

Poster Reprint

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Accurate Mass Full Spectral Monitoring and Analysis of Both the Analyte and Reference Standard with Ion Chromatography – Mass Spectrometry

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Introduction

Ion Chromatography coupled with mass spectrometry (IC/MS) is gaining traction in a wide range of industries for the detection, confirmation, and analysis of important ions existing in pharmaceuticals, industrial materials, and environmental samples. With matrix interferences from API formulations to environmental backgrounds, there is a need to accurately separate out closely located MS signals for more confident qualitative analysis and accurate quantitation. However, accessibility to this type of technology is challenging often due to their prohibitive cost or difficulty of use.

Here we present a methodology that provides not only accurate mass assignment to aid in the qualitative identification of ions but also a capability to deconvolute and spectrally resolve mutually interfering ions including isotope labelled internal standards for more accurate quantitation, all by using an easily accessible unit resolution IC/MS system.



Figure 1. Metrohm IC – Agilent MSD iQ System

Experimental

Metrohm Model 940 IC

IC column: Metrosep ASUPP19-150/4mm

Eluent A = 25mM Na_2CO_3 + 0.5mM NaHCO_3

Eluent B = 90% Water + 10% Acetonitrile

Column Flow rate = 0.7 mL/Min

Sequential Chemical Suppression with Tandem Conductivity and Mass Spectrometer detection

Agilent iQMSD

Nitrogen Gas flow = 10L/Min (Peak Scientific)

Nitrogen Gas Temperature = 325°C

ESI- ionization with OpenLab CDS 2.8 acquiring full scan profile mode MS data in m/z range 30-125 with 0.1 Da spacing (31.0 Min run time)

Experimental

Concentration and Accurate Mass Standards

10ppm H_2SO_4 and HClO_4 : injected as #3-8 in the sequence

20ppm H_2SO_4 and HClO_4 : injected as #9-13 in the sequence

Test Water Sample with ^{18}O Enriched Perchlorate (5ppb) Added as Internal Standard

Deionized water injected as #1, #2, #14 and #15 in the sequence

MassWorks Analysis and Deconvolution Software

MassWorks software from Cerno Bioscience is used to directly read the OpenLab CDS data and perform the post acquisition data analysis, both for the accurate mass calibration and ion signal deconvolution. By using a calibration standard, which may contain one or more standard ions to cover the m/z range of interest, MassWorks calibrates MS peak shape into a mathematically exact function allowing for much improved mass accuracy from 0.x to 0.00x Da while achieving 99% spectral accuracy.

As shown in Figure 2 below, in this case, the HSO_4^- standard ion (m/z 97) at 20ppm from Injection #9 is used as the sole calibration standard for calibration. Notably, the A+2 isotope of HSO_4^- , with its pronounced signature ^{34}S signal will overlap with the monoisotope of HClO_4^- (m/z 99), providing an ideal test for accurate spectral deconvolution.

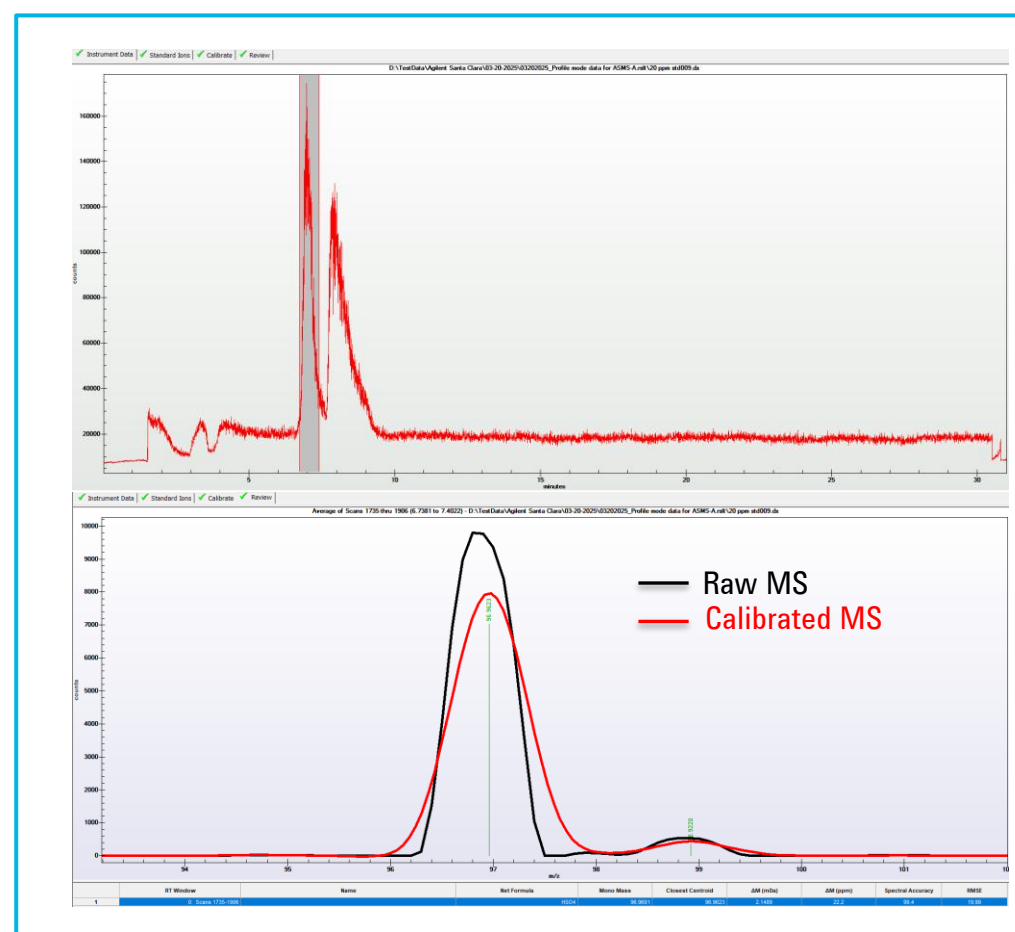


Figure 2. Cerno MassWorks Calibration with HSO_4^- (Injection #9, 20ppm)

Results and Discussion

Accurate Mass and Spectral Accuracy Evaluation of Spiked Standards across Different Concentrations and Injections

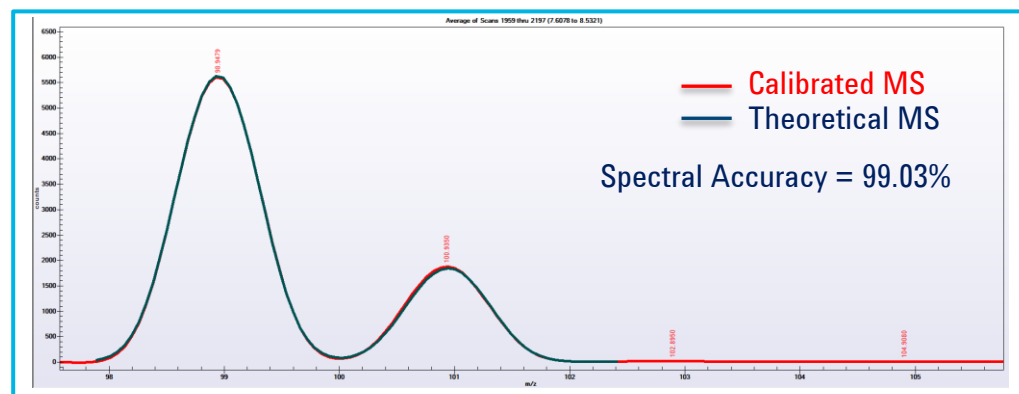


Figure 3. Accurate mass and Spectral Accuracy for perchlorate ion ClO_4^- (Injection #10, 20ppm)

Calibration accuracy and stability from injection to injection

Using the 20ppm HSO_4^- from Injection #9 as the standard to calibrate other ions including ClO_4^- from the same injection and both HSO_4^- and ClO_4^- from other injections, we tested both the calibration mass accuracy, spectral accuracy, and injection-to-injection stability throughout the whole experiment. All mass errors are found to be $<0.006\text{Da}$ with standard deviation $<0.002\text{Da}$ and all spectral accuracy $>98.5\%$.

Table 1. Accurate Mass and Spectral Accuracy for Both Standards at 10 and 20ppm over Different Injections

Injection #	Spiked-in Conc (ppm)	HSO_4^-				ClO_4^-			
		Exact Mass (Da)	Accurate Mass (Da)	Mass Error (Da)	Spectral Accuracy (%)	Exact Mass (Da)	Accurate Mass (Da)	Mass Error (Da)	Spectral Accuracy (%)
3	10	96.9601	96.9630	0.0029	99.17	98.9491	98.9509	0.0018	99.04
4	10	96.9601	96.9630	0.0029	99.25	98.9491	98.9475	-0.0016	99.19
5	10	96.9601	96.9635	0.0034	99.32	98.9491	98.9507	0.0016	98.68
6	10	96.9601	96.9639	0.0038	99.19	98.9491	98.9507	0.0016	98.52
7	10	96.9601	96.9646	0.0045	99.28	98.9491	98.9479	-0.0012	98.63
8	10	96.9601	96.9655	0.0054	99.36	98.9491	98.9473	-0.0018	99.03
Average		96.9601	96.9639	0.0038	99.26	98.9491	98.9492	0.0001	98.85
Std Dev		0.0000	0.0010	0.0010	0.07	0.0000	0.0018	0.0018	0.27
9	20	96.9601	96.9619	0.0018	99.32	98.9491	98.9476	-0.0015	98.79
10	20	96.9601	96.9624	0.0023	99.19	98.9491	98.9479	-0.0012	99.03
11	20	96.9601	96.9653	0.0052	99.31	98.9491	98.9493	0.0002	98.87
12	20	96.9601	96.9627	0.0026	99.25	98.9491	98.9468	-0.0023	99.07
13	20	96.9601	96.9662	0.0061	99.25	98.9491	98.9485	-0.0006	98.88
Average		96.9601	96.9637	0.0036	99.26	98.9491	98.9480	-0.0011	98.93
Std Dev		0.0000	0.0019	0.0019	0.05	0.0000	0.0009	0.0009	0.12

Various Forms of ^{18}O Enriched Perchlorate, Their Identification and Abundance Distribution

When analyzing deionized water (injection #1) with ^{18}O enriched perchlorate, the ^{18}O enriched perchlorate was found to contain more than just $\text{Cl}^{18}\text{O}_4^-$, which could account for only 83.78% of the spectral signal as shown in Figure 4.

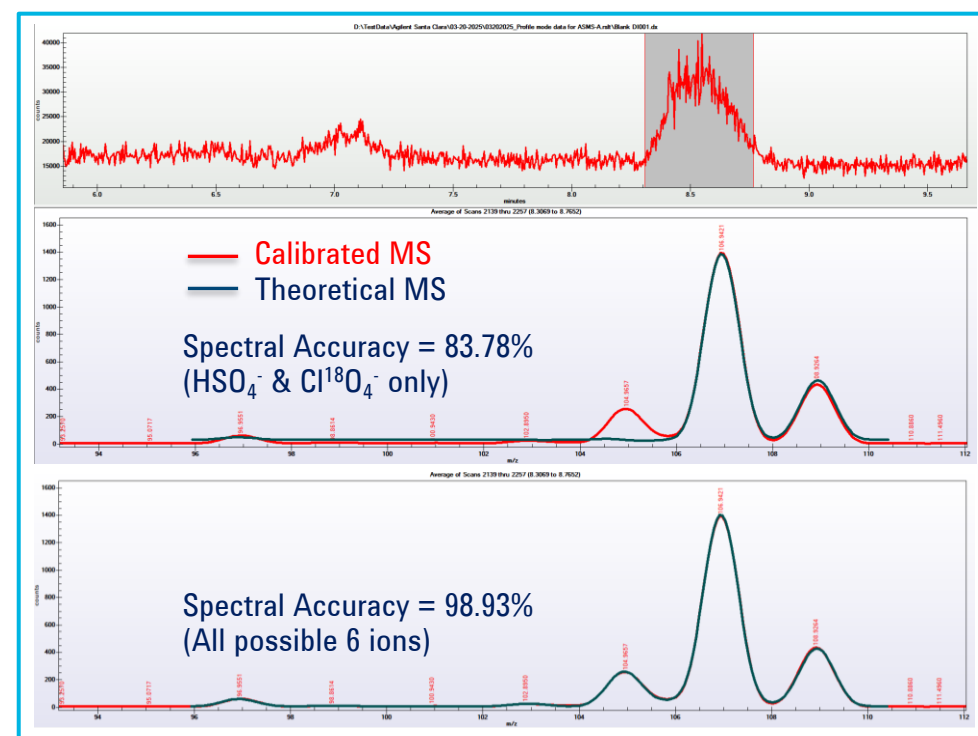


Figure 4. Full Spectral Analysis on All Possible Ions

Spectral Accuracy Ion Series Mixture Analysis

The lack of spectral accuracy, particularly the lack of spectral fit, suggests the presence of other possible spectrally interfering ions, which is corroborated by the large and systematic error ($>+20\text{mDa}$) on the accurate mass measurement at m/z 107 (Table 2). With the MS data calibrated for both mass and more importantly spectral accuracy, it is now feasible to consider these possible additional ion forms to see if the actual MS data would support the interpretation, through ion series or mixture analysis involving a total of 6 possible ions in this case.

Formula Determination / Mixture Analysis by Spectral Accuracy				
Profile Mass Range (Da)		Ion Series		
Start:	End:	Repeat Unit	Min	Max
-1.00	13.50	1 ClO4	1	1
		2 Cl[18O]4	1	1
		3 ClO[18O]3	1	1
		4 ClO2[18O]2	1	1
		5 ClO[18O]3H	1	1
Interference		0		

Results and Discussion

Perchlorate Concentration in Deionized Water Sample Determined with ^{18}O Enriched Internal Standard

Full mass spectra fit not only allows for the determination of the relative abundances of the various and differently labelled perchlorate internal standard but also the relative abundance of other ions including the perchlorate itself. This makes it possible to determine the absolute concentration of the perchlorate in each injection as shown in Table 2 below. The elevated levels found in later injections (#14 & 15) are likely due to the carry-over from the high concentration 10ppm and 20ppm standard runs. To be exact, however, one needs to be clear about the 5ppb concentration of the spiked-in ^{18}O enriched internal standard: whether it refers to the total of the ^{18}O enriched standard or a specific form. A significant and systematic error may exist due to the difference in the abundances of various ^{18}O enriched forms. The estimated perchlorate concentration reported in Table 2 assumes that the 5ppb concentration be the total of all ^{18}O enriched forms.

Table 2. Accurate Mass Measurement on m/z 107 and Full Spectral Fitting Results with All 6 Possible Ions

Injection #	$\text{Cl}^{18}\text{O}_4^-$				Relative Abundances of Various Ion Forms						ClO_4^- Conc (ppt)
	Exact Mass (Da)	Accurate Mass (Da)	Mass Error (Da)	Spectral Accuracy (%)	HSO_4^-	ClO_4^-	$\text{Cl}^{18}\text{O}_4^-$	$\text{ClO}^{18}\text{O}_3^-$	$\text{ClO}_2^{18}\text{O}_2^-$	$\text{ClO}^{18}\text{O}_3\text{H}^-$	
1	106.9649	106.9421	-0.0228	98.93	0.032	0.001	0.786	0.146	0.012	0.023	5
2	106.9649	106.9431	-0.0218	98.96	0.035	0.003	0.786	0.141	0.011	0.024	16
14	106.9649	106.9362	-0.0287	99.10	0.052	0.069	0.711	0.135	0.011	0.023	392
15	106.9649	106.9399	-0.0250	98.90	0.056	0.022	0.747	0.141	0.011	0.024	119
Average	106.9649	106.9403	-0.0246	98.97							
Std Dev	0.0000	0.0031	0.0031	0.09							

Relative Abundance among Various ^{18}O Enriched Perchlorate Ions across Different Injections

When all ^{18}O enriched perchlorate ions are normalized to 1.000 total abundance and examined across different injections, we found that the relative abundances of all these ions are remarkably consistent from injection to injection, as one would expect for the same internal standard spiked into the deionized water samples. The 81% relative abundance of the dominating form $\text{Cl}^{18}\text{O}_4^-$, the 15% of the $\text{ClO}^{18}\text{O}_3^-$, and the remaining ion forms are believed to reflect the actual ^{18}O enriched standard used in this experiment.

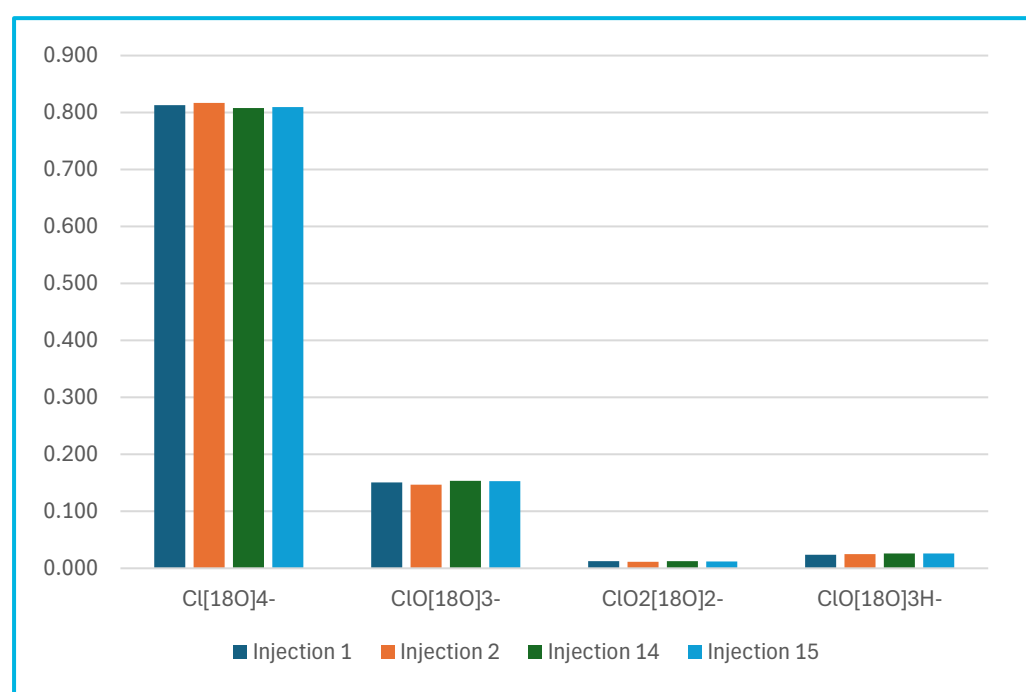


Figure 5. Precise Relative Abundance Determination for ^{18}O Enriched Ions across Different Injections

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Conclusions

Accurate Mass and Spectral Accuracy on IC-MS Easily Achievable and Useful

On an existing unit mass resolution MS

- Accurate mass can help identify the correct ion and the presence of possible ion interferences
- Spectral accuracy allows for full spectral fitting analysis by considering all possible ions present, including various and differently labelled internal standard
- Accurate quantitation through labelled internal standard can be achieved through spectral deconvolution

References

1. EPA Document #: EPA/600/R-05/049, Method 332.0, Determination of Perchlorate in Drinking Water by Ion Chromatography with Suppressed Conductivity and Electro spray Ionization Mass Spectrometry, Rev 1.0, March 2005
2. Wang, Y.; Gu, M., The Concept of Spectral Accuracy for Mass Spectrometry, Anal. Chem., 2010, 82, 17, 7055.