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Choosing internal standards for IC-MS

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Keywords

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Goal

To describe how to choose internal standards for IC-MS, using U.S. EPA Method 557 as an example to illustrate the capabilities of the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) in achieving IC-MS quantitation using the Thermo Scientific™ Dionex™ ICS-6000 system coupled to triple quadrupole mass spectrometry with electrospray ionization

Introduction

Ion chromatography (IC) coupled with mass spectrometry detection (IC-MS) is an ideal solution for determining critical ionic contaminants in environmental samples. The specificity and selectivity of ion-exchange result in better separations for ionic contaminants than reversed-phase separations. Direct coupling of IC with MS is possible with reagent-free ion chromatography (RFIC), which combines automated eluent generators and electrolytically regenerated suppressors to electrolytically create the required eluents and regenerants used for IC applications. Compatibility with MS is enabled by an electrolytic ion suppressor placed between the IC system and the electrospray ion source of the mass spectrometer. For anion separations, the potassium hydroxide eluent passes through the suppressor, and electrochemically produced hydronium is exchanged with potassium cations and pairs with the hydroxide to form water. For cation separations, the methanesulfonic acid eluent passes through the suppressor, and electrochemically produced hydroxide is exchanged with methanesulfonate and pairs with the hydronium to form water. This provides an electrospray-compatible chromatographic eluent that can be analyzed directly by the mass spectrometer. The electrochemical suppressor creates a solution that minimizes potential ion suppression during electrospray ionization in the ion source.

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However, contaminants present in the sample can interfere with analyte quantification. This is termed matrix interference, the extent of which can vary considerably depending on the nature of the sample. Matrix components can directly interfere by producing a signal at or near the retention time of an analyte peak. Matrix components can also suppress or enhance the signal of the method analytes. Suppression and enhancement effects occur during the ionization process in the electrospray source when a coeluting contaminant influences the ionization of the analyte of interest. Common anions present in drinking water samples, which can cause matrix suppression, are typically diverted from the MS.

A well-considered choice of an internal standard (IS) can improve the accuracy and precision of the method. The degree of ion suppression or enhancement caused by matrix components may vary largely between sample types and depends on the chemical structure of the analyte. Therefore, the properties of a good IS need to be considered when choosing an IS.

Internal standard characteristics

Never found in sample: This seems logical — after all, if the compound chosen as the IS is always or occasionally present in untreated samples, how can you tell the difference between the response of the native IS and the added IS? Although the absence of the IS is an ideal, if the amount of native IS in samples is much less than the amount of added IS, it is unlikely that there will be a problem. For example, if the native IS peak is never more than 2% of the added IS peak, there would only be a small impact on the results. The analyst must decide if this impact is significant as it relates to the goal(s) of their analysis.

Structure similar to the analyte: Because the IS needs to have similar extraction characteristics (e.g., aqueous and organic solvent solubilities), chromatographic retention, stability, and detector response as the analyte, the proper internal standard should be a compound of similar structure or a stable isotopically labeled (SIL) analog of the analyte. SIL internal standards are compounds in which several atoms in the analyte are replaced by their stable isotopes, such as ^{2}H (D, deuterium), ^{13}C, ^{15}N, or ^{18}O. Because SIL internal standards have identical chemical and structural properties as the analyte, the analyte and its SIL analog will be analyzed simultaneously during the entire process of sample preparation and chromatography. In general, SIL internal standards are the best choice of IS for IC-MS to reduce signal variability and improve precision. This is due primarily to the SIL IS ionizing with the same efficiency as the analyte of interest. This is also one of the assumptions made when choosing a structurally similar compound for an IS.

Well-resolved: The chromatographic system must be able to measure the size of the analyte and IS peaks independently. For most detectors, there should be baseline resolution between the peaks. For IC-MS, however, the added selectivity of the MS detector often will allow independent measurement of the analyte and IS if they do not have the same molecular weight. This is one reason why stable-label, or isotopically labeled, versions of the analyte are commonly used with IC-MS methods — the peaks can coelute, yet the MS detector can separately measure them.

Stable: At a minimum, the IS needs to be sufficiently stable so that it does not degrade during the sample preparation and chromatographic analysis processes. Although not essential, more stability will mean more convenience so that stock solutions will last longer, and refrigeration or freezing may not be needed.

Available in pure form: This requirement is misleading to some extent. It would be better to state that any impurities present will not coelute with the analyte or otherwise interfere with the process. For example, an IS with 80% purity could be used if the 20% impurity did not interfere with the method. Higher-purity internal standards usually are preferred, but 100% purity is not required and, in some cases, is very difficult to obtain. For example, in the case of stable-label or isotopically labeled internal standards that are common with IC-MS methods, 100% purity is rare. The reason is that it is nearly impossible to synthesize a stable-labeled IS without at least some unlabeled analyte present. This will be inconsequential as long as the amount of unlabeled material is so small that it does not affect the results. For example, for U.S. EPA Method 557, the requirement is that the SIL internal standards should not contain the unlabeled analytes at a concentration > 1/3 of the minimum reporting level (MRL) when added at the appropriate concentration to samples.

Number of internal standards: Given cost and availability, it is impractical to quantify each analyte with its SIL internal standard. In the example of U.S. EPA Method 557, there are 11 analytes of interest. This method requires that a minimum of the four internal standards prescribed in the method be used.[1] Each internal standard is assigned to a specific analyte or multiple analytes and is used to measure relative response.

Amount of the internal standards: Ensure that the amount of the internal standard is well above the limit of quantitation but not so high as to suppress the ionization of the analyte. It helps to know roughly how much compound is in your sample. This can be accomplished by making trial analyses of a few samples that you believe will be typical of the samples the lab will receive. This information will be very valuable when building an appropriate standard curve and in knowing how much internal standard to add. If you were trying to quantify samples in the range of 0.1 to 20 µg/L and the limit of quantitation was 0.1 µg/L, you might add 1 to 5 µg/L of internal standard to every sample. A typical approach is to target the internal standard to the lower 1/3 of the working standard curve. This is a range that will give an easily measurable response without interfering with the ionization of the analyte. An internal standard concentration of 4.0 µg/L was used for U.S. EPA Method 557.[1] Lower concentrations may be used, providing the internal standard QC criteria are met.

U.S. EPA Method 557 is a direct-inject, ion chromatography, negative-ion electrospray ionization, tandem mass spectrometry (IC-ESI-MS/MS) method for the determination of haloacetic acids in finished drinking water.[1] Here, we will use this method as an example to demonstrate the capabilities of Chromeleon CDS in performing IC-MS quantitation using internal standards. Application Note 73343 describes experimental details to perform U.S. EPA Method 557.[2]

Standards for U.S. EPA Method 557, including internal standards

- Haloacetic acid Mix (9 components), 1,000 mg/L each in methyl tert-butyl ether, Restek (P/N 31896)

- Dalapon (2,2-dichloropropionic acid), 1,000 mg/L in methanol, Restek (P/N 32253)

- Bromate standard, 1,000 mg/L, Thermo Scientific™ (P/N 303168)

- Monochloroacetic acid MCAA-2-^{13}C, 1,000 mg/L, Thermo Scientific™ (P/N 069406)

- Monobromoacetic acid MBAA-1-^{13}C, 1,000 mg/L, Thermo Scientific™ (P/N 069407)

- Dichloroacetic acid DCAA-2-^{13}C, 1,000 mg/L, Thermo Scientific™ (P/N 069408)

- Trichloroacetic acid TCAA-2-^{13}C, 1,000 mg/L, Thermo Scientific™ (P/N 069409)

Table 1. IC-MS parameters for selected reaction monitoring transitions

Compound	Start time (min)	End time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	Min dwell time (ms)	Tube lens (V)	Source fragmentation
MCAA	5	7.5	92.9	35.1	9.72	297.691	79	14.6
MCAA_IS*	5	7.5	93.9	35.1	9.09	297.691	82	13.1
MBAA	6	8	136.9	78.9	8.71	297.691	86	9.8
MBAA_IS	6	8	137.9	78.9	9.38	297.691	84	14.7
Bromate	7	11	126.9	110.9	21.68	297.691	85	24.5
Dalapon	8	14	140.9	96.9	7.7	297.681	84	13
DCAA	9	14	126.9	83.0	8.54	297.681	86	24.5
DCAA_IS	9	14	128.0	83.9	8.41	297.681	84	13.1
BCAA	12	17	172.8	128.9	9.55	297.681	89	22.8
DBAA	13	17	216.8	172.8	10.05	297.681	87	14.7
TCAA_161	22	26	160.8	116.9	5.25	373.025	81	19.5
TCAA_163	22	26	162.8	118.9	5.25	373.025	79	22.9
TCAA_IS	22	26	161.9	117.9	5.25	373.025	78	18
BDCAA	24	28	162.8	80.9	8.62	373.025	79	22.9
DBCAA	26	36	206.8	78.9	15.57	747.677	91	22.9
TBAA	28	36	250.7	78.9	19.41	747.677	87	26.1

*IS: Internal standard

Data processing in Chromeleon software

IC-MS data were acquired and processed using Chromeleon CDS, which allows the creation of the acquisition and processing methods for high-throughput quantitative analysis, data reviewing, and final reporting. Chromeleon software contains an MS Component Table to create the component list in the Data Processing Category in the Chromeleon Chromatography Studio window.

Creating an MS Component Table

The MS Component Table is part of the Processing Method Editor and contains all parameters required to identify a peak and to determine the amounts of the substances serving as standards. This requires that you have specified the compound name, retention time, and extraction window (Figure 1). For U.S. EPA Method 557, most of the information needed to complete the table can be found in the IC-MS parameters for Selected Reaction Monitoring Transitions (Table 1).

Defining an MS quantitation peak

- On the Component Table, click **Show Properties** (Figure 1, red box) and then click on **Extracted Ion Chromatograms**.

  –OR–

  Double-click the cell of **MS Quantitation Peak** of the component you want to edit (Figure 1, yellow box). The **Extracted Ion Chromatograms** page for the selected component opens.

- To create an MS quantitation peak, select the following methods:

  Type the precursor mass in the **Precursor Mass** and/or product ion mass in the **XIC Mass(es) or Range(s)** fields (Figure 2).

Viewing extracted ion chromatograms

Click **MS quantitation channel** (Figure 3), which displays all the extracted ion chromatograms of the MS quantitation peaks (Figure 4). All components from the completed Component Table are listed in the **Components** group (Figure 3).
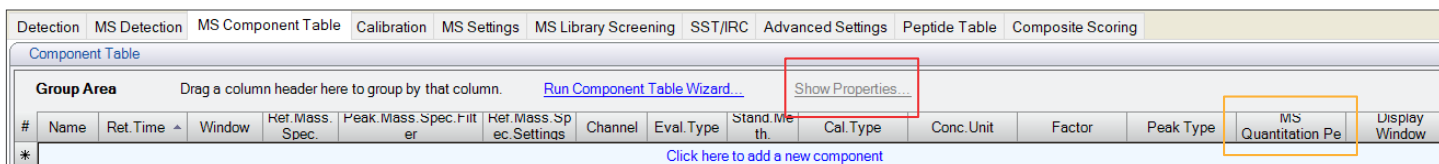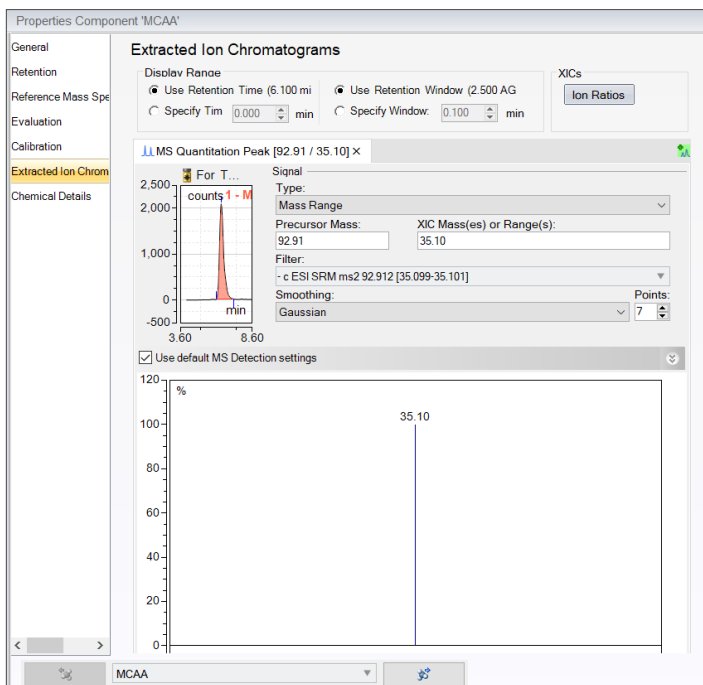

Figure 1. MS Component Table

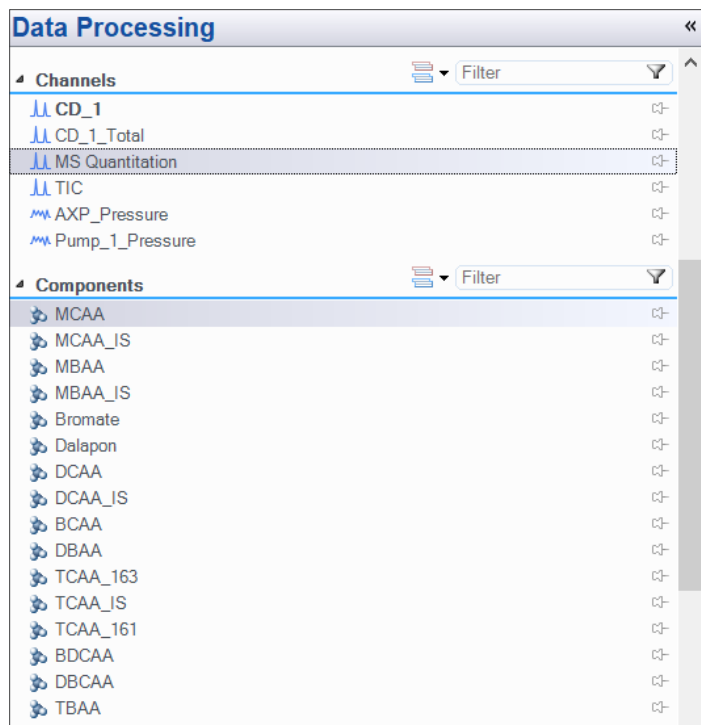Figure 2. Extracted Ion Chromatograms dialog box

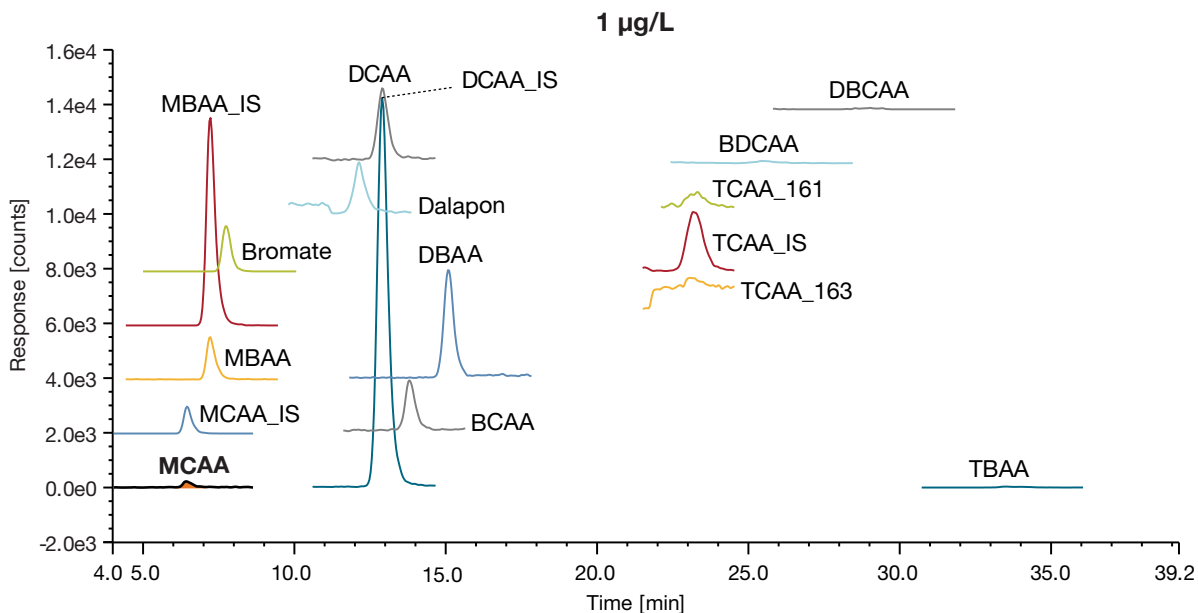Figure 3. MS Quantitation channel and component list

Figure 4. All the extracted ion chromatograms of the MS quantitation peaks

Performing a calibration using the internal standard method

1. Specify which injections are Calibration Standards.

2. Create Calibration Levels and assign these to the Calibration Standards.

3. Enter the amount or concentration of the Calibration Standards.

4. Specify which compounds are internal standards and to which compounds they are assigned for calibration.

5. Review the calibration curve after the sequence has run.

These five steps will be discussed in detail.

Specifying which injections are calibration standards

Calibration standards are marked in the injection list:

1. Click the **Injection List** category in the Chromatography Studio window (Figure 5).

2. For each Calibration Standard injection, click in the **Type** field and select **Calibration Standard** from the list of types (Figure 6, Red box).
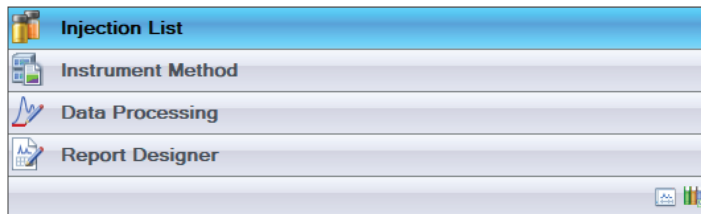

Figure 5. Injection List category in the Chromatography Studio window

Figure 6. Injection sequence with TIC MiniPlots

Creating and assigning calibration levels

1. Click the **Injection List** category in the Chromatography Studio window.

2. Click in the **Level** cell for the first calibration standard and select **Create new level**. The value 1 is added. Assign this level to all injections of this calibration standard.

3. Repeat Step 2 for all calibration levels (Figure 6, yellow box).

Entering calibration standard amounts or concentrations

1. Click the **Data Processing** category in the Chromatography Studio window (Figure 5).

2. In the **Panes** group of the **Data Processing Home** tab, click **Processing Method** (Figure 7).

3. In the processing method, select the **MS Component Table** tab (Figure 8).

4. Enter the standard amount for each level of the calibration component in the appropriate cell (Level "1", Level "2", etc. See the yellow shaded area).
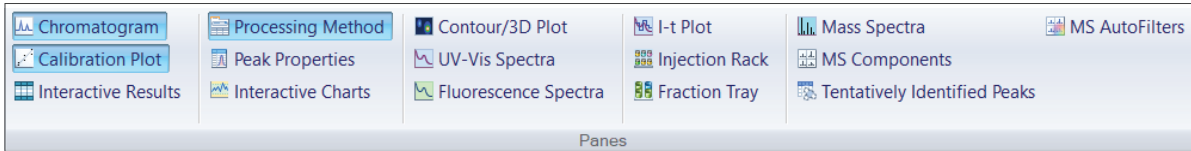

Figure 7. Processing Method in the Panes group

#	Name	Ret.Time ▲	Window	Ref.Ma	Peak.Mass.Spec.Filter	Ref.Mass.Spec.Settings	Channel	Eval.Type	Stand.Meth.	Cal.Type	Level "1"	Level "2"	Level "3"
1	MCAA	6.100	2.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal MCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
2	MCAA_IS	6.100	2.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	ISTD Internal	Lin, WithOffset	4.000000	4.000000	4.000000
3	MBAA	6.900	2.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal MBAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
4	MBAA_IS	6.900	2.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	ISTD Internal	Lin, WithOffset	4.000000	4.000000	4.000000
5	Bromate	7.500	2.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal MBAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
6	Dalapon	11.800	2.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal DCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
7	DCAA	12.600	2.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal DCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
8	DCAA_IS	12.600	2.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	ISTD Internal	Lin, WithOffset	4.000000	4.000000	4.000000
9	BCAA	13.600	2.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal DCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
10	DBAA	14.800	3.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal DCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
11	TCAA_163	23.000	1.500 AF		Quantitation	SI:0, RSI:0	All Channels	Area	Internal TCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
12	TCAA_IS	23.000	1.500 AF		Quantitation	SI:0, RSI:0	All Channels	Area	ISTD Internal	Lin, WithOffset	4.000000	4.000000	4.000000
13	TCAA_161	23.300	1.200 AF		Quantitation	SI:0, RSI:0	All Channels	Area	Internal TCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
14	BDCAA	25.400	3.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal TCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
15	DBCAA	28.800	3.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal TCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000
16	TBAA	33.700	3.000 AG		Quantitation	SI:0, RSI:0	All Channels	Area	Internal TCAA_IS (MS_Quantitation)	Lin, WithOffset	0.100000	0.250000	0.500000

Figure 8. MS Component Table

Specifying which compounds are internal standards and to which compounds they are assigned for calibration

In Chromeleon CDS, internal standard components are defined in the **Standard Method** column of the component table.

1. On the **MS Component Table** tab page, double-click the cell in the **Stand. Meth.** column (red box in Figure 8) for the *internal standard*. Select the **Use this component as Internal Standard (ISTD)** check box. Under Internal Standard Options, click **Internal** (Figure 9).

2. The line of the internal standard component has a pale yellow background and is labeled ISTD Internal (Figure 8).

3. Double-click the cell in the **Stand. Meth.** column for the *other component* (the component we wish to measure). Under Standard Method, click **Internal**, and select the name of the internal standard from the list (Figure 10). Note that internal standard assignments appropriate for the Dionex IonPac AS31 column for each analyte are presented in Table 2, and the internal standard peak must be identified by retention time in the **MS Component Table** for the used channel.

4. On the **MS Component Table** tab page, in the **Cal. Type** column, double-click the cell for the specific compound. Type Lin for linear calibration with offset.

Calibration curves are automatically built.

Figure 9. Define the internal standard

Figure 10. Assign the internal standard to a specific analyte

Table 2. Internal standard assignments for U.S. EPA Method 557

Compound	Internal standard assignment
MCAA	MCAA[2-^{13}C]
MBAA	MBAA[1-^{13}C]
Bromate	MBAA[1-^{13}C]
Dalapon	DCAA[2-^{13}C]
DCAA	DCAA[2-^{13}C]
BCAA	DCAA[2-^{13}C]
DBAA	DCAA[2-^{13}C]
TCAA	TCAA[2-^{13}C]
BDCAA	TCAA[2-^{13}C]
DBCAA	TCAA[2-^{13}C]
TBAA	TCAA[2-^{13}C]

Reviewing calibration curves

You can view the calibration curve(s) (Figure 11) in the Data Processing Category of the Chromatography Studio:

1. Click the **Data Processing** Category.

2. Go to the **Data Processing** Home tab.

3. Either:

   • Select a preset work area layout which contains a calibration pane from the **Presets** group.

   –OR–

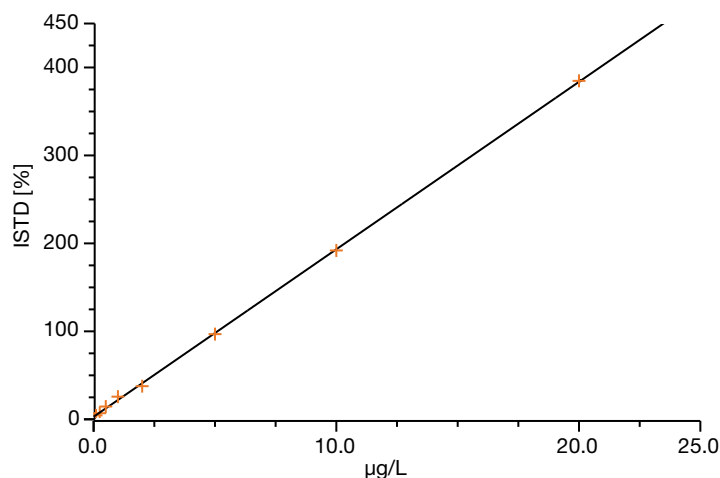   • Select **Calibration Plot** in the Panes group (Figure 7).

Figure 11. The calibration curve for MCAA ranging from 0.1 to 20 µg/L using the internal standard MCAA[2-^{13}C]

eWorkflow procedures and AppsLab Library

An eWorkflow™ procedure is an electronic template that contains all the information required to run analysis. A specific eWorkflow procedure for U.S. EPA Method 557 was generated in few steps, adding the instrument method, data processing method, report template, and view settings files. Any other documents relevant to the analysis can also be attached. After creation, it was made available for free download from the Thermo Scientific™ AppsLab Library of Analytical Applications. A simple import into Chromeleon CDS enables anyone to run the analysis, process the data, and produce the report.

The AppsLab Library is a Thermo Fisher Scientific repository comprising over 2000 applications with detailed method information, chromatograms, and related compound information.

Conclusion

The complicated matrices found in many environmental, food, and other application areas give rise to the need for internal standards for IC-MS methods. The addition of one or more internal standards can improve the precision and accuracy of an IC-MS method. This technical note describes how to choose internal standards for IC-MS and shows how to use Chromeleon CDS to set up an IC-MS method that uses internal standard calibration. Chromeleon software has built-in capabilities designed to handle the internal standard method, allowing the CDS to automatically perform the calculations, thus eliminating the need to perform them outside the software. U.S. EPA Method 557, which uses IC coupled to a triple quadrupole electrospray mass spectrometer to determine haloacetic acids in drinking water, is used as an example of how to set-up an IC-MS method that uses internal standards.

References
1. United States Environmental Protection Agency Method 557, Determination of Haloacetic Acids, Bromate, and Dalapon in Drinking Water by Ion Chromatography Electrospray Ionization Tandem Mass Spectrometry, **2009**. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005OKO.PDF?Dockey=P1005OKO.PDF

2. Thermo Scientific Application Note 73343: Fast determination of nine haloacetic acids, bromate, and dalapon at trace levels in drinking water samples by tandem IC-MS/MS. Sunnyvale, CA. https://appslab.thermofisher.com/App/4379/determination-haloacetic-acids (accessed Dec. 02, 2020).

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