

Determination of Methylamine in Drug Products

Thunyarat Phesatcha,¹ Suparek Tukkeeree,¹ and Jeff Rohrer²

¹Thermo Fisher Scientific, Bangkok, Thailand; ²Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words

Pharmaceutical, Dionex IonPac CS19 Column, Reagent-Free Ion Chromatography

Introduction

Methylamine or monomethylamine (MMA) is widely used in different stages of the preparation of various drug substances; therefore it may be retained in the drug substance. Although there is little information concerning its effect on human or animal health, its median lethal dose in a mouse is reported to be 2.5 g/kg. MMA is also controlled by the U.S. Drug Enforcement Administration as a List 1 Regulated Chemical due to its use in production of methamphetamine.¹

For any drug substance, the manufacturer must monitor the level of anticipated process-related and degradation impurities before commercial release to ensure product safety and consistency of the manufacturing process. There is one report of an ion chromatography (IC) method using manually prepared eluents and direct conductivity detection to determine MMA in drug substances.² Because poor sensitivity is an inherent weakness of direct conductivity detection, suppressed conductivity detection will improve method sensitivity.

Reagent-Free™ IC (RFIC™) systems have been successfully used to measure ionic drug-degradation products and process-related impurities. Dimethylamine, a process-related impurity of metformin hydrochloride, has been measured in a metformin hydrochloride drug product.³ N-methylpyrrolidine, a degradation product of cefipime, has been determined in cefipime and simulated cefipime for injection.^{4,5} Ethyl sulfate, another process-related impurity, has been measured in indinavir sulfate.⁶ IC also has been used to determine the process impurity ethylhexanoate in clavulanate.⁷

The work shown here uses a RFIC system with suppressed conductivity detection to determine MMA in drug products. This method uses a Thermo Scientific™ Dionex™ IonPac™ CS19 analytical column with methanesulfonic acid (MSA) eluent produced by an eluent generator. The method separates MMA from other cations typically present in drug products.



Goal

To develop an IC method to determine MMA in drug products using a RFIC system

Equipment

Thermo Scientific™ Dionex™ ICS-5000 system,* including:

- DP Dual Pump
- DC Detector/Chromatography Compartment
- EG Eluent Generator with EGC III MSA Eluent Generator Cartridges
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software version 7.10 SR or higher

*A Dionex ICS-3000 or ICS-5000+ system also may be used.

Consumables

Syringe filters, 0.45 µm

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 M Ω -cm resistivity or better
- Methylamine (CH₃NH₂) solution 40 wt. % in H₂O (Sigma-Aldrich® P/N 426466)
- Methanol (CH₃OH), HPLC grade
- Hydrochloric acid (HCl), 37%

Preparation of Solutions and Reagents

Eluent

The EG produces the eluent using the Dionex EGC III MSA Cartridge and DI water supplied by the pump. The eluent concentration is controlled by the Chromeleon CDS software. The EG degasser requires 14 MPa (2000 psi) system backpressure, which ensures optimal removal of the gas produced by the EG. For more information about adding system backpressure, refer to the Dionex ICS-5000 Ion Chromatography System Operator's Manual or any other Thermo Scientific Dionex RFIC system operator's manual.

Hydrochloric Acid, 0.5 M

Add approximately 500 mL of DI water to a 1 L volumetric flask. Slowly add 42 mL of 37% HCl to the same flask and bring to volume with DI water.

MMA Stock Standard Solution, 1000 mg/L

Add 0.25 mL of 40% MMA solution to a 100 mL volumetric flask and bring to volume with DI water.

MMA Stock Standard Solution, 10 mg/L

Add 1 mL of 1000 mg/L MMA stock standard solution to a 100 mL volumetric flask and bring to volume with DI water.

Working standard solutions

Add the appropriate volumes of 10 mg/L MMA stock standard solution (Table 1) into separate 100 mL volumetric flasks, bring to volume with DI water, and mix.

Table 1. Preparation of the working standards.

Level	Volume of MMA Stock Standard Solution (mL)	Concentration (μ g/L)
1	0.10	10
2	0.20	20
3	0.30	30
4	0.40	40
5	0.50	50

Samples

- Alfuzosin
- Sertraline Hydrochloride

Sample Preparation

Unspiked Sample

Weigh each drug tablet and grind it to a fine powder. Add DI water for the sample dissolution to make 100-fold and 200-fold dilutions of the alfuzosin and sertraline hydrochloride samples, respectively. For example, for the first tablet of each drug product listed in Table 2, add 36.6 g of DI water to 0.366 g alfuzosin and add 32.6 g of DI water to 0.163 g sertraline HCl. Filter 3 mL of sample using a 0.45 μ m syringe filter, then discard the first 2 mL while collecting the final mL sample in a sample vial. Keep the rest of the sample for spiked sample preparation.

Table 2. Weights of the drug product samples (g/tablet).

Tablet No.	Alfuzosin	Sertraline HCl
1	0.366	0.163
2	0.368	0.160
3	0.365	0.163

Spiked Sample

Because the amount of MMA in a drug tablet and each drug product may vary, spike the sample with a known amount of MMA to assess method accuracy. Prepare the sample by adding 10 μ L of 10 mg/L MMA stock standard to a 10 mL volumetric flask and bring to volume with the sample solution. Filter the spiked sample solution using a 0.45 μ m syringe filter prior to injection.

Time (min)	Pump 1 (0.5 mL/min)		Pump 2 (1.0 mL/min)		Remarks
	A: DI Water B: Methanol C: 0.5 M HCl	Injection Valve 1 Position	Conc of Eluent (mM)	Injection Valve 2 Position	
-7.0	100% A	Load	1.7	Load	Flush concentrator with DI water
-5.0		Inject	1.7		Dionex AS-AP Autosampler starts loading sample into the sample loop and flushing sample to the concentrator
0.0			1.7		Start sample analysis
1.0	90% B 10% C		1.7	Clean the Dionex IonPac NG1-10 µm guard column with 10% HCl and 90% methanol	
11.0	1.7				
12.0	1.7				
16.0	100% A	Inject	11.0	Inject	Equilibrate the Dionex IonPac NG1-10 µm guard column
25.0			11.0		

On-Line Sample Preparation

Perform off-line sample preparation as previously described. Inject the sample solution for on-line preparation by passing it through the Dionex IonPac NG1-10 µm Guard Column to trap hydrophobic compounds that would otherwise contaminate the analytical column. After MMA is trapped on the Dionex IonPac TCC-LP1 Low Pressure Cation Concentrator column, transfer it to the Dionex IonPac CS19 cation-exchange analytical column for analysis. After the transfer of MMA to the analytical column, wash the Dionex IonPac NG1-10 µm Guard Column with 0.5 M HCl and methanol. Figure 1 shows the system configuration and Figure 2 shows a DI water blank using this configuration with the program detailed in Table 3.

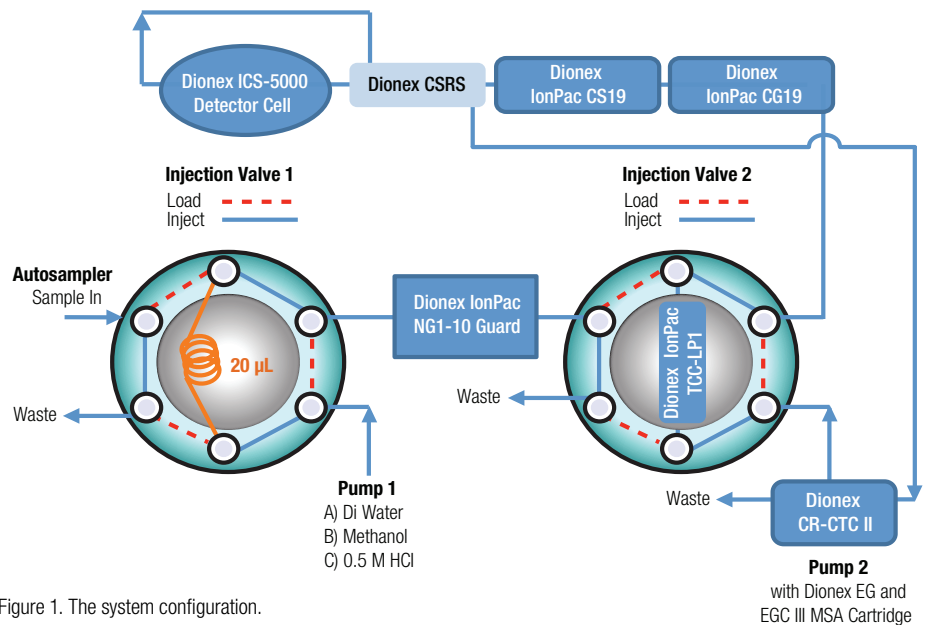
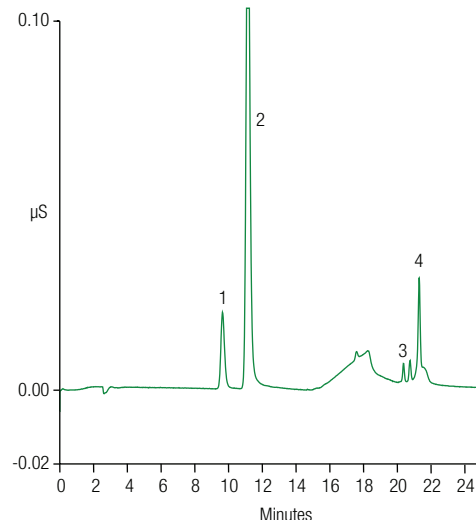


Figure 1. The system configuration.



Columns: Dionex IonPac CG19 Guard, 4 × 50 mm
 Dionex IonPac CS19 Analytical, 4 × 250 mm
 Eluent Source: Dionex EGC III MSA Cartridge with Dionex CR-CTC II
 Gradient: 1.7 mM MSA from -7 to 12 min, ramp to 11 mM MSA at 4 min, hold for 9 min
 Flow Rate: See Table 3
 Inj. Volume: 20 µL
 Temperature: 35 °C
 Detection: Suppressed conductivity, Dionex CSRS 300, Recycle mode, Current 33 mA
 Samples: DI water blank

Peaks:
 1. Sodium — mg/L
 2. Ammonium —
 3. Magnesium —
 4. Calcium —

Figure 2. A DI water blank.

Conditions

Column:	Dionex IonPac CG19 Guard, 4 × 50 mm (P/N 076027)
	Dionex IonPac CS19 Analytical, 4 × 250 mm (P/N 076026)
Concentrator:	Dionex IonPac TCC-LP1 Low Pressure Cation Concentrator, 4 × 35 mm (P/N 046027)
Trap Column:	Dionex IonPac NG1-10 μm Guard, 4 × 35 mm (P/N 039567)
Eluent Source:	Dionex EGC III MSA Cartridge (P/N 074535) with Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262)
Gradient:	See Table 3.
Flow Rate:	See Table 3.
Inj. Volume:	20 μL
Temperature:	35 °C
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ CSRS™ 300 Cation Self-Regenerating Suppressor, 4 mm (P/N 064556), Recycle mode, current 33 mA
Total Conductivity:	~0.35 μS

Columns:	Dionex IonPac CG19 Guard, 4 × 50 mm	Peaks:	1. Lithium	0.1 mg/L
	Dionex IonPac CS19 Analytical, 4 × 250 mm		2. Sodium	0.4
Eluent Source:	Dionex EGC III MSA Cartridge with Dionex CR-CTC II		3. Ammonium	0.5
Gradient:	1.7 mM MSA from -7 to 12 min, ramp to 11 mM MSA at 4 min, hold for 9 min		4. MMA	1.0
Flow Rate:	See Table 3		5. Potassium	1.0
Inj. Volume:	20 μL		6. Magnesium	0.5
Temperature:	35 °C		7. Calcium	1.0
Detection:	Suppressed conductivity, Dionex CSRS 300, Recycle mode, Current 33 mA			
Samples:	Six common cations and the MMA standard			

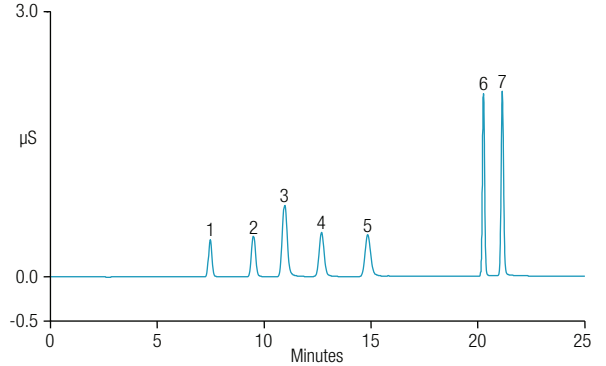


Figure 3. Six common cations and the MMA standard.

Results and Discussion

Separation

MMA is a primary amine that can be separated from six common cations using the Dionex IonPac CS19 column. As shown in Figure 3, MMA was well separated from the other common cations using a gradient of MSA. The MSA eluent was produced automatically by pumping DI water through the Dionex EGC III MSA cartridge, with the concentration controlled by Chromeleon CDS software. The Dionex IonPac CS19 column was designed to deliver good peak shapes for amines, which is evident in Figure 3.

Linearity Range

The method linearity was studied by preparing eight different concentrations of the MMA standard ranging from 10 to 125 μg/L. The prepared standard solutions were then injected and the peak area response was measured. The peak area response was plotted versus the standard concentration (Figure 4) and the data were fitted into two ranges. The results show that the plot is more linear in the range of 10–50 μg/L. The coefficients of determination (r^2) for the ranges of 10–125 μg/L (Figure 4A) and 10–50 μg/L (Figure 4B) are reported in Table 4. Although it may be possible to use the larger range, the MMA concentrations are expected to fall within the smaller range, which was used for method calibration.

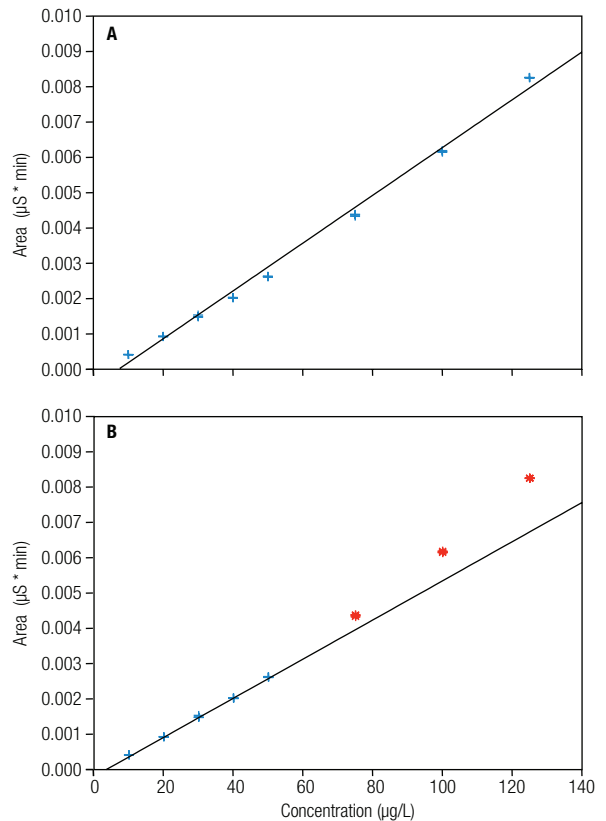


Figure 4. The plot of area response versus MMA standard concentration.

Table 4. Concentrations of MMA used for the linearity study.

Concentration (μg/L)								Result
Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	r^2
10	20	30	40	50	75	100	125	0.99429
10	20	30	40	50	—	—	—	0.99911

Table 5. Working standard concentrations and calibration results.

Concentration ($\mu\text{g/L}$)					Result			
Level 1	Level 2	Level 3	Level 4	Level 5	# Points	r^2	Offset	Slope
10	20	30	40	50	15	0.99911	-0.0003	0.0001

Method Calibration

The method was calibrated before the sample analysis using five concentrations of MMA ranging from 10 to 50 $\mu\text{g/L}$. The method shows a linear relationship between the analyte concentration and peak area of MMA. The coefficient of determination for the line was 0.9991. Figure 5 shows the overlay of chromatograms of the working (calibration) standards. Table 5 shows the concentrations of the working standards and the calibration result.

Method Detection Limit

The method detection limit (MDL) was estimated using the signal-to-noise (S/N) ratio of the spiked sample injection with the MDL defined as $3 \times \text{S/N}$. The MDLs calculated from both spiked samples of both drug products were 1.2 $\mu\text{g/L}$. Note that a 20 μL injection volume (the same as that used in Reference 2) was used, and the calculated MDL was significantly lower than the value reported for nonsuppressed conductivity detection in Reference 2. This highlights the expected sensitivity benefit of using suppressed rather than nonsuppressed conductivity detection.

Sample Analysis

Two drug products, one containing sertraline hydrochloride and one containing alfuzosin, were purchased from a pharmacy in Bangkok, Thailand. The samples were prepared three times with one tablet used for each preparation as described in the Sample Preparation section. Five sample injections were made for each preparation. MMA was found in the sertraline hydrochloride drug product sample in each sample preparation at concentrations of 16.3, 15.6, and 33.2 $\mu\text{g/L}$. There was no MMA found in the alfuzosin drug product sample.

Columns: Dionex IonPac CG19 Guard, 4 \times 50 mm
 Dionex IonPac CS19 Analytical, 4 \times 250 mm
 Eluent Source: Dionex EGC III MSA Cartridge with Dionex CR-CTC II
 Gradient: 1.7 mM MSA from -7 to 12 min, ramp to 11 mM MSA at 4 min, hold for 9 min
 Flow Rate: See Table 3
 Inj. Volume: 20 μL
 Temperature: 35 $^{\circ}\text{C}$
 Detection: Suppressed conductivity, Dionex CSRS 300, Recycle mode, Current 33 mA
 Samples: Working Standards

Peaks: 1. Sodium — $\mu\text{g/L}$
 2. Ammonium —
 3. MMA 10, 20, 30, 40, & 50
 4. Magnesium —
 5. Calcium —

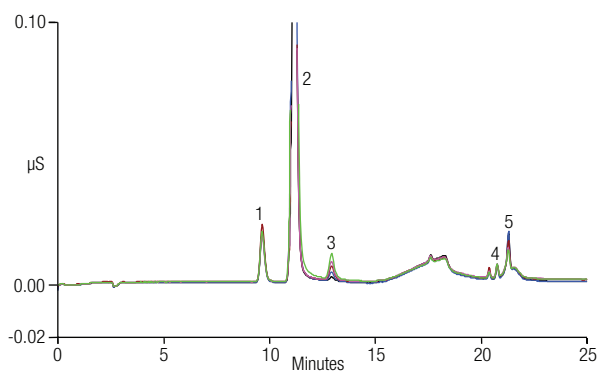


Figure 5. Overlay of chromatograms of the working standards.

Table 6. Sample and recovery results of alfuzosin.

Injection No.	Found Amount ($\mu\text{g/L}$)					
	1		2		3	
	Sample	Spiked Sample	Sample	Spiked Sample	Sample	Spiked Sample
1	—	11.3	—	10.8	—	10.3
2	—	11.4	—	10.6	—	10.7
3	—	11.4	—	10.5	—	10.5
4	—	11.3	—	10.6	—	10.1
5	—	11.1	—	10.7	—	10.6
Average	—	11.3	—	10.6	—	10.4
RSD	—	1.1	—	1.3	—	2.1
Spiked Conc ($\mu\text{g/L}$)	10		10		10	
Recovery (%)	113		106		104	

To evaluate method accuracy, the spiked samples were prepared three times and five injections of each spiked sample were made to quantify MMA. The measured concentrations were then compared to the spiked plus endogenous concentration. The analysis of spiked alfuzosin-containing samples yielded MMA recoveries of 104–113% with RSDs for each set of five injections of 1.1–2.1% (Table 6). Figure 6 shows the overlay of chromatograms of unspiked and spiked alfuzosin-containing samples. Analysis of the spiked sertraline hydrochloride product sample yielded MMA recoveries of 99.8–106% with RSDs for each set of five injections of 0.31–1.4% (Table 7). Figure 7 shows the overlay of chromatograms of unspiked and spiked sertraline hydrochloride-containing samples.

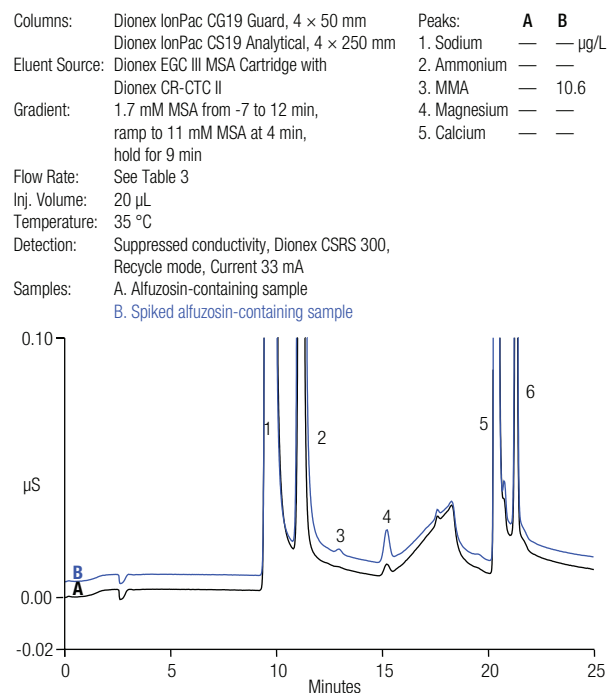


Figure 6. Overlay of chromatograms of unspiked (A) and spiked (B) alfuzosin-containing samples.

Table 7. Sample and recovery results of sertraline hydrochloride.

Injection No.	Found Amount ($\mu\text{g/L}$)					
	1		2		3	
	Sample	Spiked Sample	Sample	Spiked Sample	Sample	Spiked Sample
1	16.4	26.4	15.5	25.6	33.2	43.4
2	16.1	26.4	15.7	25.9	33.6	43.8
3	16.5	26.5	15.5	25.3	33.2	44.0
4	16.3	26.4	15.4	25.7	33.2	44.2
5	16.2	26.6	16.0	25.5	33.0	43.8
Average	16.3	26.5	15.6	25.6	33.2	43.8
RSD	1.0	0.31	1.4	0.93	0.62	0.68
Spiked Conc ($\mu\text{g/L}$)	10		10		10	
Recovery (%)	101		99.8		104	

Columns: Dionex IonPac CG19 Guard, 4×50 mm Peaks: A B
 Dionex IonPac CS19 Analytical, 4×250 mm 1. Sodium — — $\mu\text{g/L}$
 Eluent Source: Dionex EGC III MSA Cartridge with 2. Ammonium — —
 Dionex CR-CTC II 3. MMA 16.3 26.5
 Gradient: 1.7 mM MSA from -7 to 12 min, 4. Magnesium — —
 ramp to 11 mM MSA at 4 min, 5. Calcium — —
 hold for 9 min
 Flow Rate: See Table 3
 Inj. Volume: 20 μL
 Temperature: 35 $^{\circ}\text{C}$
 Detection: Suppressed conductivity, Dionex CSRS 300,
 Recycle mode, Current 33 mA
 Samples: A. Sertraline hydrochloride-containing sample
 B. Spiked sertraline hydrochloride-containing sample

Conclusion

This study demonstrates an accurate and reproducible method with a RFIC system with suppressed conductivity detection for the determination of MMA in drug products. The method uses simple off-line sample preparation followed by an on-line step to complete the sample preparation before analysis. This approach requires only 32 min per analysis. The eluent is produced by an eluent generator to preclude the labor and potential error associated with eluent preparation.

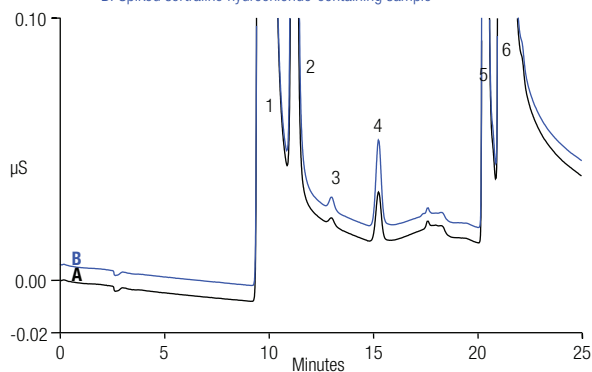


Figure 7. Overlay of chromatograms of unspiked (A) and spiked (B) sertraline hydrochloride-containing samples.

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