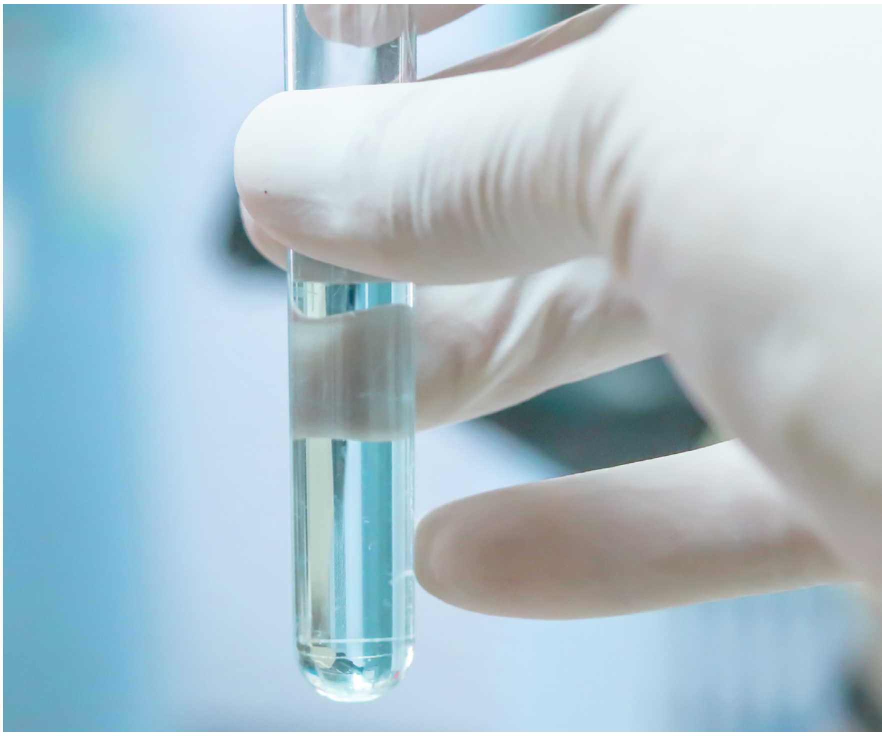

APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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Rapid Determination of Trace Amount of Aniline and Its Derivatives in Water by Direct Injection UHPLC/MS/MS

Introduction

Aniline and aniline derivatives are widely used as raw materials and intermediates in the polymer, rubber, dye, pesticide and pharmaceutical industries. Unintended releases of aniliane or its derivatives into the environment pose a serious threat, with release possible during any stage of production, storage, transport, use, or disposal. With rapid industrial development, environmental pollution caused by industrial releases has become a serious issue.

Aniline and its derivatives are considered toxic compounds because of their carcinogenic and mutagenic effects.[1-2] Therefore, it is important and necessary to develop fast, simple, sensitive, selective and efficient methods for the determination of aniline and its derivatives in drinking and environmental waters. Although a variety of analytical methods, such as gas chromatography (GC),[3-7] high performance liquid chromatography (HPLC),[8-12] capillary electrophoresis (CE),[13] and spectrophotometry[14] have been used for the determination of aniline and its derivatives in aqueous matrices, extensive sample clean up and analyte concentration steps are often necessary to achieve good separation and sensitive responses for these analytes due to the low sensitivity and selectivity of these methods.

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The aim of this study is to develop a simple, selective and sensitive LC/MS/MS method for rapid analysis of aniline and its derivatives in water samples by direct injection, without using time consuming sample preparation steps. By using a direct injection approach, this method can achieve the highest levels of sample throughput, while reducing potential analyte loss and contamination caused by various sample preparation steps. In addition, utilization of a stable isotopically labeled internal standard resulted in a method that is more accurate and robust, and can be easily applied by commercial laboratories for routine monitoring of aniline and its derivatives in water samples.

Experimental

Hardware/Software

Chromatographic separation of analytes from potential interfering components was conducted utilizing a PerkinElmer QSight® LX50 UHPLC System, and determination of analytes was achieved using the PerkinElmer QSight 220 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q software.

Method

Standards, Solvents and Sample Preparation

Aniline, its derivatives and deuterium labelled d5-aniline were obtained from Sigma-Aldrich. Water samples were obtained from local tap water resources in Guangzhou, China. LC/MS grade methanol (MeOH), formic acid, and water were obtained from Fisher Scientific.

Calibration curves were built by preparing standards at several concentration levels (0.01 to 100 µg/L) in water with internal standard to overcome any matrix effects.

1.0 mL of test sample and 10 µL of internal standard (d5-aniline with a concentration of 1 mg/L) were accurately pipetted into a centrifuge tube, and then mixed well on a vertex mixer. After centrifugation for five minutes at 15000 rpm, the supernatant was transferred directly into an autosampler vial for LC/MS/MS analysis without further treatment.

LC Method and MS Source Conditions

The LC method and MS source parameters are summarized in Table 1. The multiple reaction monitoring mode (MRM) transitions of analytes and their optimized parameters are included in Table 2.

Results and Discussion

A UHPLC/MS/MS method was successfully developed for simultaneous determination of 17 aniline compounds. As illustrated in Figure 1, all target compounds were determined with good peak shape and sensitivity. The limit of quantification (LOQs) of the method for target compounds ranged from 0.01 to 0.5 µg/L in water samples as shown in Table 3.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	Kinetex C18, 100 x 4.6 mm, 2.6 µm
Mobile Phase A	0.01% formic acid in water
Mobile Phase B	Methanol
Mobile Phase Gradient (Flow Rate: 0.5mL/min)	Start at 35% mobile phase B and perform isocratic run for 3 min, then increase B to 90% at 7.0 min and keep at 90% B for 2 mins, finally equilibrate the column at initial condition for 3 min.
Column Oven Temperature	30 °C
Auto Sampler Temperature	15 °C
Injection Volume	20 µL
MS Source Conditions	
ESI Voltage (Positive)	5500 V
Drying Gas	100
Nebulizer Gas	150
Source Temperature	500 °C
HSID Temperature	280 °C
Detection mode	Time managed MRM

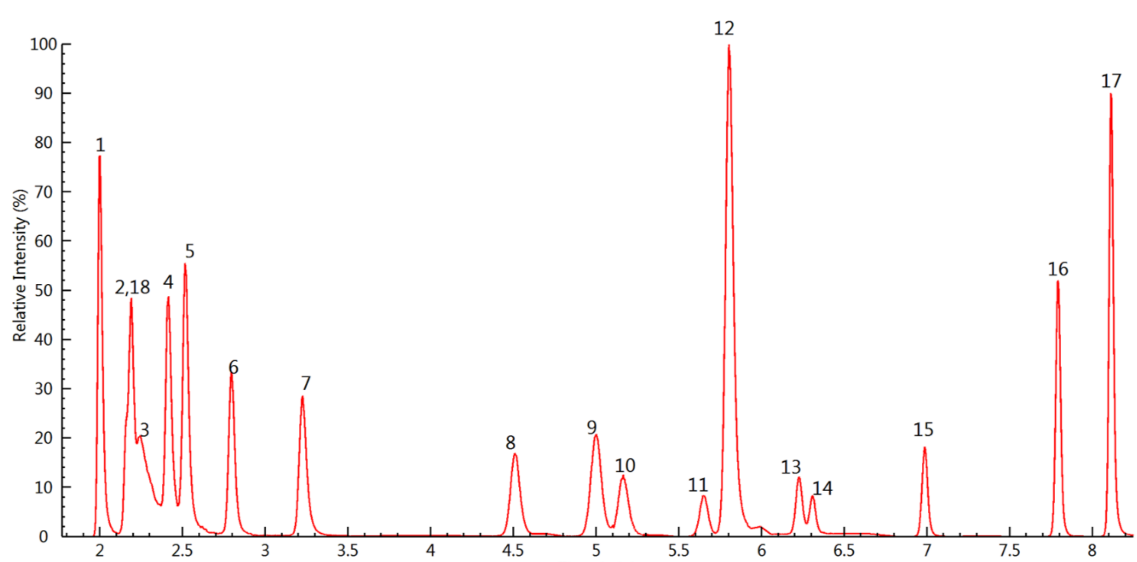

Figure 1. Total ion chromatograms of the 17 analytes at a concentration of 10 µg/L (analyte names and orders are shown in Table 2).

Table 2. Analyte retention time and optimized MRM parameters

Index	Analyte	MRM Quantifier / MRM Qualifier		RT (min)	CE (eV)
1	o-Phenylenediamine	109.2	92.0	5.45	-24
		109.2	65.0		-33
2	Aniline	94.0	77.1	2.17	-25
		94.0	50.9		-41
3	Benzidine	185.0	141.0	2.21	-33
		185.0	167.1		-38
4	p-Toluidine	108.1	91.0	2.39	-26
		108.1	65.0		-36
5	o-Anisidine	124.0	109.0	2.49	-24
		124.0	92.0		-25
6	o-Toluidine	108.1	91.0	2.77	-25
		108.1	65.0		-36
7	2,4-Dimethylaniline	122.0	107.0	3.20	-22
		122.0	79.0		-29
8	4-Nitroaniline	138.9	122.0	4.47	-21
		138.9	91.9		-33
9	3-Nitroaniline	138.9	93.1	4.95	-24
		138.9	76.0		-42
10	4-Chloroaniline	127.9	111.0	5.13	-31
		127.9	93.1		-29
11	2,6-Dimethylaniline	122.0	105.0	5.63	-24
		122.0	79.0		-29
12	2-Aminonaphthalene	144.0	127.0	5.79	-31
		144.0	76.9		-49
13	3-Chloroaniline	127.9	93.0	6.20	-27
		127.9	110.9		-31
14	2-Nitroaniline	138.9	121.0	6.29	-15
		138.9	91.0		-24
15	2-Methyl-6-ethylaniline	136.0	91.0	6.97	-31
		136.0	117.0		-27
16	2,6-Diethylaniline	150.0	105.0	7.78	-27
		150.0	91.0		-36
17	3,3-Dichlorobenzidine	253.0	217.0	8.10	-28
		253.0	182.0		-40
18	d5-Aniline	99.0	82.1	2.16	-25

Table 3. Limit of quantification (LOQ), linear concentration range and linearity (R^2).

Analyte	LOQ (µg/L)	Range (µg/L)	Linearity (R^2)
o-Phenylenediamine	0.01	0.01-100	0.995
Aniline	0.01	0.01-100	0.995
Benzidine	0.05	0.05-100	0.994
p-Toluidine	0.01	0.01-100	0.994
o-Anisidine	0.01	0.01-100	0.994
o-Toluidine	0.01	0.01-100	0.997
2,4-Dimethylaniline	0.01	0.01-100	0.999
4-Nitroaniline	0.05	0.05-100	0.997
3-Nitroaniline	0.05	0.05-100	0.998
4-Chloroaniline	0.02	0.02-100	0.997
2,6-Dimethylaniline	0.05	0.05-100	0.998
2-Aminonaphthalene	0.01	0.01-100	0.996
3-Chloroaniline	0.05	0.05-100	0.998
2-Nitroaniline	0.5	0.5-100	0.996
2-Methyl-6-ethylaniline	0.02	0.02-100	0.995
2,6-Diethylaniline	0.01	0.01-100	0.998
3,3-Dichlorobenzidine	0.01	0.01-100	0.996

During method development, the composition and ratio of the mobile phases were optimized. The effects of formic acid concentrations (such as 0.1%, 0.05%, 0.01% and 0.005%) on analyte separation and responses was evaluated, and it was found that 0.01% of formic acid gave the best results. In this study, internal standard calibrations were used for quantification to compensate for sample matrix effects. The calibration curves showed wide linear dynamic ranges (as shown in Table 3), with regression coefficients (R^2) greater than 0.99. Method accuracy was evaluated by the recovery of a known amount of analyte spiked into a water sample. In this study, recoveries of the analytes were evaluated at concentrations of 0.1, 1.0 and 10.0 µg/L for all analytes except for 2-nitroaniline, which was evaluated at 1.0 and 10.0µg/L due to its lower sensitivity. As shown in Table 4, the mean recovery values ranged from 73.1% to 127% with RSD <5% (n = 5). The intra-day and inter-day variations, expressed as RSD, were less than 8%, respectively.

The method described above was applied for the determination of aniline and its derivatives in five water samples. Results show that no analytes were found in two of the five samples. A small amount of aniline was determined from two of the remaining water samples, one with aniline at 0.23 µg/L and the other with aniline at 0.16 µg/L. In the final sample, 0.03 µg/L of 3,3-dichlorobenzidine, an aniline derivative, was detected.

Conclusions

A simple, fast, sensitive, selective, and robust analytical method has been developed and validated for simultaneous determination of trace amounts of aniline and its derivatives in water samples by coupling a QSight LX50 UHPLC and a QSight mass spectrometer. The method showed a wide linear range, and eliminated time-consuming and labor-intensive sample preparation procedures, reducing the cost and time associated with the analysis, while also preventing analyte loss and potential contamination during sample preparations. The method can be applied to the analysis of aniline and its derivatives in water samples with good precision and accuracy.

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Table 4. Results of analyte recoveries (%) and precision (RSD%) for water sample analysis.

Analyte	Spiked Level (0.1 µg/L)		Spiked Level (1.0 µg/L)		Spiked Level (10.0 µg/L)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
o-Phenylenediamine	113	4.4	119	1.6	127	1.5
Aniline	86.8	3.7	108	2.1	109	0.6
Benzidine	103	2.4	108	1.7	101	2.4
p-Toluidine	109	4.6	105	1.1	106	1.0
o-Anisidine	109	4.0	114	1.2	116	0.8
o-Toluidine	104	4.9	108	1.4	107	1.0
2,4-Dimethylaniline	103	3.9	109	0.4	109	1.1
4-Nitroaniline	101	3.3	103	1.6	104	1.2
3-Nitroaniline	73.1	4.0	92.1	3.0	88.8	0.9
4-Chloroaniline	97.6	4.8	91.1	2.9	101	4.1
2,6-Dimethylaniline	118	2.9	113	1.2	115	1.2
2-Aminonaphthalene	125	3.5	117	3.0	119	0.3
3-Chloroaniline	121	3.9	117	1.8	122	1.1
2-Nitroaniline	-	-	127	2.6	121	2.6
2-Methyl-6-ethylaniline	115	4.3	126	0.9	121	0.5
2,6-Diethylaniline	127	2.6	123	0.8	125	0.6
3,3-Dichlorobenzidine	117	3.8	121	1.3	127	0.2

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