Determination of Dimethylamine in Metformin HCl Drug Product Using IC with Suppressed Conductivity Detection

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Application Note 298

Key Words
Type 2 Diabetes, Reagent-Free Ion Chromatography (RFIC), Dionex IonPac CS19 Column, Pharmaceuticals

Introduction
Metformin (*N,N*-dimethylimidodicarbonimidic diamide) is one of the most widely prescribed orally administered antidiabetic drug for the treatment of type 2 diabetes. It is also used for treatment of polycystic ovary syndrome.[1]

Metformin is prepared from the reaction of dimethylamine hydrochloride and 2-cyanoguanidine. Metformin drug preparations must be tested for residual dimethylamine, because the weakly acidic conditions required for the reaction can promote formation of dimethylnitrosamine, a suspected human carcinogen.[2] To confirm the consistency of the manufacturing process, drug product manufacturers must monitor the level of anticipated process-related and degradation impurities before commercial release of a drug product. Dimethylamine is not monitored in the U.S. Pharmacopeia and National Formulary Metformin Hydrochloride (HCl) monograph.[3]

Ion chromatography (IC) has been successfully used to measure ionic drug degradation products and process-related impurities. *N*-methylpyrrolidine, a degradation product of cefipime, has been measured in cefipime and simulated cefipime for injection.[4,5] Ethylsulfate, a process-related impurity, has been measured in indinavir sulfate.[6] IC was also used to determine the process impurity ethylhexanoate in clavulanate.[7]

Here, an IC method is used for quantitation of dimethylamine in metformin hydrochloride. This method uses a Thermo Scientific Dionex IonPac CS19 cation-exchange analytical column with methanesulfonic acid eluent produced by an eluent generator. The method separates dimethylamine from other cations typically present in drug products.

Equipment
- Thermo Scientific Dionex ICS-2000 Reagent-Free™ IC (RFIC™) System
- Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software Version 6.80 SR9 or higher

* This application can be run on any Dionex IC system capable of eluent generation or, if eluents are manually prepared, any Dionex IC system.

Reagents and Standards
- Deionized water (DI), Type I reagent-grade, 18 MΩ-cm resistivity or better
- Dimethylamine hydrochloride 99% (C_2H_7N HCl, Sigma-Aldrich)
- Thermo Scientific Dionex Six Cation-II Standard (P/N 046070)

Preparation of Solutions and Reagents
Eluent
The eluent generator produces the eluent using the Thermo Scientific Dionex EGC III MSA EluGen Methanesulfonic Acid Cartridge and DI water (18 MΩ-cm resistivity or better) supplied by the pump. The eluent concentration is controlled by Chromeleon™ CDS software.

Note: The eluent generator degasser requires 14 MPa (2000 psi) of system backpressure to ensure optimum removal of electrolytic gas produced by the Dionex EGC III MSA Cartridge. For more information about adding system backpressure, refer to the ICS-2000 Operator's Manual (document no 031857) or any other RFIC system Operator's Manual.

1000 mg/L Dimethylamine Stock Standard Solution
Place 0.183 g of dimethylamine hydrochloride, $(CH_3)_2NH{\cdot}HCl$, in a 100 mL volumetric flask, dissolve in DI water, bring to volume, and mix.

100 mg/L Dimethylamine Standard Solution
Add 10 mL of 1000 mg/L dimethylamine stock standard solution to a 100 mL volumetric flask and bring to volume with DI water.

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10 µg/L Dimethylamine Standard Solution (Method Detection Limit Study)

Add 10 µL of 100 mg/L dimethylamine standard solution to a 100 mL volumetric flask and bring to volume with DI water.

Working Standard Solutions

Prepare dimethylamine standards at concentrations of 0.1, 0.25, 0.5, 1.0 and 2.0 mg/L by adding 0.1, 0.25, 0.5, 1.0 and 2.0 mL of 100 mg/L dimethylamine standard solution into separate 100 mL volumetric flasks, and bring to volume with DI water.

Sample Preparation

Grind three tablets of metformin HCl (500 mg) to fine powder. Add 0.1 g of metformin HCl fine powder to a 100 mL volumetric flask, dissolve in DI water, and bring to volume. Filter the sample with 0.2 µm syringe filter.

To prepare a spiked sample, add 10 µL of 1000 mg/L dimethylamine stock standard solution to a 100 mL volumetric flask containing 0.1 g of metformin HCl fine powder before dissolution.

Conditions

IC Conditions

Column:	Dionex IonPac™ CS19 Analytical, 4 × 250 mm (P/N 076026)
Guard:	Dionex IonPac CG19 Guard, 4 × 50 mm (P/N 076027)
Eluent Source:	Dionex EGC III MSA EluGen Cartridge (P/N 074535) with a Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262)
Gradient:	Time (min) / Concentration MSA (mM)
	-7.0 / 2
	0.0 / 2
	12.0 / 2
	16.0 / 12
	18.0 / 12
	20.0 / 40
	30.0 / 40
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
Temperature:	40 °C
Pressure:	~2100 psi
Detection:	Suppressed conductivity, Thermo Scientific Dionex CSRS 300 Cation Self-Regenerating Suppressor, 4 mm (P/N 064556), recycle mode, suppressor current 120 mA

Results and Discussion

Separation

Dimethylamine is a secondary amine that can be separated from the six common cations using a Dionex IonPac CS19 Analytical Column with gradient elution.

Figure 1 shows separation of dimethylamine and six common cations that can be present in drug products. This separation was developed by starting with the conditions in Section 5.6 (p 27) of the Dionex IonPac CS19 Product Manual, then adjusting the final eluent concentration to elute metformin from the column.

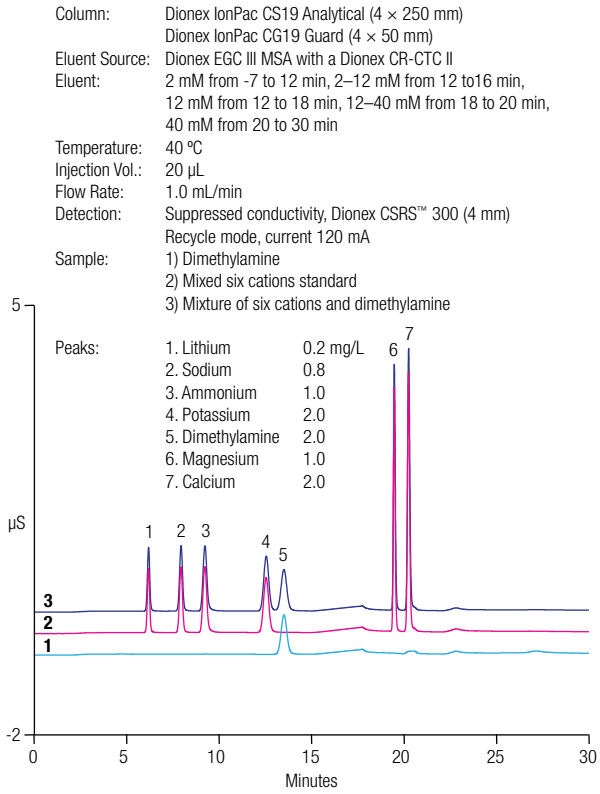

Figure 1. Overlay of chromatograms of 1) dimethylamine, 2) common cations, and 3) a mixture of cations and dimethylamine.

Method Detection Limit (MDL)

The MDL was determined by preparing 10 µg/L dimethylamine and making seven consecutive injections. The peak areas from seven consecutive injections were then used for the MDL calculation following the equation MDL = t(n-1, 0.99) (S), where: t(n-1, 0.99) = the Student's t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom, and S = standard deviation of the replicate analyses. The MDL obtained from the experiment was 1.5 µg/L. Figure 2 shows the chromatogram of 10 µg/L dimethylamine and blank (DI water). The blank chromatogram shows there are no interfering peaks at the retention time of dimethylamine. The signal-to-noise ratio (S/N) of the dimethylamine peak in Figure 2 is 17. Therefore the 3 × S/N estimate of the MDL is 1.8 µg/L, which is similar to the initial estimate.

Column:	Dionex IonPac CS19 Analytical (4 × 250 mm) Dionex IonPac CG19 Guard (4 × 50 mm)
Eluent Source:	Dionex EGC III MSA with a Dionex CR-CTC II
Eluent:	2 mM from -7 to 12 min, 2–12 mM from 12 to16 min, 12 mM from 12 to 18 min, 12–40 mM from 18 to 20 min, 40 mM from 20 to 30 min
Temperature:	40 °C
Injection Vol.:	20 µL
Flow Rate:	1.0 mL/min
Detection:	Suppressed conductivity, Dionex CSRS 300 (4 mm) Recycle mode, current 120 mA
Sample:	1) Blank DI Water 2) 0.01 mg/L Dimethylamine
Peak:	1. Dimethylamine 0.01 mg/L

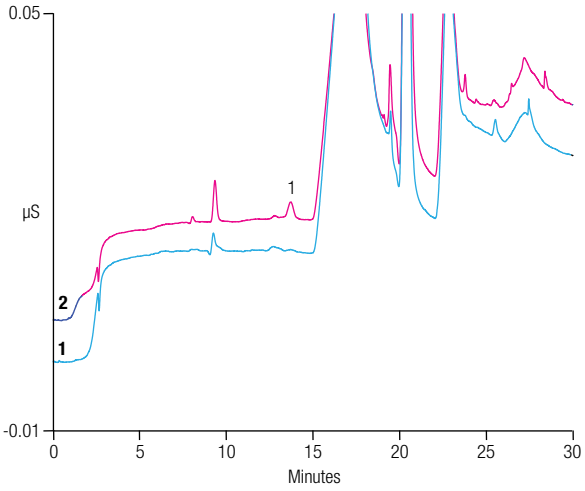

Figure 2. Overlay of chromatograms of MDL standard (0.01mg/L dimethylamine) and a DI water injection (blank).

Method Calibration

The method was calibrated before the sample analysis using five different concentrations of dimethylamine standard and three injections of each standard. Figure 3 shows the chromatogram overlay of the five concentrations of calibration standards. Figure 4 shows the peak area versus amount injected for the method calibration. The method calibration shows the linear plot of detector response versus concentration.

Column:	Dionex IonPac CS19 Analytical (4 × 250 mm) Dionex IonPac CG19 Guard (4 × 50 mm)
Eluent Source:	Dionex EGC III MSA with a Dionex CR-CTC II
Eluent:	2 mM from -7 to 12 min, 2–12 mM from 12 to 16 min, 12 mM from 12 to 18 min, 12–40 mM from 18 to 20 min, 40 mM from 20 to 30 min
Temperature:	40 °C
Injection Vol.:	20 µL
Flow Rate:	1.0 mL/min
Detection:	Suppressed conductivity, Dionex CSRS 300 (4 mm) Recycle mode, current 120 mA
Sample:	Calibration standard
Peak:	1. Dimethylamine 0.1, 0.25, 0.5, 1.0, and 2.0 mg/L

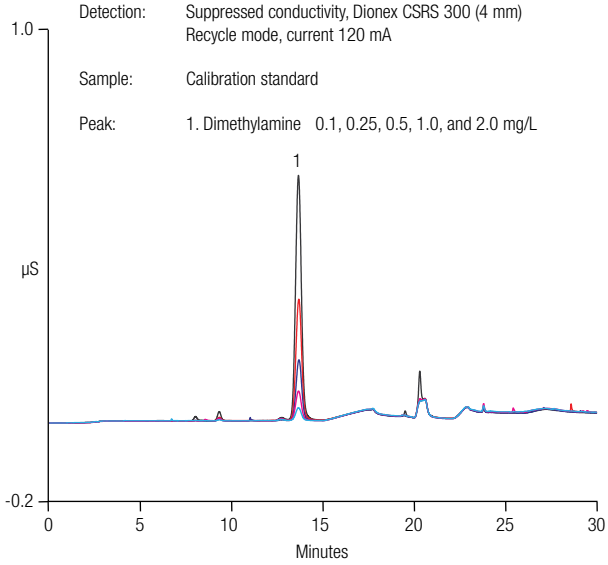

Figure 3. Overlay of calibration standards chromatograms.

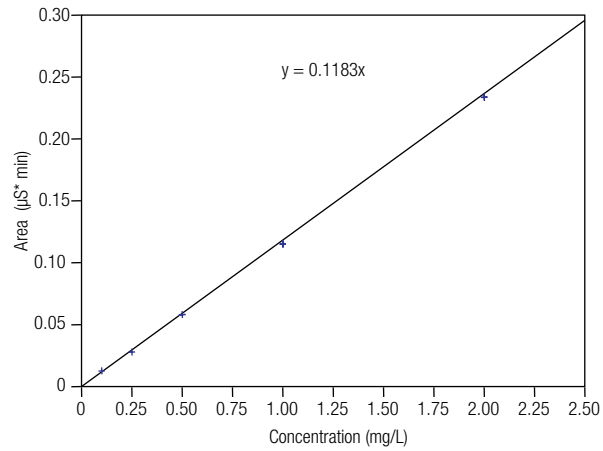

Figure 4. Calibration plot of dimethylamine (forced through the origin).

Table 1. Amount of dimethylamine in the drug sample and the spike recovery results.

Injection No.	Amount (mg/L)					
	Sample			Spiked Sample		
	1	2	3	1	2	3
1	0.5098	0.4846	0.4717	0.5706	0.5927	0.5823
2	0.4855	0.4857	0.4803	0.5707	0.5968	0.5970
3	0.4665	0.4854	0.4814	0.5534	0.6038	0.5842
4	0.4884	0.4743	0.4792	0.5701	0.6047	0.5856
5	0.4829	0.4796	0.4788	0.5659	0.5918	0.5825
Average	**0.487**	**0.482**	**0.478**	**0.566**	**0.598**	**0.586**
RSD	**3.18**	**1.03**	**0.80**	**1.31**	**1.01**	**1.05**
Average (N = 3)	**0.482**			**0.583**		
Spiked Concentration (mg/L)	**0.1**					
Recovery (%)	**101**					

Sample Analysis

Metformin hydrochloride (500 mg/tablet) was purchased from a local pharmacy. The sample was prepared in triplicate and five injections were made for each sample preparation. The results in Table 1 show the reproducibility of sample preparation and sample injection. Dimethylamine was found at a concentration of 0.482 mg/L in the prepared sample. This is 0.0482% relative to 500 mg metformin.

The method accuracy was determined by performing a recovery experiment. Dimethylamine standard was added into the sample before the dissolution to achieve 0.1 mg/L dimethylamine after preparation. Spiked samples were prepared in triplicate and five injections were made for each preparation. The average concentration was used for recovery calculation. The recovery result obtained from the experiment was 101%, confirming the method is accurate. Figure 5 shows the chromatogram overlay of sample and spiked sample. Note the large sodium peak at approximately seven minutes (does not interfere with the determination). Table 2 shows sample analysis and recovery results.

Conclusion

This document shows an ion chromatography method with suppressed conductivity detection that is more sensitive than nonsuppressed conductivity detection methods for determining dimethylamine in metformin hydrochloride drug products. The combination of a Dionex IonPac CS19 Analytical Column and gradient elution using a Dionex RFIC system provides good resolution of dimethylamine and common cations found in drug products. The results confirm that the method is accurate and reproducible for determination of dimethylamine in metformin drug products.

Column: Dionex IonPac CS19 Analytical (4 × 250 mm)
Dionex IonPac CG19 Guard (4 × 50 mm)
Eluent Source: Dionex EGC III MSA with a Dionex CR-CTC II
Eluent: 2 mM from -7 to 12 min, 2–12 mM from 12 to16 min, 12 mM from 12 to 18 min, 12–40 mM from 18 to 20 min, 40 mM from 20 to 30 min
Temperature: 40 °C
Injection Vol.: 20 µL
Flow Rate: 1.0 mL/min
Detection: Suppressed conductivity, Dionex CSRS 300 (4 mm) Recycle mode, current 120 mA

Sample: Sample and spiked sample

Peak:	Conc (mg/L)	
	Sample	Spiked Sample
1. Dimethylamine	0.482	0.583

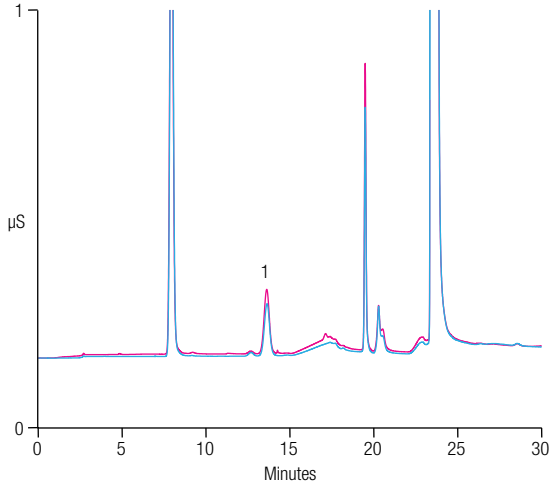

Figure 5. Overlay of the sample and spiked sample chromatograms. The spiked sample is the red trace.

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