

How to Get the Best Mass Accuracies with HR-Cl on the Pegasus® GC-HRT

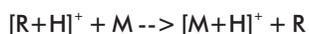
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Introduction

A chemical ionization (CI) source is available for the Pegasus GC-HRT. The combination of CI and a high resolution GC-HRT mass spectrometer (HR-Cl) provides a powerful tool for the generation and analysis of pseudo-molecular ions and molecular adducts for analytes that do not readily produce molecular ions by electron ionization (EI). This document will provide general operating guidelines for obtaining the best possible mass accuracies using HR-Cl on the Pegasus GC-HRT.

CI Mechanism and CI Reagent Gas Ionization Coverage

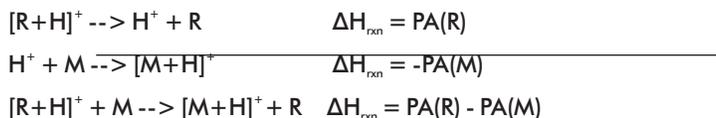
Chemical ionization generates analyte ions by a series of ion/molecule reactions between reagent CI gas ions and neutral analyte molecules. The most common reaction type in CI is a proton transfer reaction to create the $[M+H]^+$ ion. The following reaction is an example of a generic proton transfer reaction between reagent gas R and analyte M:



The thermodynamics of this reaction are driven by the proton affinity of the analyte—PA(M)—and the proton affinity of the reagent gas—PA(R). In general, the proton affinity for analyte M is the ΔH_{rxn} for the following reaction:



The proton transfer reaction can be written as the sum of the protonated reagent gas losing a proton and the neutral analyte gaining a proton:



If the PA(M) is greater than the PA(R), then the proton transfer reaction will be exothermic and will occur inside the CI source. The difference between the two proton affinities is also important. The excess energy created in the exothermic reaction is mostly transferred to the analyte ion as internal energy, which can cause fragmentation of the analyte ion. The larger the difference in the proton affinities of the reagent gas and the analyte (i.e. $PA(M) \gg PA(R)$), the more internal energy that is transferred to the analyte ion and the more fragmentation that is observed. Conversely, if the PA(M) is less than the PA(R), the reaction is endothermic and will not occur inside the CI source.

Because of the relationship between PA(M) and PA(R), the choice of reagent gas determines whether adduct formation occurs (exothermic process) and governs the amount of observed fragmentation in the ion source. Reagent gases with lower PA will ionize more compounds, but they will also provide more fragmentation. Reagent gases with higher PA will provide softer ionization with less fragmentation, but they will not ionize as many compounds. The Pegasus GC-HRT provides the following four choices for CI reagent gas:

- Methane: PA = 127 kcal/mol
- Isobutane: PA = 195 kcal/mol
- Ammonia: PA = 207 kcal/mol
- Other: User customizable

CI Reagent Gas Selection

LECO recommends the following procedure to select the appropriate CI reagent gas for your application. Start with methane gas because it has a low PA and will ionize the most compounds. If there is too much fragmentation of the compounds of interest with methane CI, then switch to a reagent gas with higher PA that will provide softer ionization—*isobutane* or *ammonia*.

- Notes:
- *Isobutane will not ionize any compounds with a PA < 195 kcal/mol, and ammonia will not ionize any compounds with a PA < 207 kcal/mol.*
 - *Methane is a CI reagent gas that will ionize saturated hydrocarbons.*

If methane, isobutane, and ammonia are not sufficient for your application, there is the option of using a custom CI reagent gas. Before using a custom reagent gas, contact your LECO Service Representative to ensure the gas is compatible with the Pegasus GC-HRT, and it is also your responsibility to ensure all Local and National Safety Codes and Regulations are followed.



Mass Accuracy on the Pegasus GC-HRT with HR-Cl (Internal vs External Calibration)

When acquiring EI data on the Pegasus GC-HRT, it is recommended that PFTBA be infused during the run. The consistent background ions from PFTBA provide two benefits to improving the mass accuracy of the Pegasus GC-HRT. First, it provides an internal calibrant that can be used for mass calibration in the data processing method. Second, it provides ions that are used for drift correction by the software during acquisition. When acquiring HR-Cl data with methane as the Cl reagent gas, it is also recommended that PFTBA be infused during the acquisition because the same two benefits will be realized.

When isobutane and ammonia are used as the Cl reagent gas, internal mass calibration and drift correction with PFTBA is not possible because these two Cl reagent gases will not ionize PFTBA. When isobutane or ammonia are used as Cl reagent gases, the instrument will be tuned and mass calibrated without reagent gas added to the ion source, causing the PFTBA to be ionized by the EI mechanism in the Cl source. During a sample acquisition with isobutane or ammonia as the Cl reagent gas, there will not be any PFTBA present in the background to be used for internal mass calibration in the data processing method. Therefore, in order to use internal mass calibration with isobutane or ammonia as the Cl reagent gas, two or more ions from the chromatographic peaks of known compounds from the sample can be used to build the mass calibration matrix.

Recommended Low Acquisition m/z for each Cl Reagent Gas

In chemical ionization not all of the reagent Cl gas ions that are generated are used up in the ion/molecule reactions which create the analyte ions. The excess Cl reagent gas ions are extracted from the ion source along with the analyte ions because both are positively charged. The signals for the Cl reagent gas ions are generally low m/z and very high intensity. Therefore, the low m/z used in the Cl mass spectrometer method is important to ensure that the high-intensity Cl reagent gas ions are deflected away to avoid exposing the ion detector to high intensity Cl reagent gas ions. The following table lists the recommended low m/z for acquisition depending on which Cl reagent gas is used.

Cl Reagent Gas	Recommended Low m/z for Acquisition
Methane	≥ 50
Isobutane	$= 65$
Ammonia	$= 45$
Other	Varies—depending on the custom gas used

As mentioned above in "Mass Accuracy on the Pegasus GC-HRT with HR-Cl (Internal vs External Calibration)", having persistent background ions during sample acquisition will improve mass accuracy through drift correction in the software. In EI and methane Cl acquisition, PFTBA can be infused during the entire run to provide the persistent background ions. In the table above the low m/z for acquisition in methane Cl can be anything $\geq m/z 50$. For isobutane and ammonia Cl, the recommended low m/z for acquisition is 65 and 45, respectively, for all high resolution acquisitions. These low m/z for acquisition will allow deflection of high-intensity Cl reagent gas ions, but will also allow other larger m/z, lower intensity Cl reagent gas ions to be collected. These lower intensity Cl reagent gas ions can be used for drift correction in the absence of PFTBA ions that will not be generated by isobutane or ammonia Cl.

Acquisitions in Ultra High Resolution (UHR) mode have a limited mass range where the largest acquired m/z is a function of the low m/z. Therefore, the low m/z recommendations for isobutane and ammonia acquisitions from the table above might not apply depending on the desired highest acquired m/z. In UHR mode where no Cl reagent gas ions are in the acquisition mass range, there might not be any persistent background ions for drift correction. In this case, the best mass accuracy will be achieved by using two or more known chromatographic compounds to build a mass calibration matrix that can be used for post-acquisition mass calibration in the data processing method.

