APPLICATION NOTE 000164

Determination of histamine, agmatine, and putrescine in wine by ion chromatography-single quadrupole mass spectroscopy

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Keywords: Dionex IonPac CS19-4µm column, RFIC, Reagent-Free IC, IC-MS, biogenic amines

Goal

Demonstrate determinations of three biogenic amines in wine by ion chromatography coupled with electrospray ionization single quadrupole mass spectrometry

Introduction

Biogenic amines have important biological functions, such as regulating growth, controlling blood pressure, and facilitating neural transmittance. Although biogenic amines can occur naturally in many foods (fish, meat, dairy, fruits, vegetables, chocolate, and wine), they are usually present due to microbial activity.[1-3] Vintners use malolactic fermentation to create the desired flavors. However, certain strains of the lactic acid bacteria necessary for this fermentation can also produce biogenic amines by decarboxylating amino acids. Putrescine, cadaverine, histamine, and agmatine have been found in red wines, and to a lesser extent, in white wines. In healthy persons, dietary histamine is rapidly detoxified by diamine oxidase and other amine oxidases.[3,4] However, people with histamine intolerance or with low amine oxidase activity can experience minor to serious health effects, ranging from headaches, flushing, rhinitis, and urticaria, to changes in blood pressure (hyper- or hypotension), heart rate (increased heart rate or arrhythmia), and breathing (asthma symptoms).[2,3,5] Multiple factors have been surmised to impact concentration and type of biogenic amines occurring in wine, such as storage time and conditions, grape varietals, and contamination by wild type and certain strains of lactic acid bacteria; therefore, it is important to have suitable analytical methods for determining biogenic amines.

Biogenic amines are positively charged ions, but they typically contain poor chromophores and are therefore unsuitable for absorbance detection. Cation-exchange chromatography with suppressed conductivity detection (cation ion chromatography (IC)) is a well-established analytical method for determining positively charged ionic species and is frequently used to determine biogenic amines.[6,7] Cation IC with serial detection has also been demonstrated.[8-12] In references 8–12, the authors demonstrated separation of 11 biogenic amines by cation-exchange chromatography followed by suppressed conductivity detection and integrated pulsed amperometry detection (IPAD). IPAD is for the less conductive amines. Cation IC followed by mass spectrometry is also well-established but less commonly used because some biogenic amines produce ions in the positive electrospray interface below the mass limit of many mass spectrometers. However, the Thermo Scientific™ Dionex™ ISQ™ EC single quadrupole mass spectrometer was designed for IC, and charged ions can be detected as low as *m/z* 19.[13,14] Additionally, mass spectrometry can confirm the ion by the charged molecular weight and has the advantages of providing different selectivity, and sometimes lower detection limits than suppressed conductivity.

In this application note, three biogenic amines (putrescine, histamine, and agmatine) were determined in wine samples using IC with suppressed conductivity and mass spectrometry detections. The biogenic amines were resolved on the Thermo Scientific™ Dionex™ IonPac™ CS19-4µm cation-exchange column, detected by suppressed conductivity with the suppressed eluent delivered to electrospray interface of the Thermo Scientific™ ISQ™ EC for detection by MS in selected ion monitoring (SIM) mode. The results from recently opened and 3-day old wine samples were compared. The lowest biogenic amine concentrations were found in the sparkling wine and chardonnay samples and the highest results in the cabernet sauvignon sample. Biogenic amine concentration increased after exposure to air.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system*
  - Dionex ICS-6000 Dual Pump DP, isocratic configuration (PN 22181-60011)
  - Dionex ICS-6000 Eluent Generator EG module (PN 22181-60019)
  - Dionex ICS-6000 Detector Chromatography DC module with two 6-port injection valves (P/N 22181-60045)
  - Dionex ICS-6000 CD Conductivity Detector (P/N 079829)
- Thermo Scientific™ Dionex™ AS-AP autosampler with temperature control option (P/N 074926) and 100 µL syringe (syringe P/N 074305)
  - Dionex AS-AP trays: 19-position 10 mL tray (P/N 074938), 8-position 10 mL tray for standards (P/N 069877), 40-position 0.3 or 1.5 mL vials (P/N 074936)
- Thermo Scientific ISQ EC single quadrupole mass spectrometer with HESI-II probe (P/N ISQEC-IC)

* Or Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, RFIC model (P/N 22153-60301) with Dionex Integrion CD Conductivity Detector (P/N 22153-62034), extra 6-port valve to use as a diverter valve (P/N 22153-62027), and Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump for suppressor regen flow (P/N 060684)

Table 1 lists the consumable products needed for the IC-MS system.

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2.10

Table 1. Consumables list for the IC-MS system using Dionex ICS-6000 HPIC and ISQ-EC single quadrupole mass spectrometer instruments

Product name	Description	P/N
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kit	Dionex IC Viper fitting assembly kit for the Dionex ICS-6000 HPIC system with CD Detector	302965
RFIC eluent generation	Thermo Scientific™ Dionex™ EGC 500 MSA eluent generator cartridge	075779
	Thermo Scientific™ Dionex™ CR-CTC™ 600 continuously regenerated cation trap column	088663
	Thermo Scientific™ Dionex™ analytical EG kit with degasser module and tubing for use with this IC system	075522
Thermo Scientific™ Dionex™ CDRS™ 600 suppressor, 2 mm	Suppressor for 2 mm cation columns	088670
Dionex IonPac CS19-4µm columns	Cation guard column, 2 × 50 mm	078839
	Cation analytical column, 2 × 250 mm	078836
Thermo Scientific™ Dionex™ AS-AP autosampler vial kit options	1.5 mL polypropylene, package of 100 vials and caps	079812
	10 mL polystyrene, package of 100 caps and septa	055058
Thermo Scientific™ Dionex™ IC PEEK Viper™ sample loop	2.5 µL PEEK Viper sample injection loop for push full mode	302899
IC-MS installation kit	IC-MS installation kit includes tubing, mixing tee	22153-62049
ISQ EC mass spectrometer calibration solution	Replacement calibrant solution, 250 mL	1R120590-6204
ISQ EC HESI II needle	Replacement HESI II needle	80000-60317
20 mL HDPE scintillation vials	Recommended storage vials	03-337-23A

Conditions

Columns	Dionex IonPac CG19-4µm guard (2 × 50 mm) and Dionex IonPac CS19-4µm analytical column (2 × 250 mm)
MSA gradient	9 mM MSA (-3 to 0.1 min), 9–65 mM (0.1–10.7 min), 65–75 mM (10.7–13 min) 75 mM (13–16 min), 9 mM (16–20 min)
Eluent source	Dionex EGC 500 MSA eluent cartridge, Dionex CR-CTC 600 trap column, and high pressure degas module
Flow rate	0.325 mL/min
Injection volume	2.5 µL in push full mode
Autosampler tray temperature	10 °C
Column temperature	20 °C
Detection/ suppressor compartment temperature	20 °C

Detection 1	Suppressed conductivity, Dionex CDRS 600 suppressor, 2 mm, 72 mA, constant current, and external water mode at 0.325 mL/min by the second pump of the DP	
Conductance background	<1 µS/cm	
Conductance noise	<1 nS/cm	
IC-MS system backpressure	~3,700 psi (100 psi = 0.6894 MPa)	
IC-MS run time	20 min	
Inject valve 2 (as an IC diverter valve)	Timing (min)	Valve position
	-3	DC.InjectValve_2. InjectPosition
	0 start run	DC.InjectValve_2. LoadPosition
	19.8	DC.InjectValve_2. InjectPosition
	20.0 stop run	No additional commands

Detection 2	ISQ EC mass spectrometer, +ESI, +2,500 V, full scan 80–250 *m/z*, CID: 10 V. SIM: see SIM table		
Flow (N_2)	Sheath: 40 psi, Aux: 2 psi, Sweep: 0.5 psi		
MS temperatures	Vaporizer: 280 °C, Ion transfer tube: 355 °C		
Chromatography width, SIM filter	10 points at 15 s, 0.3 amu		
Desolvation solution	None		
SIMs in component mode	Biogenic amine	Mass (*m/z*)	SIM window (min)
	Putrescine	89.1	0–20
	Histamine	112	0–20
	Agmatine	131	0–20
	Agmatine-$^{15}N_2$,^{13}C	133	0–20
	Histamine-d_4	97.2	0–20
CID	10 V		

Reagents

- Degassed ASTM Type I deionized (DI) water[15,16]
- Agmatine sulfate. Alpha Aesar™ (Fisher Scientific P/N AAH5536306), FW: 228.67
- Histamine dihydrochloride. Alpha Aesar™ (Fisher Scientific P/N AAL0919814), FW: 184.6
- 1,4 Diaminobutane (Putrescine dihydrochloride), ACROS Organics™ (Fisher Scientific P/N AC215110250), FW: 161.07
- Histamine-d_4 dichloride ($C_5H_5D_4N_3 \cdot 2HCl$), Toronto Research Chemicals P/N H436502, FW: 115.17
- Agmatine sulfate-$^{15}N_2$,^{13}C sulfuric acid ($C_4{}^{13}CH_{16}N_2{}^{15}N_2O_4S$), Toronto Research Chemicals P/N A426903, FW: 231.25

Column conditioning reagents

- Acetonitrile, Optima™ (Fisher Scientific P/N A996-1)
- Methanesulfonic acid, ACS grade. ACROS Organics™ (Fisher Scientific P/N AC432970010)

Preparation of column conditioning solutions

Offline conditioning is preferred because the acetonitrile solution can temporarily damage the suppressor used on the analytical instrument. The recommended offline conditioning is using an IC instrument configured with multiple eluent lines.

- Option 1: Using multiple eluent lines

  If the offline IC instrument is configured for multiple eluent lines, designate Eluent A for DI water, Eluent B for 80 mM MSA solution, and Eluent C for 95% acetonitrile solution.

  – Eluent A: DI water

  – Eluent B: 80 mM methanesulfonic acid (MSA)

  To prepare 1 L of 80 mM MSA from the reagent MSA, pipette 80 mL of the reagent MSA into a 1 L volumetric flask containing ~500 mL DI water and dilute to the mark with DI water. Cap the flask and invert multiple times to mix the 80 mM MSA solution. Transfer to a 2 L eluent bottle labeled Eluent B: 80 mM MSA.

  – Eluent C: 95% acetonitrile solution

  To prepare 250 mL of 95% acetonitrile, transfer 237.5 mL of reagent acetonitrile into a 250 mL volumetric flask. Add DI water to the mark, and mix as described for the 80 mM MSA solution. Transfer the solution to a 2 L eluent bottle labeled Eluent C: 95% acetonitrile.

- Option 2: Using a single eluent line

  Conditioning the column can be done serially with two solutions: 8 mM MSA and 8 mM MSA with 9.5% acetonitrile. Run 10 min with 8 mM MSA, followed by 55 min with 8 mM MSA with 9.5% acetonitrile, and 15 min with 8 mM MSA.

  – 8 mM MSA

  To prepare 1 L of 8 mM MSA, pipette 8 mL of the reagent MSA into a 1 L volumetric flask containing ~500 mL of DI water. Dilute to the mark with DI water. Cap and mix as described for the 80 mM MSA solution. Transfer to a 2 L eluent bottle labeled 8 mM MSA.

– 8 mM MSA with 9.5% acetonitrile

To prepare 1 L of 8 mM MSA 9.5% acetonitrile, pipette 8 mL of the reagent MSA into a 1 L volumetric flask containing ~500 mL of DI water and 95 mL of acetonitrile. Dilute to the mark with DI water. Cap and mix as described for the 80 mM MSA solution. Transfer to a 2 L eluent bottle labeled 8 mM MSA 9.5% acetonitrile.

Standard and sample preparation

Standards

To prepare a 1,000 mg/L agmatine stock standard, dissolve 172 mg of agmatine sulfate in a 125 mL HDPE bottle containing 100 mL DI water. Shake until dissolved. Prepare a 1,000 mg histamine standard similarly by dissolving 162 mg of histamine dichloride in 100 mL DI water. Prepare a 1,000 mg putrescine standard similarly by dissolving 101 mg of putrescine dichloride in 100 mL DI water. Prepare a 10 mg/L intermediate mixed standard by pipetting 200 µL of each stock standard into a 20 mL scintillation vial and adding DI water to a total weight of 20 g.

To prepare stock standards of the ISTD, pipette 1 mL of DI water into the 1-2 mL vial containing 1 mg of the reagent. The resulting concentrations are 384 mg/L of histamine-d_4 and 564 mg/L agmatine-$^{15}N_2$,^{13}C .

Working (five standards from 0.5, to 1.0, 5.0, 10, and 25 mg/L) and spiking standards were prepared from stock standards by diluting with DI water. A combined 10 mg/L ISTD was prepared from stock stable isotopic standards and added to each sample at 10 µg/L final concentration.

Samples

Three wine samples were opened and immediately sampled and then stored in the refrigerator: a sparkling wine (white), a cabernet sauvignon (red), and a chardonnay (white). In addition, a portion of each wine sample was exposed to ambient conditions for three days in a coffee cup to increase the exposed surface area. After three days, the samples were stored in the refrigerator. All wine samples were diluted 3-fold with DI water prior to analysis.

Instrument setup and installation

Physical and electronic configuration

The Dionex ICS-6000 is a modular, high pressure, Reagent-Free IC™ (RFIC™) ion chromatography system that can be configured as a dual system, as it is here. This application runs on System 1 with the second pump (Pump 2) and second injection valve (Valve 2), facilitating the flow to the suppressor regenerant (Inject position) and the MS (Load position) (Figure 1). Valve 2 in the Load position directs the suppressed effluent exiting the CD detector to the ISQ EC mass spectrometer and directs the Pump 2 flow of the DI water to the suppressor Regen In. Valve 2 in the Inject position directs the DI water to the MS, and the CD suppressed effluent to the suppressor Regen In.

For the best results, position the Dionex ICS-6000 system near the MS source. Install the power and USB cables, and power up the IC, autosampler, and computer. Add DI water to the eluent bottles and prime the pumps.

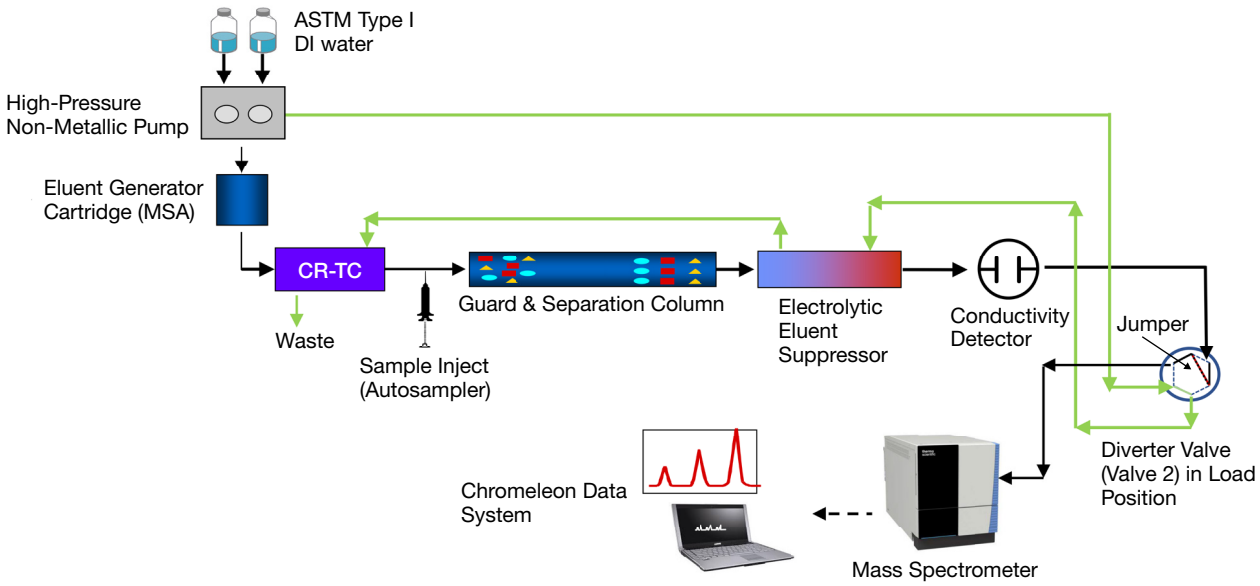


Figure 1. IC-MS flow diagram

Plumbing the Dionex ICS-6000 HPIC system

Plumb the Dionex ICS-6000 IC system as a standard Reagent-Free IC (RFIC) system as shown in Figure 1. Use the IC PEEK Viper fittings as indicated on their labels. Temporarily direct the liquid flow away from the ISQ EC mass spectrometer until the IC and IC consumables are fully conditioned. The schematics are also illustrated on the inside doors of the Dionex ICS-6000 IC system. Further information can be found in the operator's and installation manuals.[17-19] Direct the waste lines to waste containers.

Electronic configuration

To electronically configure the IC system, start the Thermo Scientific™ Chromeleon™ Instrument Services program, then start the Instrument Controller program by selecting the *Configure instruments* link. Add the Dionex ICS-6000 system DP module, EG module, DC module, and the AS-AP autosampler modules as described in Table 2. Check and correct any errors in the configuration. Save and close the instrument configuration program.

Power up the ISQ EC single quadrupole MS for electronic configuration. Power up the nitrogen generator so that the N_2 pressure output is ~100 psi. The foreline vacuum pump is plugged into the mass spectrometer and will automatically start-up with the mass spectrometer. Re-open the Instrument Configuration program as described above. Add the ISQ EC mass spectrometer module to the instrument configuration by selecting the ISQ EC/EM family under the mass spectrometer category as described in Table 2. Save and close the instrument configuration. Temporarily remove the tubing connecting the IC to the mass spectrometer. The fluid can be directed to waste. The ISQ EC mass spectrometer can remain in idle with the turbo pump running and the vaporizer temperature set below 100 °C while the IC is being conditioned.

Table 2. Electronic configuration parameters

Module	Tab	Action
DP	General	Select *Browse*, select serial number to link module to Instrument.
	Device	Link pump to Instrument.
EG	General	Select serial number to link module to Instrument.
	Cartridges	Link to instrument. Check EGC_1 and link to Pump 1.
DC	General	Select serial number to link module to Instrument, Select *Instrument*.
	Detectors	Double click on CD, Link to Pump 1.
	Thermo controls	Check Compartment_TC and Column_TC.
	Suppressors	Double click Suppressor1, Link to Pump 1.
	High pressure valves	• Double click InjectValve_1, Link to Pump 1, select control by autosampler. • Double click InjectValve_2, Link to Pump 1, select control by DC.
AS-AP autosampler	General	Select serial number to link module to Instrument.
	Sharing	This option is present, select *Instrument*.
	Segments / Pump link	Select 10 mL PolyVials or 1.5 mL vials for "Red", "Blue", and "Green". Leave the pump and TTL links empty.
	Options	Select 1,200 buffer loop, 250 µL syringe, temperature control, and push mode. Enter 2.5 µL in sample loop for full push mode.
ISQ EC mass spectrometer	Add module	Mass spectrometry, ISQ ICMS family.
	General	• Select ISQ EC module. • Deselect the hardware inject synchronization. This is not needed for Chromeleon CDS. • Select *ActiveLo*w for Remote Start. (Setting for Dionex AS-AP autosampler) • Deselect *split flow*, *fraction collection*, *Warn on source change* and *Simulation mode* boxes.
	Maintenance	Enable all boxes. Select OK.
	Associate pump flow	• Optional: Select *Associate* — a pump box will automatically enter flow rate when setting source conditions. If using this option, select pump used for eluent flow. • Select *OK*.

Conditioning electrolytic devices and columns

Important: Do not remove consumable tracking tags on the columns and consumable devices. These tags are required for consumables monitoring functionality.

Set the ISQ EC mass spectrometer to standby mode (vaporizer temperature = “0”, ion transfer temperature = 150 °C, and sheath = 20 psi), and direct the flow from the IC to waste.

Hydrate and condition the Dionex EGC 500 MSA eluent generator cartridge and Dionex CR-CTC 600 continuously regenerated trap column according to the product manuals or the instructions in the drop-down menu (Chromeleon Console, under Consumables drop down menu).[20, 21]

Caution: Precondition the Dionex IonPac CS19-4µm columns using an offline pump before installing the columns. To do so, follow the instructions described earlier.

Note: Residual acetonitrile can damage the suppressor. If the same Dionex ICS-6000 DP module used for preconditioning will be used for the application, extend the 8 mM MSA conditioning while delaying the conditioning of the suppressor for several hours.) Condition at the QAR conditions (8 mM MSA at 0.25 mL/min and 30 °C) for at least 30 min.

To hydrate the Dionex CDRS 300 suppressor, follow the Suppressor Installation Checklist instructions that are delivered with the suppressor.[23] The operations of the Dionex CDRS 600 suppressor are thoroughly discussed in the suppressor manual.[24] The suppressor has its best performance if the IC is started soon after the suppressor is hydrated and allowed to rest for 20 min.

Starting and conditioning the IC-MS system

For optimum results, the IC and mass spectrometer are initially conditioned separately. To condition the IC system, open Chromeleon CDS console, turn on Pump 1, the Dionex EGC 500 MSA cartridge, Dionex CR-CTC 500 trap column, and prime and turn on Pump 2. Set Pump 2 to 0.25 mL/min. Turn on the suppressor and set Valve 2 (DC panel) to the “Inject” position so that the DI water flows from Pump 2 to the suppressor Regen In port. Temporarily disconnect the tubing from the IC to the mass spectrometer and redirect it to waste. Set the IC conditions to the Quality Assurance Report (QAR) conditions (8 mM MSA, 0.25 mL/min, 30 °C separation temperature, and suppressor to constant current and 6 mA).

The ISQ EC single quadrupole mass spectrometer has already been started during electronic configuration. (Starting conditions: Power up the mass spectrometer, nitrogen generator, and foreline vacuum pump. After the foreline pump vacuum is reached, the turbo pump inside the ISQ EC MS will automatically start and bring the vacuum to the operating level as signaled by the “yes” in the “vacuum ready” box on the e-Panel.) Select the ISQ EC panel in the Chromeleon CDS console. Temporarily set the nitrogen conditions to 20 psi for sheath gas, and 2 psi for the aux and sweep gas. Set the vaporizer temperature to 150 °C and the ion transfer temperature to 0 °C. Ensure that the IC flow is diverted to waste.

Equilibrate the IC system separately using an instrument method for the IC system only, or with the ISQ EC temporarily removed from the method (deselect the “to remove from method” box on the ISQ EC page.) Direct the IC flow temporarily to waste. Condition the IC system using the Quality Assurance Report (QAR) conditions for the Dionex IonPac CS19-4µm column until the total conductivity <1 µS/cm. Verify that the QAR conditions are met.

Creating IC-MS instrument methods with emergency shutdown subprograms

Enter the N_2 flow conditions and the MS temperatures into the ISQ EC panel. Redirect the IC effluent to the MS as shown in Figure 1.

To create an IC-MS method, use the Chromeleon Instrument Method Wizard program and enter the parameters listed in the Conditions section, including the InjectValve_2 timing. Uncheck the “to remove from method” box on the ISQ EC page. Manually insert commands for Injection valve 2 positions: Inject (DC.InjectValve_2.InjectPosition) in the prerun conditions and at the end of the program and Load in the start run time (DC.InjectValve_2.LoadPosition).

Unexpected failures due to the suppressor or interrupted regenerant flow to the suppressor can result in unsuppressed eluent damaging the mass spectrometer. At the end of each run the diverter valve should be rotated to direct the CD effluent away from the mass spectrometer. In this configuration, the Inject Valve 2 is in the Inject position directing DI water to the MS and the suppressor is in the recycle mode. This has the additional benefit of rinsing the diverter valve.

The high conductivity trigger, using the Chromeleon Conditional Trigger function, implements emergency actions when the total conductivity signal exceeds a high level (50 µS/cm) for a set time (180 s). This could be due to an unexpected suppressor failure. The action commands are intended to halt un-suppressed eluent flow to the ISQ EC mass spectrometer by directing Valve_2 to rotate to the injection position, thereby directing external water flow to the mass spectrometer and the CD flow to the suppressor Regen In port. (Note: when the trigger conditions are met, the pump will turn off, which turns off the RFIC consumables and suppressor.) These conditions were selected for the application and can be adjusted to lower or higher conductivity or a different elapsed time. The commands for the Dionex Integrion HPIC system will be slightly different.

To create an emergency trigger for the Dionex ICS-6000 system:

1. Open the IC-MS Chromeleon instrument program.
2. Open the Chromeleon Script Editor.
3. Insert a Conditional Trigger before the equilibration time, -4.000 Equilibration.
4. Place the cursor on the End Trigger row and Command column. Select Insert Command and enter the conditions of the HighConductivity trigger.

Name	"HighConductivity"
Condition	CDet1.CD_1_total.signal.value>=50
TrueTime	180
Delay	5
Allow Immediate	yes

5. Enter the commands to divert IC flow away from the mass spectrometer while diverting DI water to the mass spectrometer and to stop the pumps. The "system stop" mode stops the pump flow and places the system on hold. The "pump motor-off" commands are redundant.

Action Commands	Value
DC.InjectValve_2.InjectPosition	No entry
System.StopMode	on
DP.Pump_2.motor	off
DP.Pump_1.motor	off

6. Save the trigger and save the instrument method.

More details on creating emergency triggers can be found in Thermo Scientific Application Note 73339.[25]

```
◢ Trigger                                   "highconductivity",
                                            CDet1.CD_1_Total.Signal.Value>=50,
                                            TrueTime=180,
                                            Delay=5,
                                            AllowImmediateExecution=Yes
                  DC.InjectValve_2.InjectPosition
                  System.StopMode           on
                  DP.Pump_2.Motor           off
                  DP.Pump_1.Motor           off
    End Trigger
◢ -4.000          Equilibration             Duration = 4.000 [min]
```

Figure 2. Emergency trigger script

Results and discussion

The Dionex IonPac CS19-4µm column is a high-capacity (600 µequiv/column) column composed of ethylvinyl benzene, cross-linked with 55% divinyl benzene supermacroporous particles with 4 µm average diameter. The ion-exchange sites are carboxylic acid groups. The column was selected for this application because it is optimized for polar amines, including biogenic amines. Although the inorganic cations are shown in this separation, the gradient separation was optimized for the separation of putrescine, histamine, and agmatine.

The separation temperature was set to 20 °C for optimum column life and biogenic amines stability (Figure 3A). The autosampler temperature was set to 10 ºC to maintain analyte, standard, and sample stability.

After optimizing the chromatography, the MS conditions (source voltage, temperatures, and nitrogen flow rates) were optimized on the ISQ EC mass spectrometer using a 10 mg/L mixed biogenic amines standard. The results shown in Figure 3B, show a strong response for 10 mg/L putrescine, histamine, and agmatine.

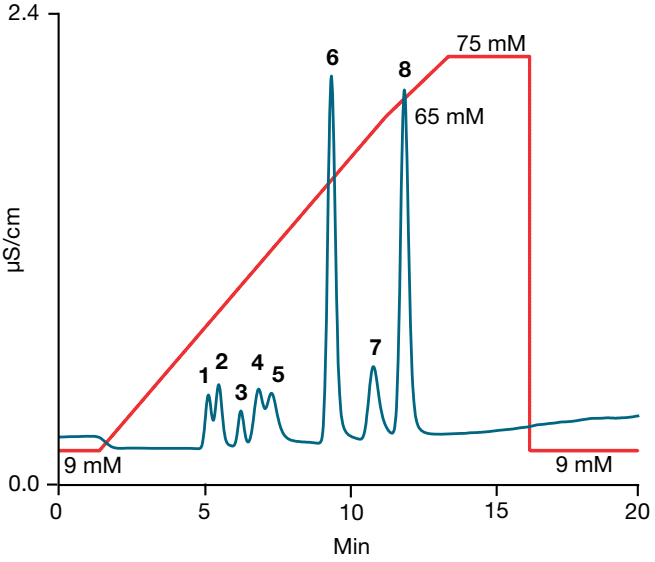


Figure 3A. Cations and biogenic amines by suppressed conductivity

Columns:	Dionex IonPac CG19-4µm, Dionex IonPac CS19-4µm, 2 mm i.d.
MSA gradient:	9 mM MSA (-4–0.1 min), 9–65 mM (0.1–10.7 min), 65–75 mM (10.7–13 min), 75 mM (13–17 min), 9 mM (17–20.0 min)
Eluent source:	Dionex EGC-500 MSA cartridge, Dionex CR-CTC 600 trap column, Dionex high pressure degasser
Flow rate:	0.3 mL/min
Injection volume:	2.5 µL full loop
Column temp.:	20 °C
Detector oven temp.:	20 °C
First detection:	Suppressed conductivity, Dionex CDRS 600, 2 mm, constant current and external water modes at 0.325 mL/min, 66 mA

Peaks:

	(mg/L)		(mg/L)
1. Sodium	0.5	5. Calcium	0.5
2. Ammonium	0.5	6. Putrescine	10
3. Potassium	0.5	7. Histamine	10
4. Magnesium	0.5	8. Agmatine	10

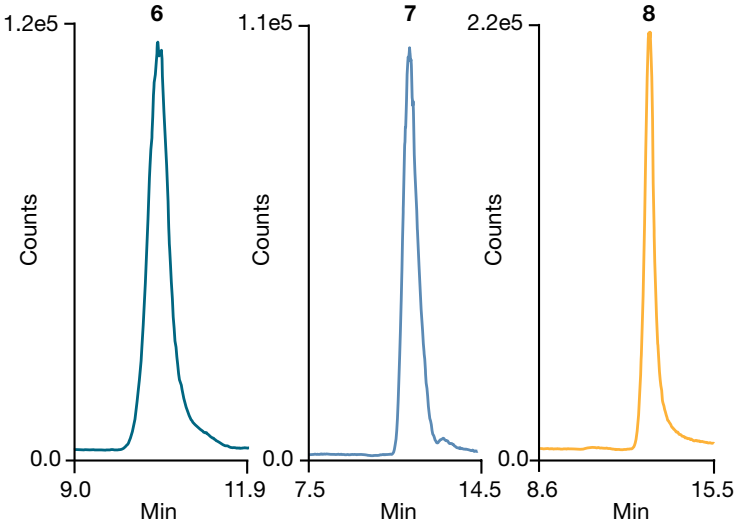


Figure 3B. Biogenic amines by mass spectrometry

Second detection:	ISQ EC mass spectrometer, +ESI, +2,900 V, Component mode
Scan mode:	Full scan: 80–250 *m/z,* SIM (see below)
MS temperature:	Vaporizer: 280 °C, Ion transfer: 355 °C
N_2 gas (psi):	Sheath: 40, Aux: 2, Sweep: 0.5
Standard:	10 mg/L

Peaks:

	CID (V)	SIM (*m/z*)
6. Putrescine	10	89.1
7. Histamine	10	112
8. Agmatine	10	131

To evaluate the application method, the MS response to concentration was determined using five standards from 0.5 to 25 mg/L in triplicate, the method detection limit (MDL) was determined at 3x S/N, and the retention time and peak area reproducibilities were determined in triplicate. For calibrating the IC-MS, a second order fit was chosen (Table 3). The MDLs are 0.1 to 0.14 mg/L. The peak area reproducibilities are acceptable with <2% RSDs for agmatine and putrescine. Histamine shows more variability with 7.3% RSD.

Samples

The method was applied to three wine samples—a sparkling wine, a chardonnay, and a cabernet sauvignon. The wines were sampled upon opening and after three days of exposure to the room temperature environment. The samples were diluted 3-fold for analysis. The results in Table 4 and Figure 4 confirm that the cabernet sauvignon (red) has higher concentrations of putrescine and histamine than the sparkling wine and chardonnay wine samples. Putrescine concentrations were about three times higher in the red wine than the other two samples, and the histamine concentration was about three times the chardonnay value. Agmatine was below the MDL in all three wine samples, and histamine was not detected in the sparkling wine sample.

Table 3. Summary of IC-MS linearity, MDL, and reproducibility results

		Coefficient of determination (r^2)	MDL (mg/L)	Peak area reproducibility (RSD)
Putrescine	Quadratic with offset	0.9992	0.14	1.8
Histamine	Quadratic with offset	0.9985	0.11	7.3
Agmatine	Quadratic with offset	0.9990	0.14	1.8

Table 4. Summary of the biogenic amine analysis and recovery results

		Putrescine				Histamine				Agmatine		
	Sample	Found (mg/L)	RSD	Added (mg/L)	Recovery (%)	Found (mg/L)	RSD	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (mg/L)	Recovery (%)
Sparkling wine 3-fold diluted	Initial	1.64	1.7	1.0	108	< MDL	--	1.0	109	< MDL	1.0	130
	After 3 days	1.94	1.6	1.0	111	< MDL	--	1.0	111	< MDL	1.0	134
Chardonnay 3-fold diluted	Initial	1.48	3.2	1.0	113	0.98	3.8	1.0	117	< MDL	1.0	116
	After 3 days	2.33	5.7	1.0	110	2.46	1.9	1.0	117	< MDL	1.0	116
Cabernet Sauvignon 3-fold diluted	Initial	6.08	1.2	1.0	98.7	3.06	1.5	1.0	106	~0.26	1.0	115
	After 3 days	10.5	2.9	1.0	96.0	6.03	1.1	1.0	101	~0.38	1.0	112

Effect of exposure to air

The biogenic amines were compared in 3-fold diluted wine samples from freshly opened bottles and after exposing the sample to air for three days. Figure 4 compares the results. Putrescine concentrations increased for the sparkling wine, chardonnay, and cabernet sauvignon samples from 1.6 to 1.9, 1.5 to 2.3, and 6 to 10.5 mg/L, respectively. The histamine concentrations in the chardonnay and cabernet sauvignon samples also increased. Agmatine concentrations were below the MDL for all samples.

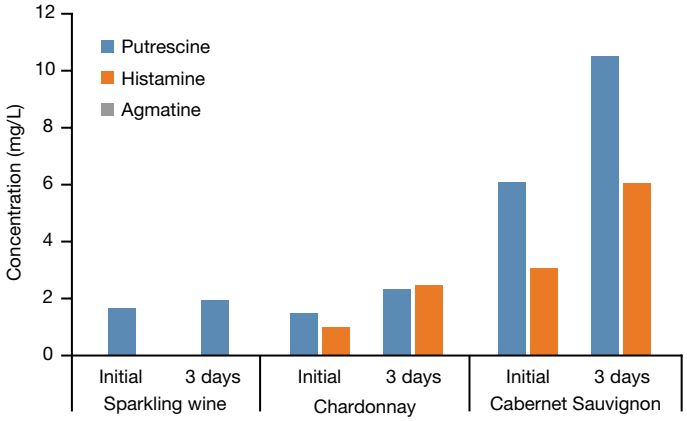
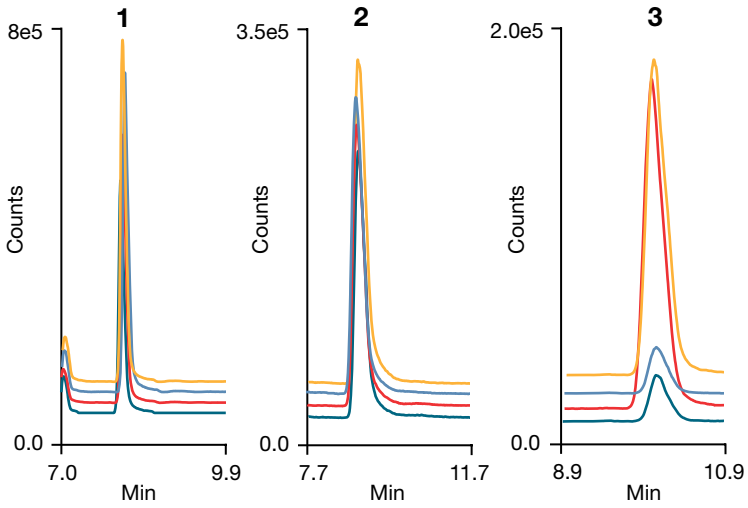


Figure 4. Comparison of biogenic amines in the 3-fold diluted wine samples

Recovery

To evaluate the accuracy of the method, 1 mg/L of putrescine, histamine, and agmatine mixed standard was added to samples. The recoveries summarized in Table 4 show 96 to 117% recoveries for histamine and putrescine. Figure 5 shows SIM chromatograms of putrescine and histamine in the red wine sample. The recoveries for agmatine were high, 112 to 130%.



Conditions: See Figures 3A and 3B
CID (V): 10
SIMs (*m/z*): Putrescine: 89.1, Histamine: 112, Agmatine 131
Samples: 3-fold diluted Cabernet Sauvignon

Peaks:	A (mg/L)	B (mg/L)	C (mg/L)	D (mg/L)
1. Putrescine	6.08	6.99	10.05	11.04
2. Histamine	3.06	6.39	6.03	6.09
3. Agmatine	~0.26	1.32	~0.38	1.42

Figure 5. Biogenic amines in 3-fold diluted cabernet sauvignon wine sample

Conclusion

This application note demonstrates putrescine, histamine, and agmatine determinations in three wine samples using a Dionex IonPac CS19-4µm column with serial detection by suppressed conductivity and MS.

Sample analysis confirms the presence of biogenic amines in the wine samples and that these concentrations increased when the wine was exposed to air. The red wine had higher concentrations of putrescine and histamine than the two white wine samples.

More information on this application and other related applications can found in the Thermo Scientific™ AppsLab Library of Analytical Applications.[26]

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