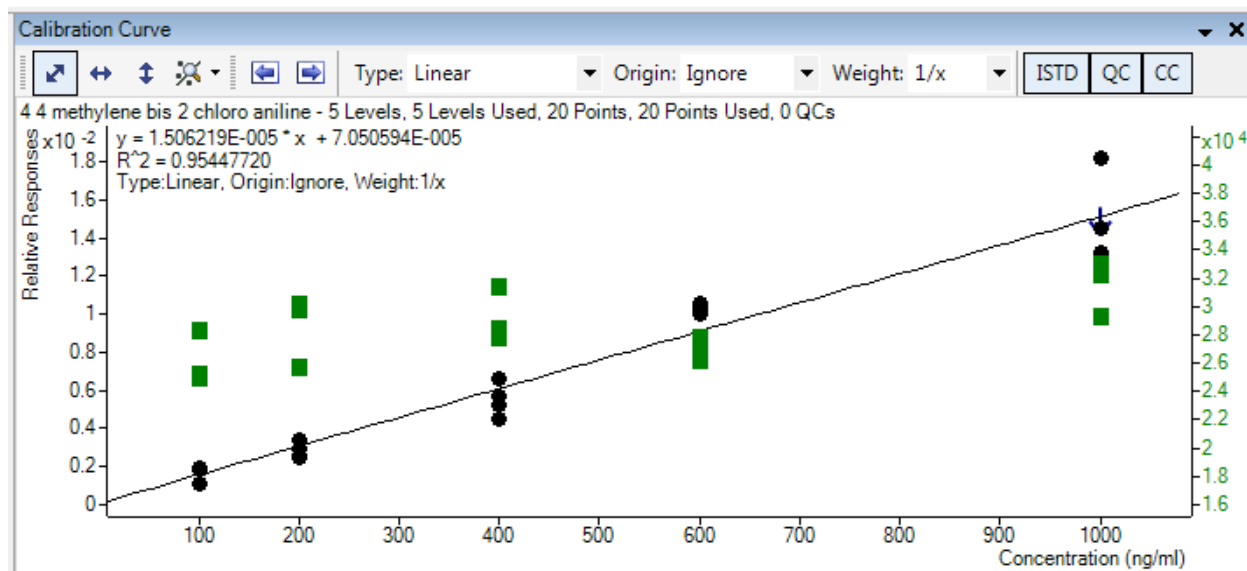
Figure 13: Calibration curve prepared for *4-4'-Methylene-bis-2-chloroaniline*. The green points represent the relative internal standard response.
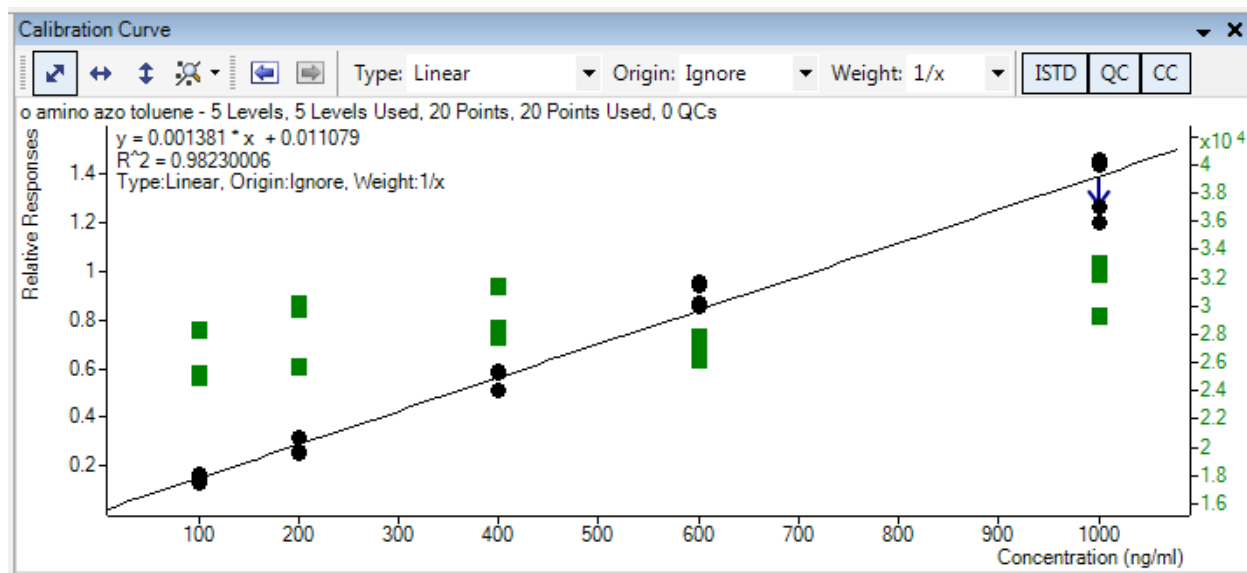

Figure 14: Calibration curve prepared for *o-Amino-azo-toluene*. The green points represent the relative internal standard response.

Analysis Conditions

QqQ-UHPLC

The following system was used for the quantitative QqQ-UHPLC analysis:

Instrument: Agilent 6470 QqQ with Agilent 1290 UHPLC system
Source: AJS ESI Source
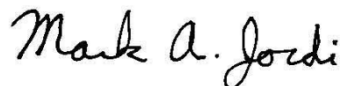Column: Extend C18, 1.8 µm, 2.1 x 150mm

Closing Comments

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Jordi Labs specializes in polymer testing and has 30 years experience doing complete polymer deformulations. We are one of the few labs in the country specialized in this type of testing. We will work closely with you to help explain your test results and solve your problem. We appreciate your business and are looking forward to speaking with you concerning these results.

Sincerely,

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Chemist
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