Designing ion chromatography methods for determining amines in pharmaceuticals

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Goal

Describe how to develop an ion chromatography method to determine an amine in pharmaceutical samples

Introduction

Amines are aliphatic and aromatic derivatives of ammonia. Like ammonia, amines are weak bases, which makes them amenable to analysis by ion chromatography (IC). Some amines are used as pharmaceuticals (drug products) or used to synthesize drug substances. Many amine drugs are designed for mimicking or interfering with the action of natural amine neurotransmitters. For example, chlorpheniramine is an antihistamine to relieve allergic disorders. Chlorpromazine is a tranquilizer that sedates without inducing sleep and is used to relieve anxiety and mental disorders. Ephedrine and phenylephrine are used as decongestants.

Ion chromatography is especially valuable to the pharmaceutical industry for ionic analytes in products containing non-ionic components. IC-based methods are included in several USP monographs and have been applied to all aspects of pharmaceutical product manufacturing, including the determination of active ingredients, degradation products, and impurities.[1] For most pharmaceutical samples, little or no sample preparation is required, and analyte derivatization is unnecessary. IC of aliphatic amines using a cation-exchange column has been reported by several authors. The determination of bethanechol and its degradation product using a cation exchange IC method was included as part of the USP Bethanechol Chloride monograph.[2]

This technical note will provide method development guidance for determining amines in pharmaceuticals. Tools including the IC Column Selection Guide, AppsLab, and Virtual Column will be described. The determination of dimethylamine in pharmaceuticals will be used to demonstrate how to apply the described tools.

Amine IC separation method development guidance

The typical eluent for cation-exchange ion chromatography is methanesulfonic acid. Amines are cations in acidic solutions. In cation-exchange ion chromatography, ion exchange is the major mode of separation. However, other modes such as hydrophobic and hydrogen bonding interactions also contribute to the retention of polar organic molecules on these phases. The relative impact of these interactions was evaluated for three Thermo Scientific™ Dionex™ IonPac™ cation-exchange columns (Dionex IonPac CS19, Dionex IonPac CS17, and Dionex IonPac CS12A columns) using the retention of 15 structurally similar biogenic amines and amino acids. The separation mechanism for these three stationary phases included electrostatic, hydrophobic, hydrogen bonding, ion-dipole, and possibly, π-π interactions between aromatic rings of the probes and EVB-DVB matrix of the resin.[3]

The Dionex IonPac CS12A, Dionex IonPac CS17, and Dionex IonPac CS19 columns have carboxylic acid cation-exchange groups. Carboxylic acid functional groups on macro-porous polymer substrate beads were developed by Thermo Fisher Scientific laboratories as cation-exchange stationary phases to be used with suppressed conductivity detection. Cation-exchange phases for IC were first created to obtain more efficient peaks for the Group I and II cations. Later, some phases were developed specifically for amine separations. The complete list of cation-exchange columns and their target applications can be found in the IC Column Selection Guide.[4]

A high number of carboxylic acid groups were grafted onto polymeric macroporous resin to create the Thermo Scientific™ Dionex™ IonPac™ CS16 stationary phase, which resulted in a much higher capacity than previously achieved. This high-capacity column allows quantitation of a 10000:1 concentration ratio of sodium to ammonium as well as disparate concentration ratios of other adjacent cation peaks. It also offers better selectivity of the common cations and monovalent small amines. The application of the Dionex IonPac CS16 column to ammonia determination in pharmaceuticals is demonstrated in Application Note 1073 (AN1073) (ammonia in sodium bicarbonate)[5] and AN73482 (ammonia in potassium bitartrate)[6].

Polyvalent amines such as the biogenic amines are tightly bound to cation-exchange stationary phases. Very high acid concentrations or a divalent ion are required to elute them effectively from many cation-exchange columns. Hydrophobic amines, such as long-carbon-chained amines, can partition into the polymeric substrate of the carboxylated stationary phase, and therefore organic solvent is required to elute then effectively from many cation-exchange columns. Eluent that does not contain organic solvent is preferred for IC because the electrolytic suppressor can be used in eluent recycle mode, and detection sensitivity is improved. The Dionex IonPac CS17 column was developed to separate amines, especially polyvalent and/or hydrophobic amines using a simple acidic eluent. The column is packed with solvent-compatible particles of ethylvinylbenzene crosslinked with 55% divinylbenzene. The surface of the bead is coated with a non-functional monomer followed by functionalization through grafting. This gives the Dionex IonPac CS17 stationary phase its hydrophilic nature. As a result, a simple acidic eluent is sufficient to elute polyvalent and hydrophobic amines, and divalent cations. The application of Dionex IonPac CS17 columns to pharmaceutically relevant polyvalent and hydrophobic amines is demonstrated in AN194 (carbachol in ophthalmic solutions)[7], AN199 (N-methylpyrrolidine in cephalosporins)[8], and AN249 (methacholine chloride, other impurities in methacholine chloride)[9].

The Dionex IonPac CS19 column is a relatively new cation exchange column designed to analyze six common cations, small polar amines (including alkanolamines and methylamines) and moderately hydrophobic and polyvalent amines (including biogenic amines and alkyl diamines). The column has 65% higher capacity and 50% higher efficiency than the Dionex IonPac CS17 column, and the stationary phase is more hydrophobic than the Dionex IonPac CS17 column. The high capacity allows the analysis of peak pairs with widely disparate concentrations, and high ionic strength matrix without overloading the column. High capacity is essential for impurity determinations of pharmaceuticals. The application of Dionex IonPac CS19 columns to amine analysis is demonstrated in AN298 (dimethylamine in metformin)[10], AN1057 (methylamine in alfuzosin, sertraline hydrochloride)[11], AN1062 (morpholine in linezolid)[12], and AN72649 (choline in succinylcholine chloride)[13]. The Dionex IonPac CS18 and Dionex IonPac CS20 columns were also designed for determining amines.

Table 1 summarizes the determination of ammonia and amines in pharmaceuticals using Dionex IonPac CS16, Dionex IonPac CS17, and Dionex IonPac CS19 columns. The application details can be downloaded from the Thermo Scientific™ AppsLab Library of Analytical Applications (https://appslab.thermofisher.com). AppsLab is a web portal to Thermo Fisher Scientific chromatography, including HPLC, GC, and IC, and other analytical applications. New applications from Thermo Fisher Scientific are uploaded to AppsLab as they become available. You can easily search, filter, and download complete methods. If your application of interest has a Thermo Scientific™ Chromeleon™ Chromatography Data System eWorkflow, you can directly download it to a Chromeleon CDS software for immediate execution.

Seeking the best column and separation conditions for an IC analysis has traditionally been an arduous process. You had to guess what column to use and which separation parameters to choose. Now you can search for the desired separation in AppsLab and the Column Selection Guide to pick a column and starting conditions. To guide you to find the best starting conditions for your application, Thermo Fisher Scientific has a tool called "Virtual Column". It is an option available for the Chromeleon Management System software (release 6.6 and above). A free version with fewer graphical features is available in AppsLab. It can predict chromatography under a wide range of experimental conditions such as column type, eluent concentration, column temperature, flow rate, and gradient steps. You can immediately see the effects of varying conditions. Using the column you picked using the Column Selection Guide and AppsLab, specify the analytes you want to separate, and the Virtual Column Separation Simulator can find and display the optimal separation. You can select over 100 cations on the most popular cation exchange columns. Figure 1 shows an example separation of methylamine, dimethylamine, and trimethylamine with the Dionex IonPac CS16 column. The cation exchange columns in the simulator include the Dionex IonPac CS12A, Dionex IonPac Dionex IonPac CS16, Dionex IonPac CS17, Dionex IonPac CS18, and Dionex IonPac CS19 columns.

Application example - determination of dimethylamine in pharmaceuticals

N-nitrosodimethylamine (NDMA) is an organic compound with the formula $(CH_3)_2NNO$. NDMA is a known carcinogen that is present in some fermented foods.[14-15] Since July 2018, a few drug products—including losartan, ranitidine, and metformin—have been recalled by the United States Food and Drug Administration (FDA) due to the presence of NDMA. Therefore, the FDA has provided industry guidance for controlling NDMA in human drugs.[16-21] This has increased interest in the understanding of potential NDMA formation during pharmaceutical manufacturing. The use of nitrite in a manufacturing process can represent a risk for NDMA formation if a secondary or tertiary amine is present (Figure 2).[22-23] Therefore, it is important to limit the nitrite and amine levels in drug substances and drug products. The determination of nitrite in pharmaceuticals by IC was discussed in AN73987.[24] Here, we describe how to develop an IC method for determining an amine in pharmaceutical samples. We use dimethylamine (DMA) as the example and delineate the steps to developing the IC method based on the nature of the sample.

Table 1. Dionex IonPac cation exchange columns for ammonia and amines in pharmaceuticals applications

Column	Capacity	Target applications	Application Note #	Application
Dionex IonPac CS16	Highest (5270 µeq)	Short-chained alkylamine and alkanolamines	1073	Ammonia in sodium bicarbonate
		Low ammonium in the presence of high sodium	73482	Ammonia in potassium bitartrate
Dionex IonPac CS17	Moderate (1450 µeq)	Polyvalent and hydrophobic amine (alkanolamines and methylamines)	194	Carbachol in ophthalmic solutions
			199	N-Methylpyrrolidine in cephalosporins
			249	Methacholine chloride and impurities in methacholine chloride
Dionex IonPac CS19	Moderate (2410 µeq)	Small polar amine (alkanolamines and methylamine)	298, 1057	Dimethylamine in metformin
		Moderately hydrophobic and polyvalent amines (biogenic amines and alkyl diamines)	1062	Methylamine in alfuzosin, sertraline hydrochloride
			72649	Morpholine in linezolid Choline in succinylcholine chloride

Note: The capacity value is based on the 4 mm column. For the Dionex IonPac CS16 column the 4 mm format has a 4 µm particle size.

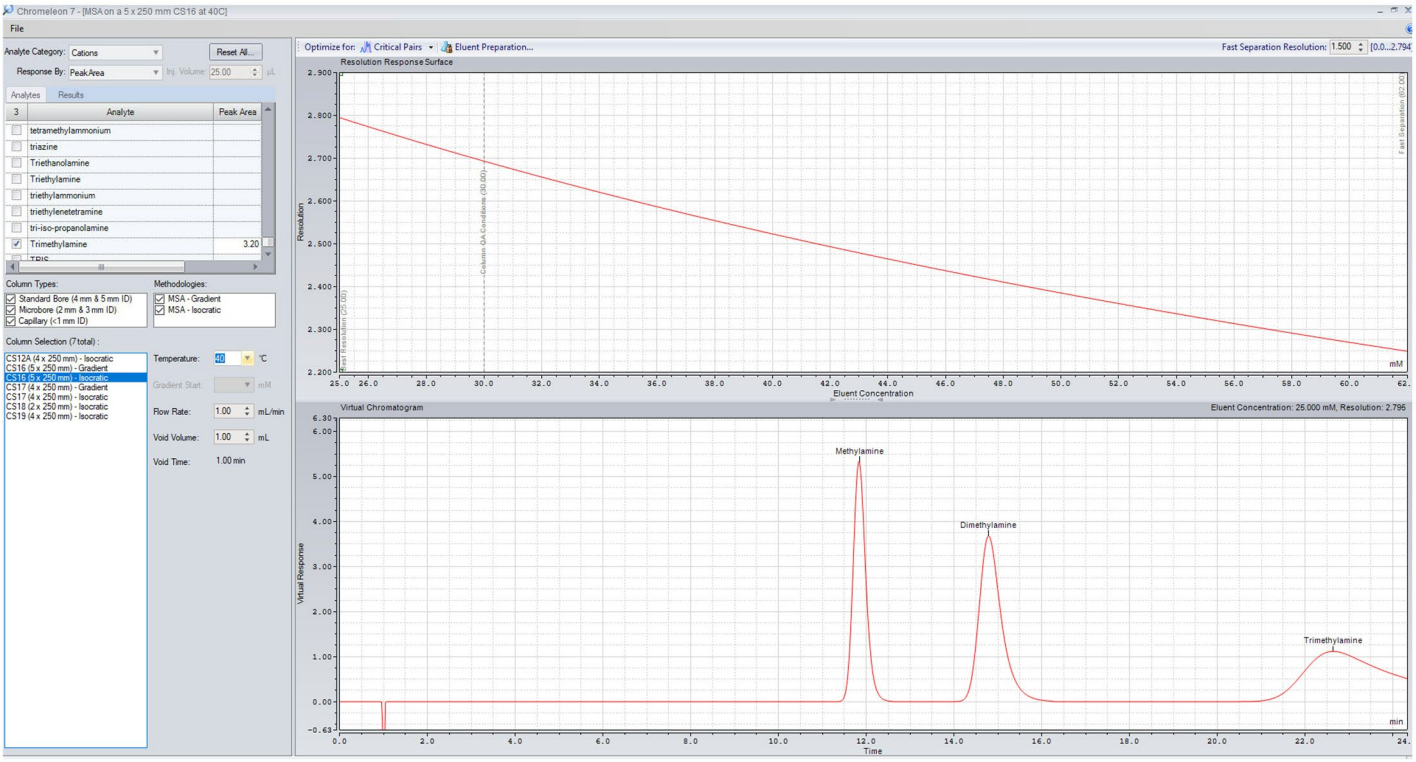

Figure 1. Virtual Column simulator

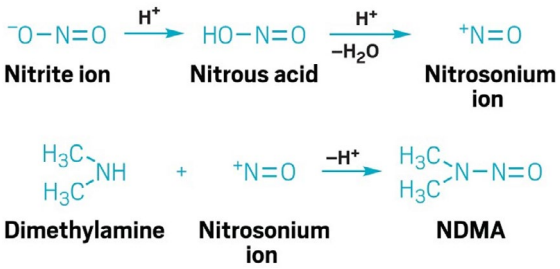

Figure 2. NDMA formation

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC system including*:
  - Thermo Scientific™ Dionex™ ICS-5000⁺ DP Pump module
  - Thermo Scientific™ Dionex™ ICS-5000⁺ DC Detector/ Chromatography module with Conductivity Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306), and 10 mL vial trays
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.9

*These methods are applicable to any Dionex ICS system that uses eluent generation, including the Thermo Scientific™ Dionex™ IonPac™ ICS-6000 HPIC system.

Consumables

- Thermo Scientific™ Dionex™ EGC 500 MSA Cartridge (P/N 075779)
- Thermo Scientific™ Dionex™ CR-CTC 500 Continuously Regenerated Anion Trap Column (P/N 075551)
- Thermo Scientific™ Dionex™ CDRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088670)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)
- Fisherbrand™ Narrow-mouth field sample bottles, high density polyethylene (HDPE), 125 mL, 250 mL sizes for storage of standards and samples (Fisher Scientific P/N 02-895A, B)
- Thermo Fisher™ Nalgene™ Syringe Filter 0.2 µm PES (Fisher Scientific P/N 09-740-113)

Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Chloride salts, A.C.S. reagent grade or better, for preparing cation standards
- Dimethylamine hydrochloride, 99% (Sigma-Aldrich P/N 126365-100G)
- Losartan potassium (Sigma-Aldrich P/ N PHR1602-1G)
- Metformin hydrochloride (Sigma-Aldrich PHR1084-500MG)

Samples

The pharmaceutical samples used in this study are listed in Table 2.

Chromatographic conditions (Dionex IonPac CS16 column method)

Parameter	Value
Columns:	Dionex IonPac CG16 Guard Column, 3 × 50 mm (P/N 079931) Dionex IonPac CS16 Analytical Column, 3 × 250 mm (P/N 059596)
Eluent:	25 mM MSA
Eluent source:	Dionex EGC 500 MSA cartridge with Dionex CR-CTC 500
Flow rate:	0.5 mL/min
Injection volume:	25 µL in Push-Full mode
Column temperature:	20 ˚C
Detection:	Suppressed conductivity
Suppressor:	Dionex CDRS 600 (2 mm) suppressor, AutoSuppression Recycle Mode, 37 mA current
Detection/suppressor compartment:	20 °C
Cell temperature:	25 ˚C
Background conductance:	<0.2 µS/cm
System backpressure:	~3,600 psi (100 psi = 689.5 kPa)
Noise:	<1 nS/cm
Run time:	30 min

Chromatographic conditions (Dionex IonPac CS19 column method)

Parameter	Value
Columns:	Dionex IonPac CG19 Guard Column, 2 × 50 mm (P/N 076029) Dionex IonPac CS19 Analytical Column, 2 × 250 mm (P/N 076028)
Eluent:	3 mM MSA from 0 to 12 min 3–40 mM KOH from 12 to 16 min 40 mM from 16 to 21 min 3 mM from 21.1 to 30 min
Eluent source:	Dionex EGC 500 MSA cartridge with Dionex CR-CTC 500
Flow rate:	0.25 mL/min
Injection volume:	25 µL in Push-Full mode
Column temperature:	10 ˚C
Detection:	Suppressed conductivity
Suppressor:	Dionex CDRS 600 (2 mm) suppressor, AutoSuppression Recycle Mode, 30 mA current
Detection/suppressor compartment:	30 °C
Cell temperature:	35 ˚C
Background conductance:	<0.2 µS/cm
System backpressure:	~2,700 psi (100 psi = 689.5 kPa)
Noise:	<1 nS/cm
Run time:	30 min

System preparation and setup

Figure 3 shows the flow diagram of the IC system. The Dionex ICS 5000⁺ HPIC system is plumbed as a Reagent-Free IC (RFIC) system using eluent generation following the Dionex ICS 5000⁺ installation and operator's manual.[25] Install the suppressor in AutoSuppression Recycle Mode.

Table 2. Pharmaceutical samples. OTC = over-the-counter; Rx = prescription.

#	Drug product name	API	Type	API/pill (mg)	Source	Indication
1	NA	Losartan potassium	Substance	NA	Sigma	NA
2	NA	Metformin hydrochloride	Substance	NA	Sigma	NA
3	Nytol™ Quickcap™	Diphenhydramine HCl	Product	25	OTC	Sleep aid
4	Benadryl™	Diphenhydramine HCl	Product	25	OTC	Allergy
5	Metformin	Metformin hydrochloride	Product	500	Rx	Diabetes
6	Losartan	Losartan potassium	Product	50	Rx	High blood pressure
7	Ranitidine	Ranitidine hydrochloride	Product	300	Rx	Diabetes

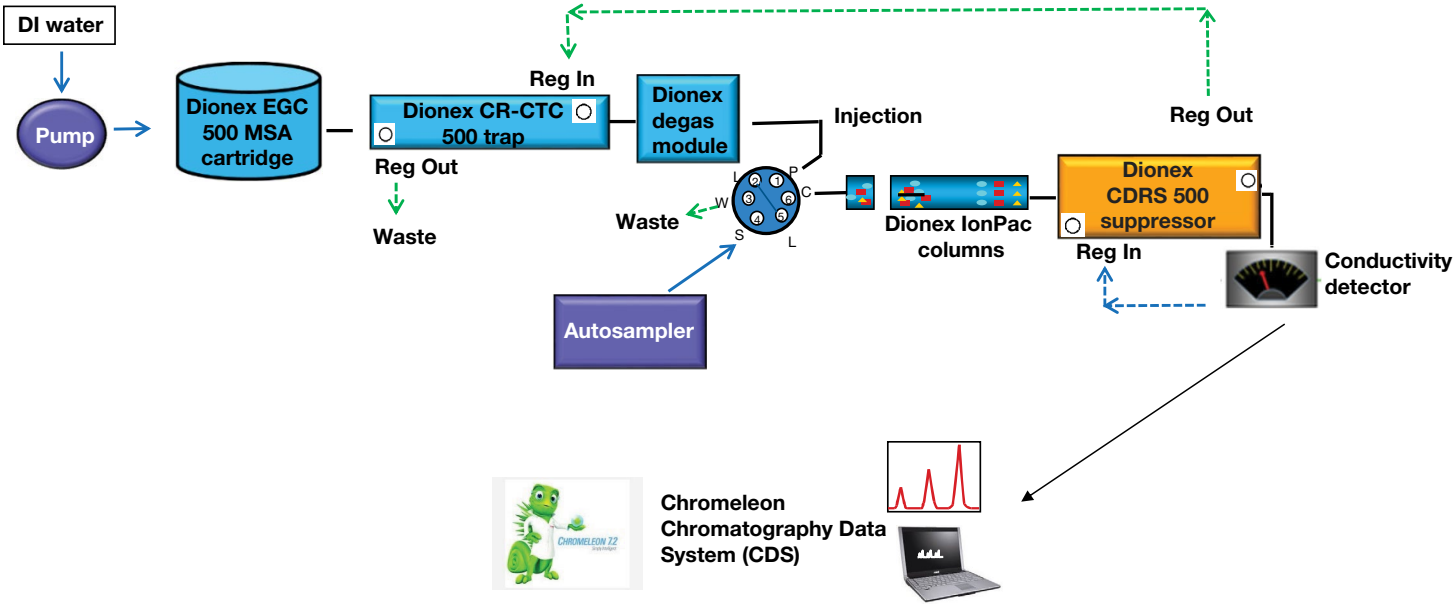

Figure 3. Flow diagram for IC using conductivity detection with the suppressor in AutoSuppression Recycle Mode

Preparation of solutions and reagents

Common cations stock standard solutions

Stock standard solutions (1,000 mg/L) can be prepared by dissolving the appropriate amounts of the required analytes in 100 mL of DI water, according to Table 3.

Table 3. Masses of compounds used to prepare 100 mL of 1,000 mg/L ion standards

Analyte	Compound	Amount (mg)
Lithium	Lithium chloride	610.8
Sodium	Sodium chloride	254.2
Ammonium	Ammonium chloride	296.5
Potassium	Potassium chloride	190.7
Dimethylamine	Dimethylamine hydrochloride	180.9
Magnesium	Magnesium chloride hexahydrate	836.5
Calcium	Calcium chloride	366.8

DMA stock standard solution

A DMA stock standard solution (1,000 mg/L) can be prepared by dissolving 180.9 mg of dimethylamine hydrochloride in 100 mL of DI water.

Pharmaceutical samples preparation

Two drug substances and five drug products (Table 2) were used as samples in this study to demonstrate DMA separation in pharmaceuticals. Drug substance samples were prepared at 1 mg/mL by dissolving 50 mg of powder in 50 mL of DI water. Drug product samples were prepared by dissolving the whole pill into 50 mL or 10 mL of DI water to yield a 2.5–10 mg/mL solution based on the active pharmaceutical ingredient (API) weight. Sonicate the pill with DI water until dissolved and centrifuge the sample extract at 8,000 x g for 15 min. Dilute the sample solution to 1 mg/mL with DI water and filter the solution with a 0.2 µm PES filter.

Results and discussion

Ion chromatography separation method development

Cation exchange chromatography with suppressed conductivity detection (cation IC) is a well-established method to determine µg/L to mg/L concentrations of common cations and many amines. A search for DMA and IC instrument type in the AppsLab library generates four application documents: AU155, AN94, AN222, and AN298. Of these, the pharmaceutical application is AN298: *Determination of dimethylamine in metformin HCl drug product using IC with suppressed conductivity detection.* Figure 4 shows the conditions and separation of AN298.[10] The method uses a Dionex IonPac CS19 cation-exchange column with a methanesulfonic acid gradient eluent produced by an eluent generator. AN298 gives us an idea that the Dionex IonPac CS19 column is an appropriate column for pharmaceutical applications. The IC Column Selection Guide shows that the Dionex IonPac CS19 column is suitable for moderately hydrophobic amines, such as DMA, and the column capacity is moderate.

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
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 and 40 mM from 20 to 30 min
Column temp.:	40 °C
Injection volume:	20 µL
Flow rate:	1.0 mL/min
Detection:	Suppressed conductivity, Dionex CSRS 300, 4 mm, recycle mode, current 120 mA
Samples:	1) Dimethylamine 2) Mixed six cations standard 3) Mixture of six cations and dimethylamine

Peaks:	mg/L
1. Lithium	0.2
2. Sodium	0.8
3. Ammonium	1.0
4. Potassium	2.0
5. Dimethylamine	2.0
6. Magnesium	1.0
7. Calcium	2.0

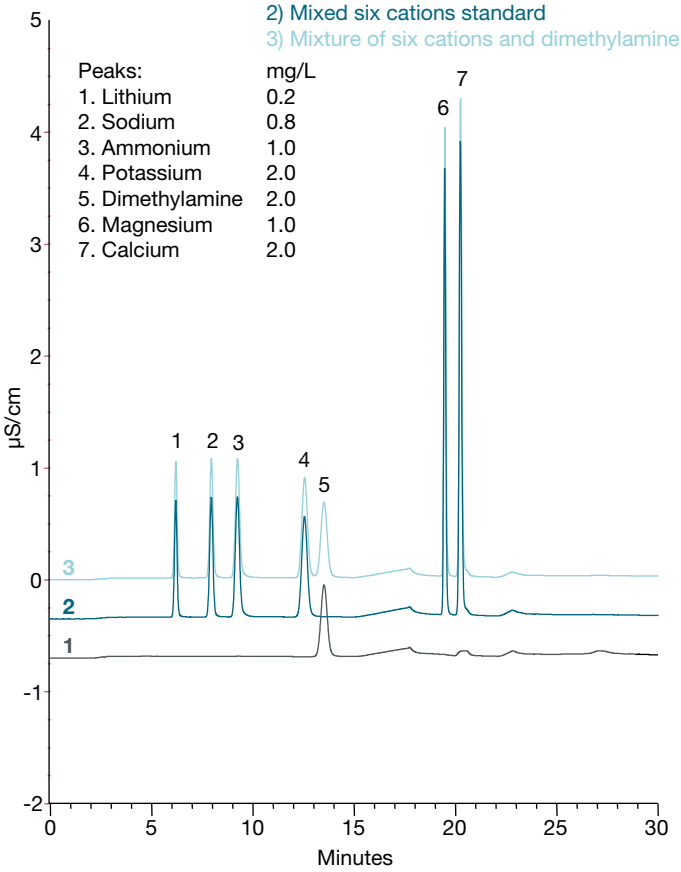

Figure 4. Separation of DMA and six common cations (AN298)

Our goal is to develop IC methods to determine DMA in pharmaceuticals. To ensure our method can be applied to a wide range of samples, a few drugs products with different APIs and formulations were selected. Losartan, ranitidine, and metformin were selected due to their FDA recall. Benadryl™ and Nytol™ were selected because they are the most common amine-containing OTC drugs.
Of the common inorganic cations, it is potassium that typically elutes close to DMA. Potassium is the counterion of losartan potassium and is expected to be at a high concentration relative to DMA in losartan samples. Therefore, the method development for DMA in losartan potassium will be more challenging than drug substances and drug products with other counterions or without a counterion (i.e., the acid form).

Figure 4 shows that with AN298 conditions, potassium elutes before DMA and they are just baseline-resolved. If a high amount of potassium is present in samples, DMA may not be detected. To separate a low amount of DMA from a high amount of potassium, we need to select a high-capacity cation-exchange column, and ideally, we also need potassium to elute later than DMA and be well-resolved from DMA. The IC Column Selection Guide shows that the Dionex IonPac CS16 column is the highest capacity cation exchange column suitable for short-chained alkylamines such as DMA.

Before method development using a Dionex IonPac CS16 column, we used the Virtual Column feature of the Chromeleon CDS to find conditions where DMA elutes before potassium. Figure 5 shows the Virtual Column screenshot of a DMA and potassium separation at a column temperature of 40 °C using a Dionex IonPac CS16 column (5 × 250 mm). Dimethylamine elutes before potassium, and the resolution of the pair increases when the eluent concentration decreases in the range of 25 to 62 mM MSA. However, the resolution is still <1.5. Figure 6 shows the Virtual Column screenshot of DMA and potassium separation at a column temperature of 23 °C. The resolution of this pair is about 2.2 with 25 mM MSA. This shows that the resolution of the pair improves at lower eluent concentration and lower column temperature. Figure 7 shows the Virtual Column screenshot of DMA and six common cations using 25 mM MSA, a column temperature of 23 °C, and a flow rate of 1 mL/min. DMA is separated from other cations and all peaks elute within 40 min. The total run time can be reduced to 30 min if the flow rate is increased from 1 mL/min to 1.4 mL/min (Figure 8). We used a Dionex IonPac CS16 3 mm column for this application; therefore, the flow rate of 1.4 mL/min for 5 mm column was converted to a flow rate of 0.5 mL/min for a 3 mm column [Calculated flow rate = $1.4 \times (3/5)^2$]. We chose an eluent concentration of 25 mM MSA and column temperature of 20 °C to achieve a resolution >3 between DMA and potassium in losartan potassium samples and to keep the run time within 30 min.

Therefore, to develop this method, we first searched AppsLab, found a column choice, and used the IC Column Selection Guide to confirm a column choice and identify other possible columns. Understanding the nature of some of our samples (high potassium concentration) led us to pick one of the other possible columns and then use Virtual Column to identify appropriate separation conditions.

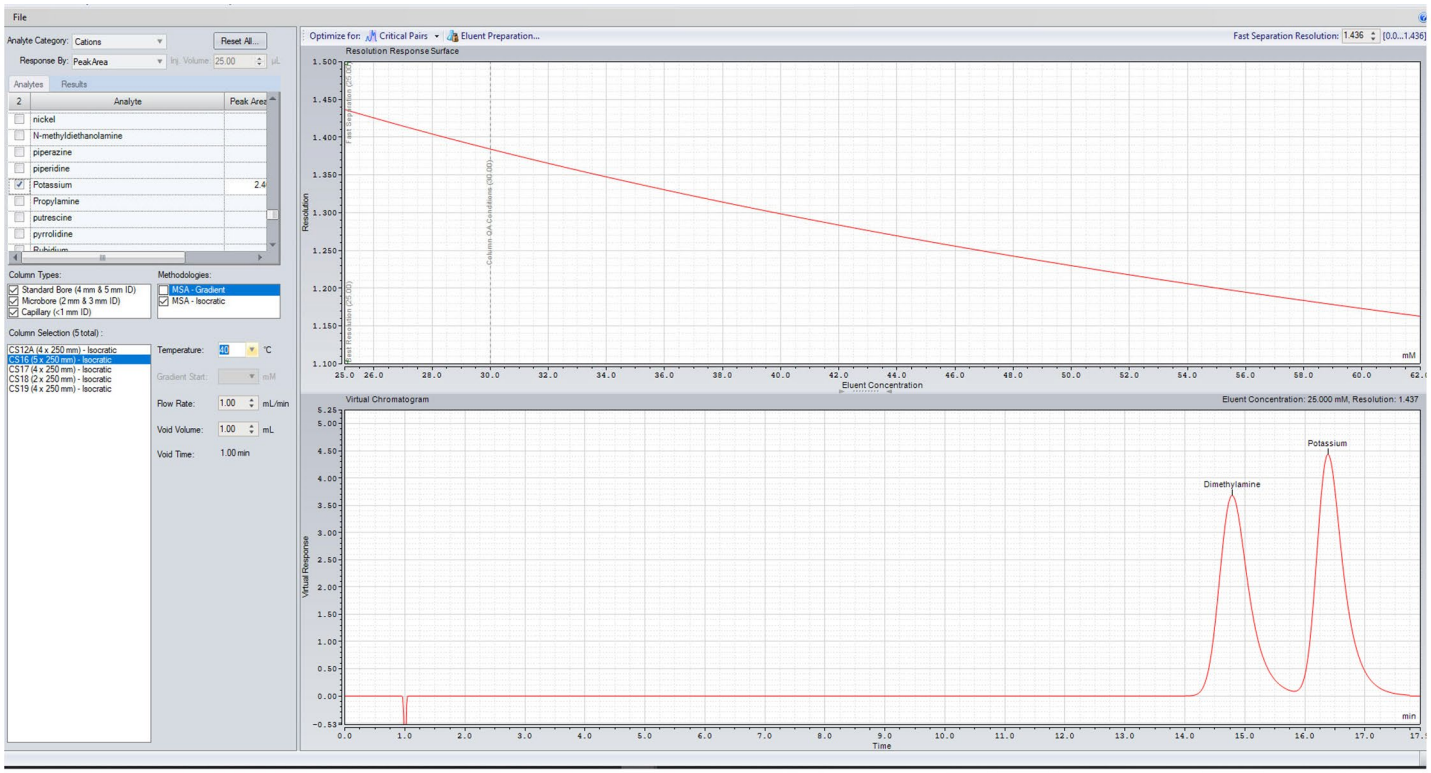

Figure 5. Virtual Column screenshot. DMA and potassium are separated at a column temperature of 40 °C using a Dionex IonPac CS16 column.

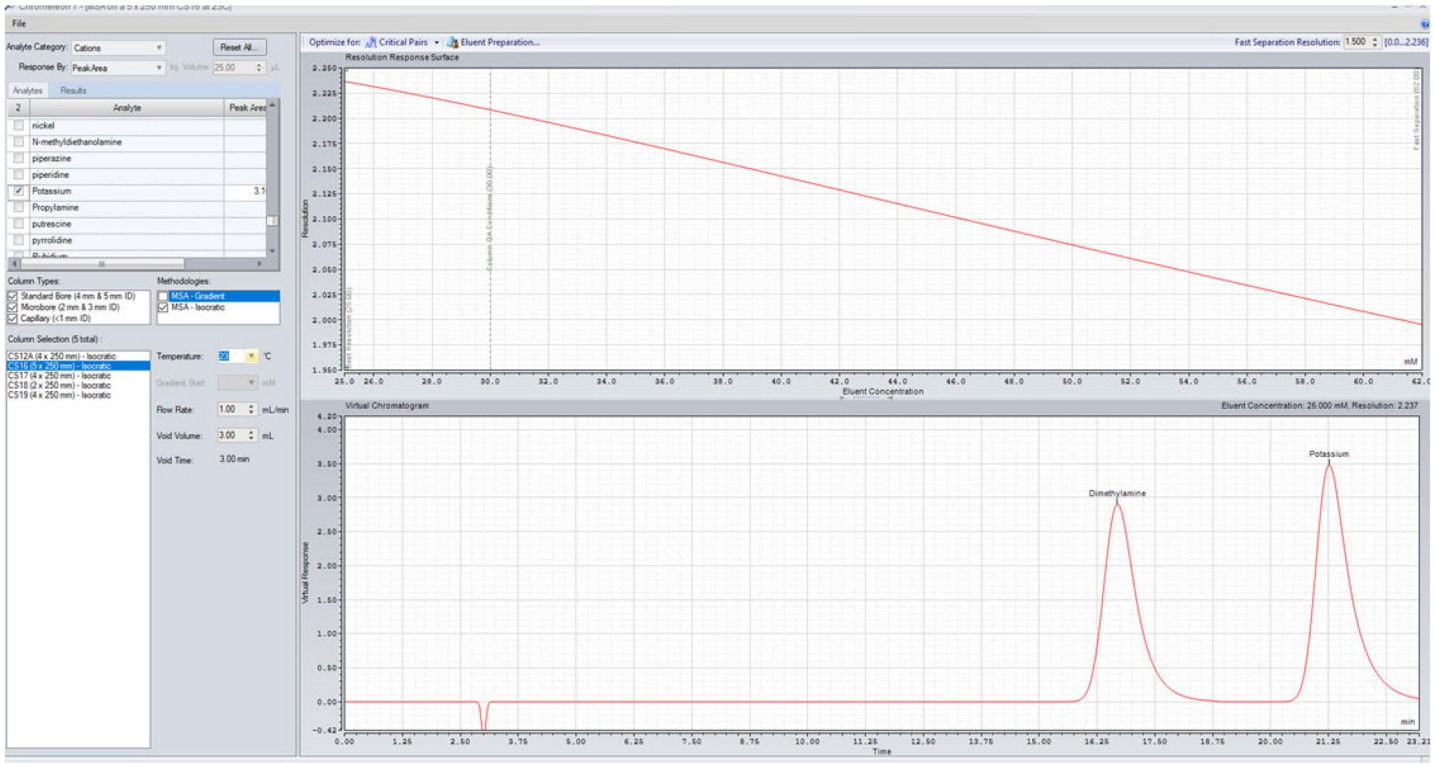

Figure 6. Virtual Column screenshot. DMA and potassium are separated at a column temperature of 23 °C using a Dionex IonPac CS16 column.

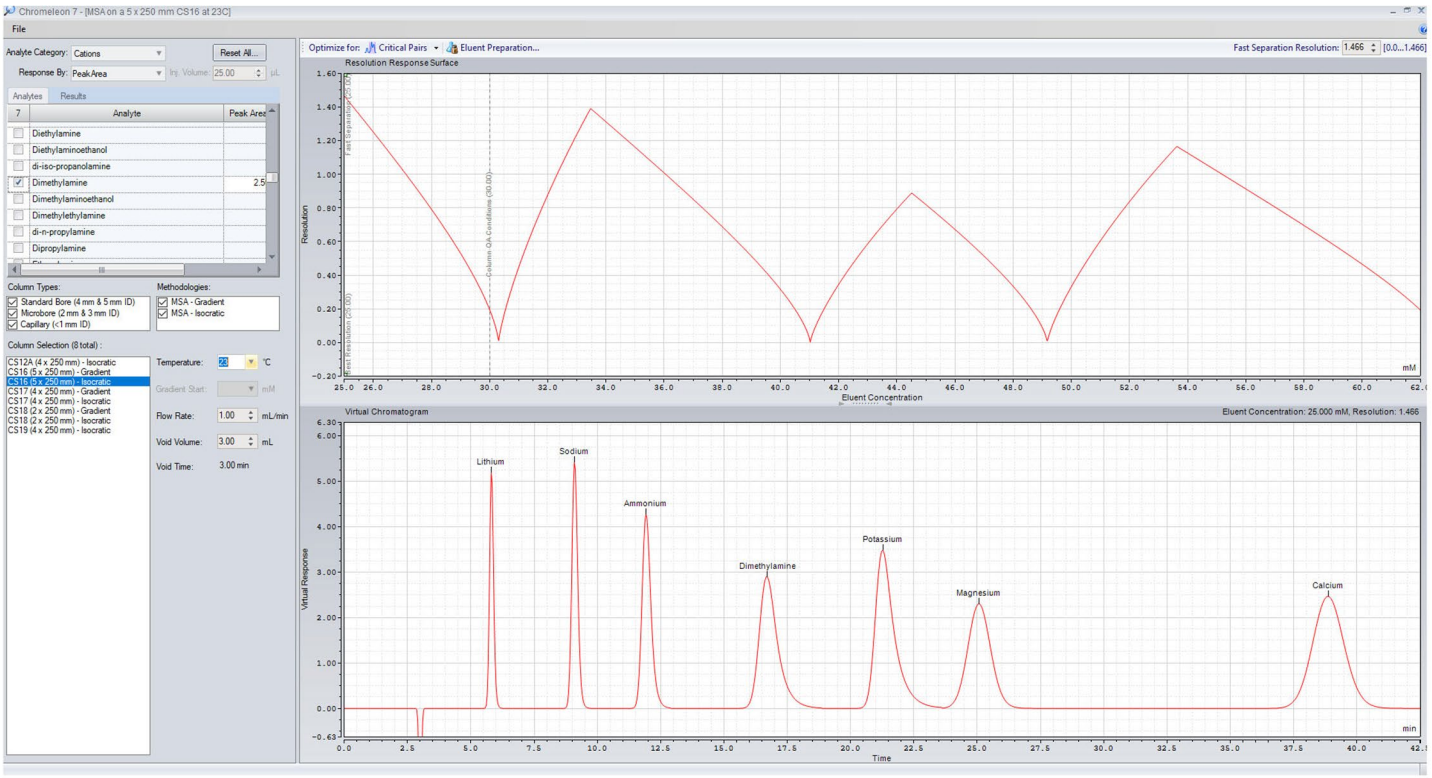

Figure 7. Virtual Column screenshot. DMA and six common cations are separated at a column temperature of 23 °C, 25 mM MSA eluent, and a flow rate of 1 mL/min using a Dionex IonPac CS16 column.

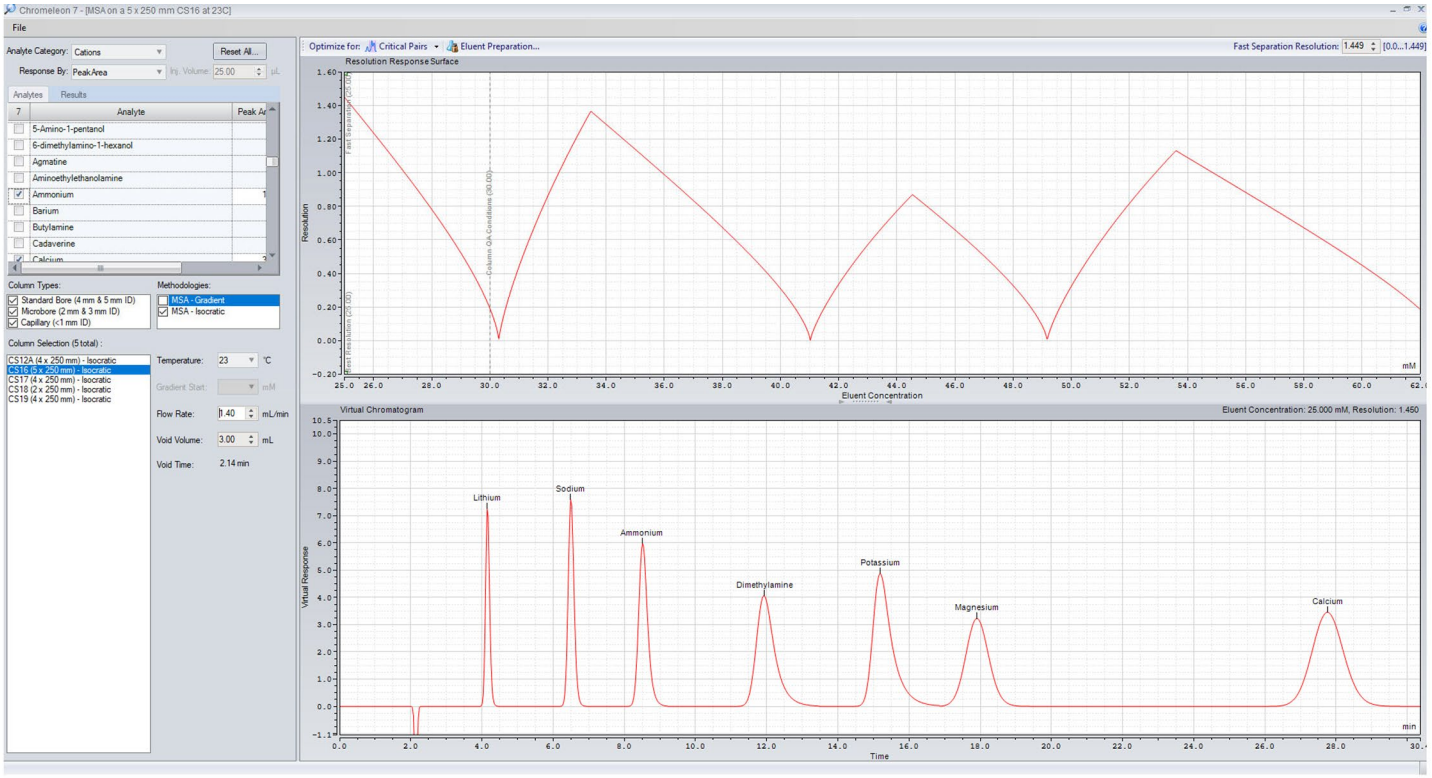

Figure 8. Virtual Column screenshot. DMA and six common cations are separated at a column temperature of 23 °C, 25 mM MSA eluent, and a flow rate of 1.4 mL/min using a Dionex IonPac CS16 column.

The Dionex IonPac CS16 column method was successfully applied to some of our pharmaceutical samples such as ranitidine and Benadryl. However, when this method was applied to the metformin drug product, a large peak appeared in the next injection. We believe the large peak is the metformin that was not eluted from the column during the separation time. We found that this peak was not eluted within 30 min when the eluent concentration was increased to 100 mM MSA, the maximum concentration the eluent generator can produce. Metformin is a polyamine that has strong interaction with most cation exchange columns.

The Dionex IonPac CS19 column is specifically designed for the fast separation of inorganic cations, small polar amines (including alkanolamines and methylamines), and moderately hydrophobic and polyvalent amines (including biogenic amines and alkyl diamines). Therefore, the Dionex IonPac CS19 column we initially identified from our AppsLab search was chosen for the method development for determining DMA in metformin drug products. Chromeleon CDS Virtual Column shows that DMA and potassium coelute at a column temperature of 30 °C at any eluent concentration (Figure 9). The column temperature needs to be lower or higher than 30 °C to achieve separation of this pair. Potassium elutes later than DMA when the column temperature is lower than 30 °C and elutes earlier than DMA when the column temperature is higher than 30 °C. High potassium concentrations were not expected in metformin drug products. Therefore, designing a separation where potassium elutes after DMA was not critical.

AN298 demonstrated that DMA in metformin can be determined using a Dionex IonPac CS19 column at a column temperature of 40 °C.[10] A lower column temperature is usually not preferred because the total system pressure will be higher and limit the opportunity to use a higher flow rate and decrease analysis time. However, the column temperature should not exceed 30 °C when using the Dionex IonPac CS19 column as this will reduce its lifetime.[26] Figure 10 shows that the resolution of this pair improves when the column temperature decreases from 15 °C to 10 °C. Therefore, in this study, we used a column temperature 10 °C to improve the resolution between DMA and potassium. The resolution of DMA and potassium in metformin samples is >2.0 at 10 °C. The Dionex IonPac CS19 column method is a 30 min gradient method that separates common cations and DMA at a low eluent concentration of 3 mM MSA that is gradually increased to 40 mM to elute the metformin. Figure 11 shows a separation of DMA and common cations within 30 min using either a Dionex IonPac CS16 (upper) or Dionex IonPac CS19 column (lower). As this figure shows, DMA is well resolved from other common inorganic

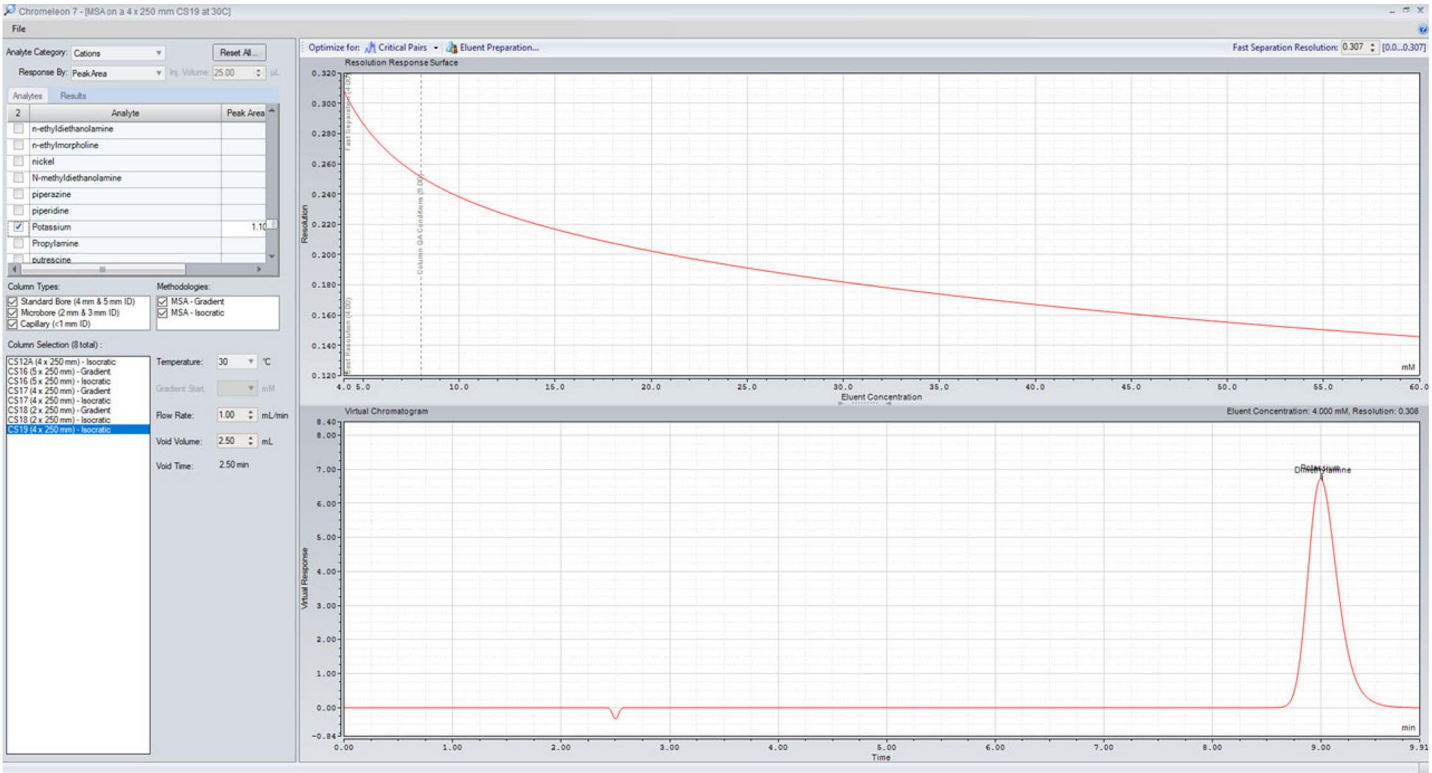

Figure 9. Virtual Column screenshot. DMA and potassium are not separated at a column temperature of 30 °C using a Dionex IonPac CS19 column.

cations such as sodium, potassium, and magnesium that can be found in pharmaceuticals. The choice of these two methods depends on the chemical nature of the drug substance. Using AppsLab, the IC Column Selection Guide, and Virtual Column, IC methods for other amines in pharmaceuticals can be developed.

Evaluating the separation methods

Calibration

To cover a wide DMA concentration range in tested samples, the calibration of DMA conductivity response to concentration was investigated in the concentration range of 5 to 250 µg/L for the Dionex IonPac CS16 column method, and 5 to 500 µg/L for the Dionex IonPac CS19 column method. Each calibration reference solution was measured in triplicate. The data were best modeled with a quadratic function (Figure 12). The coefficients of determination (r^2) were 0.9999 and 0.9997, respectively, for the Dionex IonPac CS16 column and Dionex IonPac CS19 column methods. After calibration, samples were analyzed with the two methods.

Sample analysis

The amount of DMA in the three recalled prescription drug products (losartan, metformin, and ranitidine) can provide some information for scientific researchers investigating the formation of nitrosamine. Therefore, the three drug products and two of the corresponding drug substances (losartan potassium, metformin hydrochloride) were included as samples in this study. Two common over-the-counter (OTC) drug products (Benadryl and Nytol) were also included to evaluate if this method can be applied to other amine drugs. These seven samples were previously evaluated for their nitrite content.[15]

The amounts of DMA in the seven pharmaceutical samples are summarized in Table 4. DMA was detected in all samples except losartan potassium drug substance (S1) and formulated product (S6). The highest DMA amount, 363 ppm (µg/g), was detected in sample 2 (metformin hydrochloride drug substance). DMA is detected in samples 3–5 in a range of 18.3 to 48.7 ppm. Figure 13 shows the analysis of a Benadryl drug product. DMA is separated from other cations in the drug, demonstrating that the separation conditions are appropriate.

Table 4. Amount of DMA in pharmaceutical samples, ppm (µg/g API)

Sample	DMA	RSD (n=6)
1	<LOD	NA
2	371	1.8
3	27.3	0.6
4	48.7	1.1
5	42.6	1.8
6	<LOD	NA
7	18.3	2.4

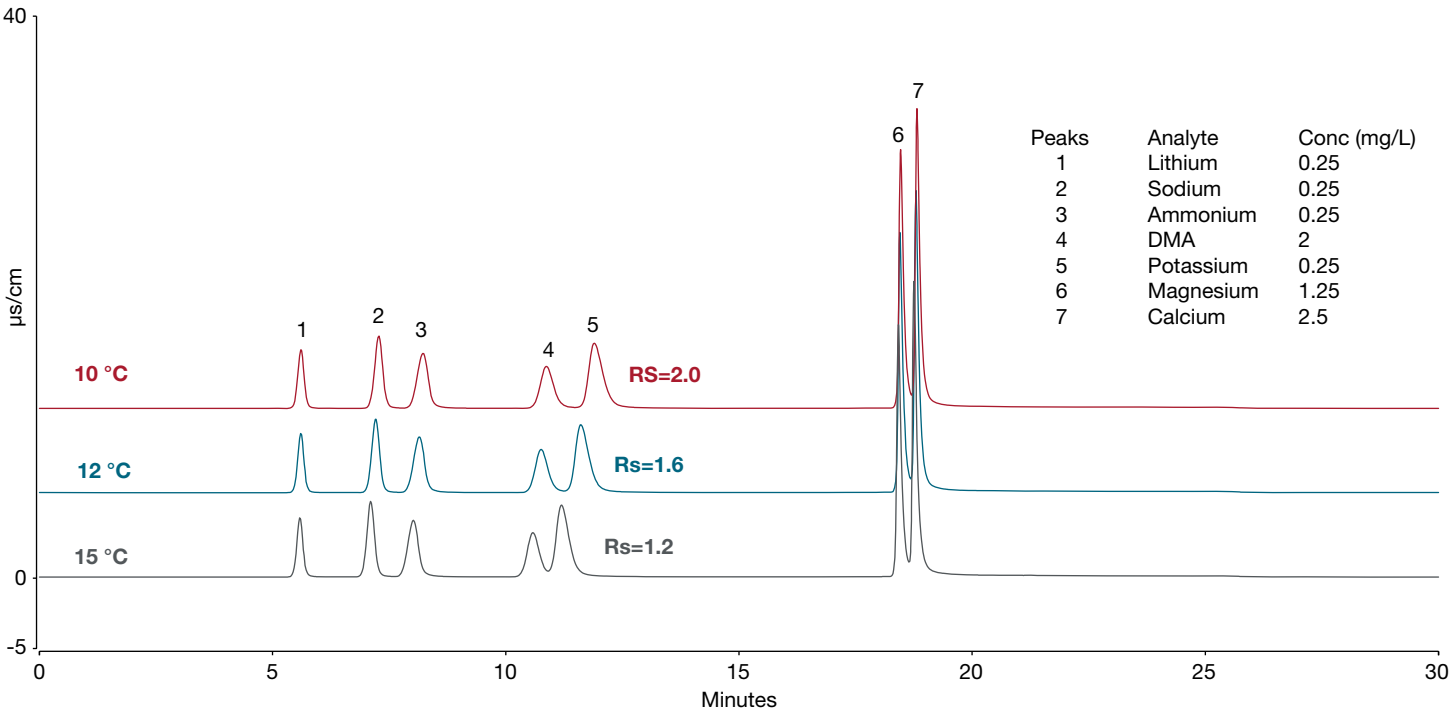

Figure 10. The effect of column temperature on the separation of DMA and potassium using a Dionex IonPac CS19 column

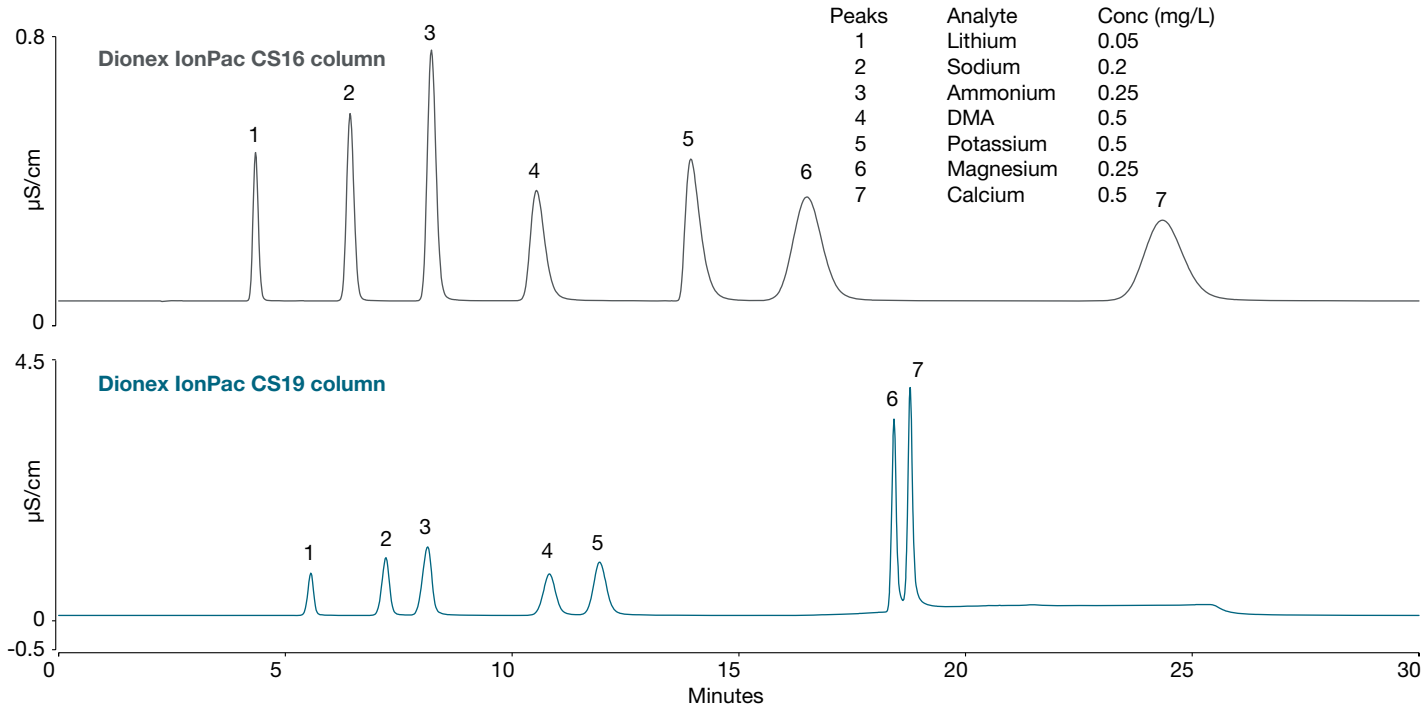

Figure 11. Separation of seven common cations using a Dionex IonPac CS19 column and a Dionex IonPac CS16 column

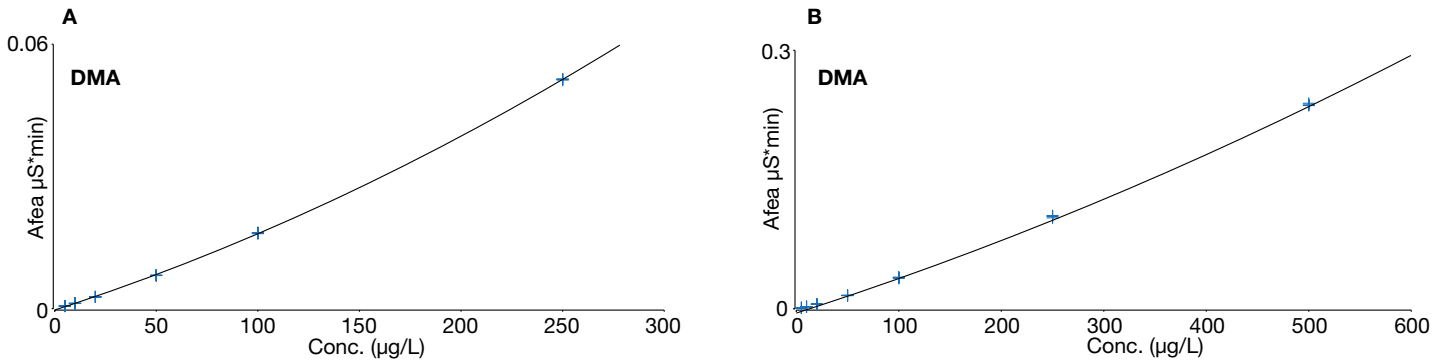

Figure 12. (A) DMA calibration using a Dionex IonPac CS16 column and (B) using a Dionex IonPac CS19 column

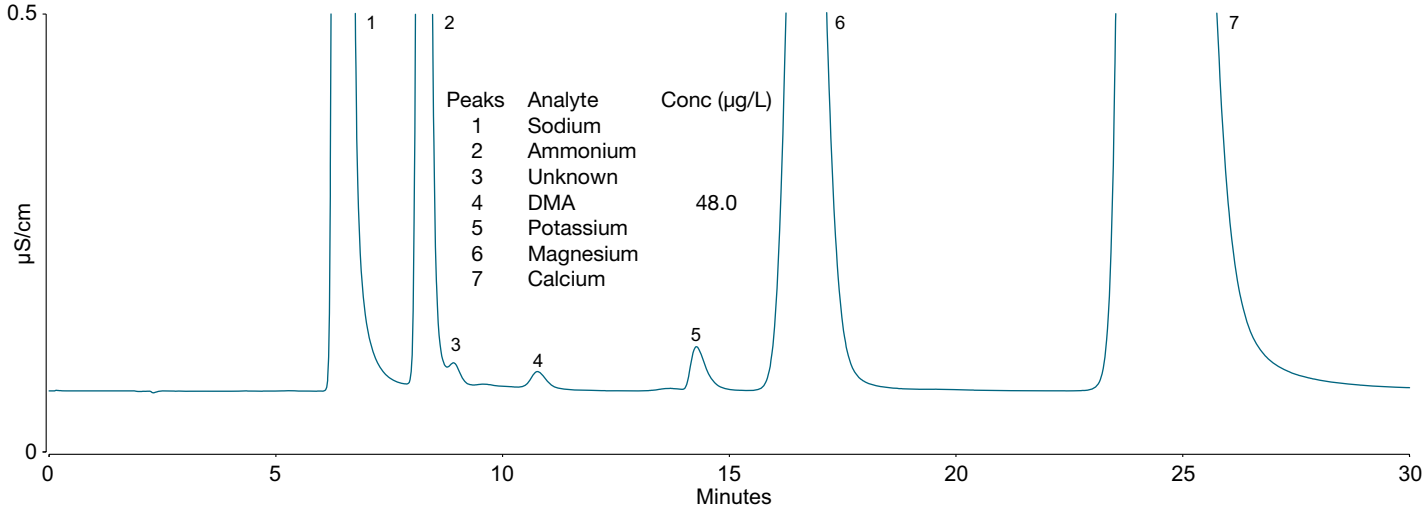

Figure 13. DMA in a Benadryl drug product (S4) using a Dionex IonPac CS16 column

Potassium is the counterion of losartan and is expected to be present at a high concentration in samples. DMA was spiked to sample 6 solution at 10 µg/L to confirm that DMA can be separated from the high concentration of potassium and the column is not overloaded. Figure 14 shows an overlay of a chromatogram of a losartan drug product sample and that sample spiked with 10 µg/L of DMA. Potassium elutes after DMA and does not interfere with DMA quantification. Dimethylamine is fully recovered, and the size of the peak suggests that the LOD estimated with standards is applicable to samples. The Dionex IonPac CS16 column can be applied to other samples in the sample list except for metformin. Metformin is a polyvalent amine that has a strong interaction with the Dionex IonPac CS16 stationary phase. As a result, the Dionex IonPac CS19 column needs to be used for this type of amine. Figure 15 shows the determination of DMA in metformin drug product using the Dionex IonPac CS19 column. Dimethylamine is separated from other cations with a MSA gradient (4–40 mM) from 0 to 16 min, and then metformin is eluted at a higher concentration MSA eluent (40 mM) from 16 to 25 min as a large peak at 20 to 25 min. This analysis, together with an analysis for nitrite, allows the analyst to determine the possibility of nitrosamine formation in the drug substance or drug product. This method for DMA determination could also be used for other components of a pharmaceutical formulation (e.g., an excipient).

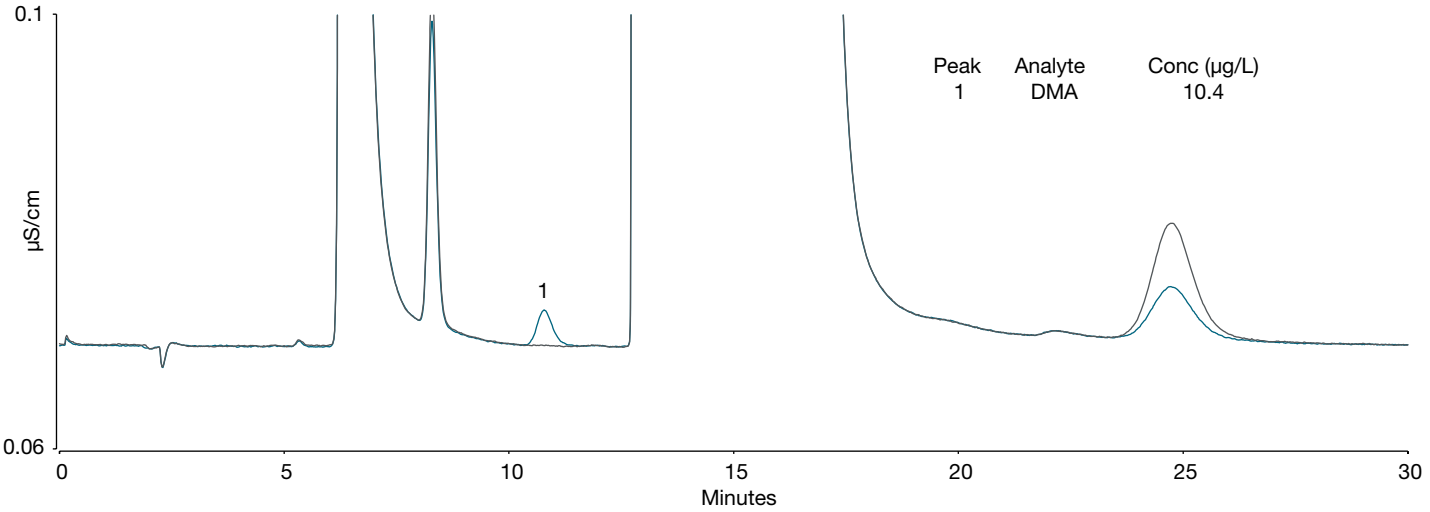

Figure 14. DMA in a losartan drug product (S6) and S6 spike 10 µg/L using a Dionex IonPac CS16 column

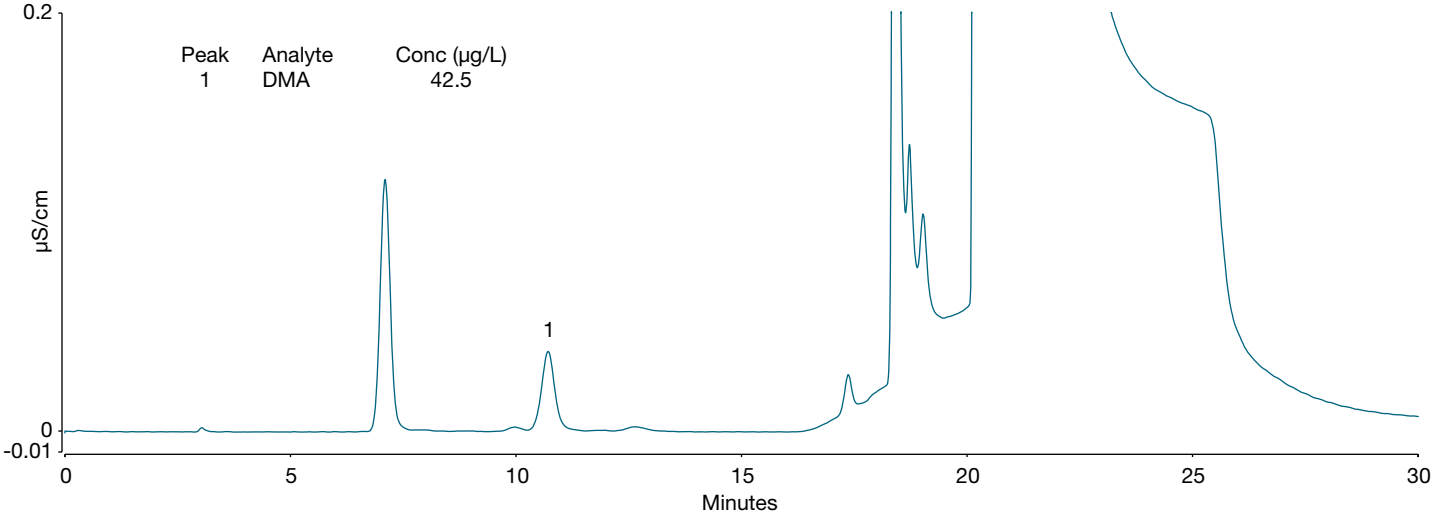

Figure 15. DMA in metformin drug product (S5) using a Dionex IonPac CS19 column

Method accuracy and precision

Method accuracy was evaluated through spike recovery studies. Dimethylamine was spiked into sample at 10 µg/L except sample #2, spiked at 100 µg/L. The recovery for DMA in the seven samples ranged from 96.0 to 104%. (Table 5). Method precision was determined by three injections of the 50 µg/L calibration standard on three separate days. The peak area precision was 1.53%, and retention time precision was 0.67% using the Dionex IonPac CS16 column. The peak area precision was 0.65%, and retention time precision was 0.07% using the Dionex IonPac CS19 column.

Table 5. Spike recovery of DMA in pharmaceutical samples

Sample	DMA recovery (%)	RSD (n=6)
1	104	2.6
2	96.0	2.3
3	100	1.8
4	101	1.7
5	104	1.4
6	104	2.6
7	96.3	2.7

Limit of detection (LOD)

The determination of LOD was based on the signal-to-noise (S/N) ratio. Determination of the S/N ratio is performed by comparing measured signal from a standard with a low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A S/N =3 is used for estimating LOD and a S/N=10 is used for estimating the quantification limit (LOQ).[27] In this study, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average height of three injections of standard (1 µg/L). The calculated LOD of DMA in sample solution was 0.960 and 0.718 µg/L using the Dionex IonPac CS16 and Dionex IonPac CS19 columns, respectively, which corresponds to 0.960 µg/g API and 0.718 µg/g (ppm) of the active pharmaceutical ingredient (API) as the pharmaceutical samples were prepared at 1 mg/mL based on API weight.

Conclusion

Following the guidance in this technical note, two IC methods were developed to determine DMA in seven pharmaceutical samples. The choice of method depends on the chemical nature of the drug substance and the formulation of the drug product, though no drug product formulations in the technical note impacted the choice of method. The LOD of DMA in a pharmaceutical sample is <1 ppm (µg/g API). The methods are accurate and precise due to the high reproducibly of the Reagent-Free IC system. The methods should be applicable to the determination of DMA throughout the manufacturing process of a drug product to assess the likelihood of nitrosamine formation. The method development strategy described here can be applied to developing IC methods for other amines in pharmaceutical samples.

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