

Automatic Drift Correction for High Resolution Mass Spectrometry

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Introduction

The most important property of the high resolution mass spectrometers is their ability to accurately measure mass to charge ratio of the ions in mass spectrum. Sufficient mass accuracy allows the unique assignment of elemental formula further progressing an analyte's identification. The plot in Figure 1 demonstrates an example of dependency of the number of possibly assigned elemental compositions from the measured mass accuracy available on the particular instrument and sample. It is quite obvious that lower (i.e. better) mass accuracy values dramatically reduce number of possible hits and narrow down selection for the accurate analyte assignment.

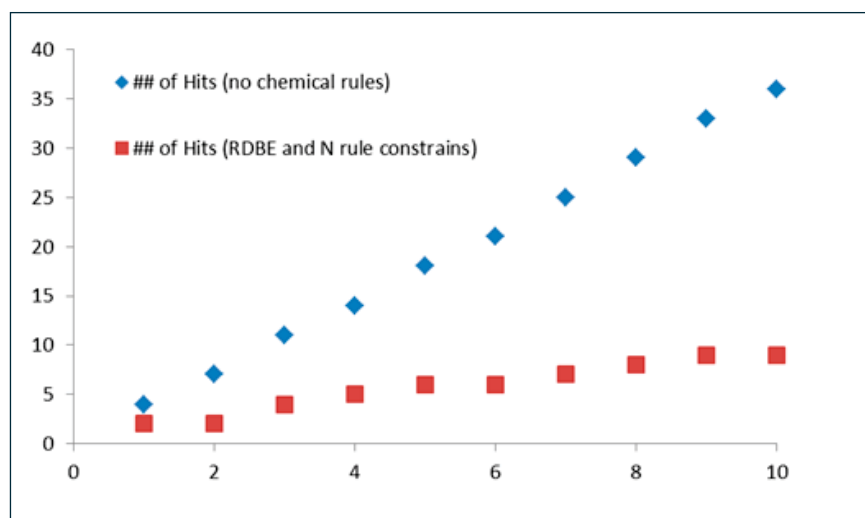


Figure 1. Example of the number of possible elemental compositions of the compound with $m/z=276.09327$ vs mass error tolerance ($C_cH_hO_oN_nS_sP_p$, $c=1-30$, $h=1-30$, $o=0-8$, $n=0-5$, $s=0-4$, $p=0-2$).

Calculations of the Mass Accuracy

The mass accuracy is usually measured in the units of millidaltons (mDa) or parts-per-million (ppm). The following formulae are used for mass accuracy calculations:

$$MA=(M_m-M_t)*1000, \text{ [mDa]} \quad (1)$$

$$MA=((M_m-M_t)/ M_t)*10^6, \text{ [ppm]}, \quad (2)$$

where M_m - measured m/z of the ion of interest, M_t - theoretically calculated m/z based on the elemental composition of the ion of interest.

For example, the molecular ion in mass spectrum of benzo(ghi)perylene (see Figure 2) is showing a measured value of m/z $M_m=276.09327$. The theoretically calculated value for $C_{22}H_{12}^+$ is $M_t=276.09335$ (using ChromaTOF-HRT Mass Calculator tool). The resulted mass accuracy calculated using formulae (1) and (2), correspondingly, is -0.08 mDa or -0.29 ppm.

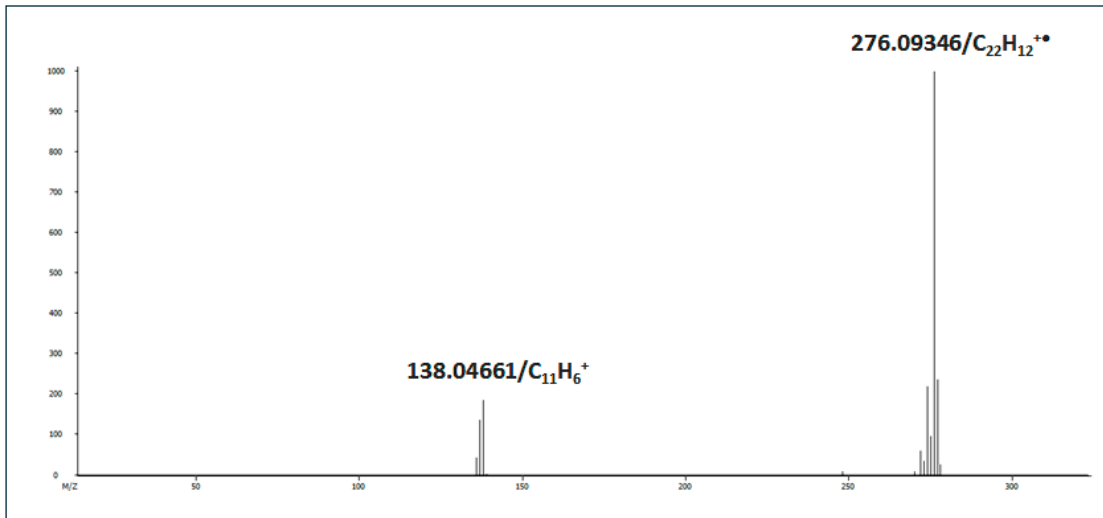


Figure 2. The mass spectrum of benzo(ghi)perylene ($C_{22}H_{14}$) acquired on GC-HRT.

Sources of Mass Accuracy Errors

There are several main sources of mass measurement errors: Mass Spectrometer resolving power; Mass Calibration errors; time-of-flight (TOF) drift of the ions during sample run and between the runs; ion peaks TOF shifts due to signal saturation; and poor statistics and thus increased uncertainty of the ion peaks TOF measurements from low ion signal.

This paper will be focused on improving mass accuracy of the data by addressing the ion TOF drift and by using the methods of automatic drift correction.

Mass Calibration Procedures

The very first step to achieving good mass accuracy is providing adequate mass calibration. The mass calibration is performed using information about known ions in mass spectrum of the calibration compound, which is Perfluorobutylamine (PFTBA, $C_{12}F_{27}N$, Figure 3). The TOF – m/z pairs data from the known PFTBA ions in the acquired mass spectrum are fitted with second-order polynomial function to define the mass calibration function coefficients C0, C1 and C2 (Figure 4).

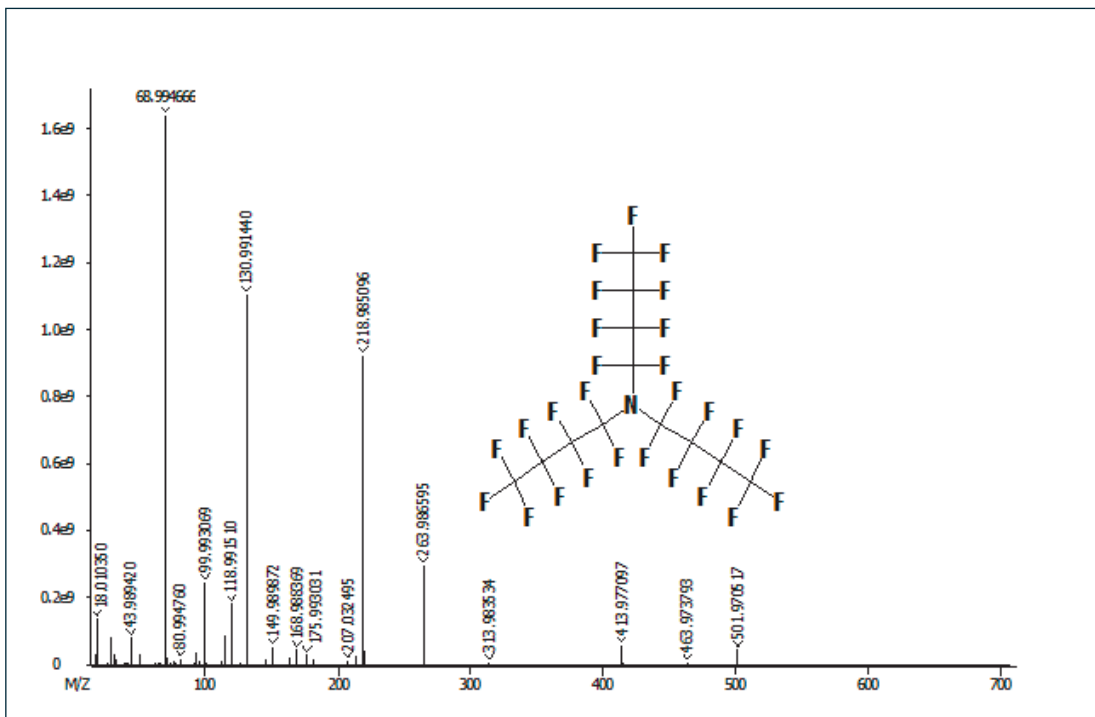


Figure 3. PFTBA electron ionization mass spectrum, obtained on GC-HRT.

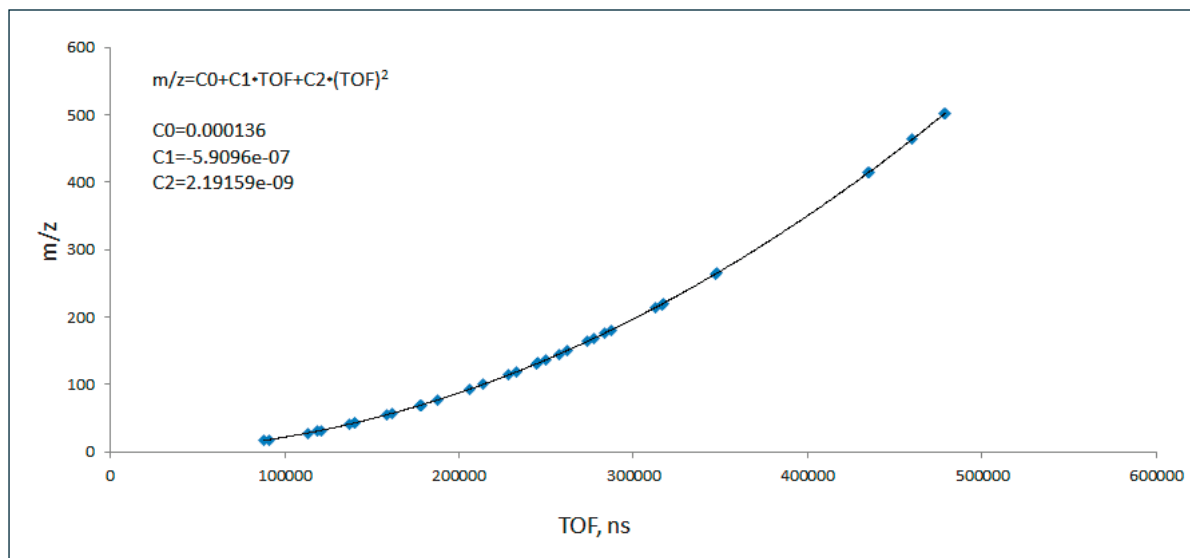


Figure 4. Plot m/z vs TOF for the calibrant (PFTBA) ions and the second order polynomial curve fitting the data. The calibration function $m/z=f(TOF)$ is defined from the second-order polynomial curve fit.

The external mass calibration can be performed as the automated Mass Calibration routine before starting the analysis. The mass calibration coefficients will be then stored with the corresponding MS method and automatically applied during data acquisition to convert ion TOFs to their corresponding m/z s.

The internal mass calibration is performed as a part of the Data Processing routine on already collected data. The mass calibration matrix may include any known ion types: PFTBA ions, persistent background ions (chemical background, column bleeds), and sample ions.

The results of the standard (external or internal) calibration routines are presented in the Mass Calibration Table view (Figure 5).

Mass calibration results table

##	Name	Charge	Priority	Formula	Expected M/Z	Observed M/Z	Mass Delta (mDa)	Mass Accuracy (ppm)	Mass Window, Da(+/-)	Resolution	TOF (ns)	Area
1	PFTBA069	1	1	CF3	68.994661	68.99467	0.01	0.07	10	27870	177565	1.649E+09
2	PFTBA119	1	Check Mass	C2F5	118.991467	118.9915	0.04	0.36	0.01	29974	233147	183728640
3	PFTBA131	1	1	C3F5	130.991467	130.9914	-0.03	-0.2	10	30913	244614	1.108E+09
4	PFTBA219	1	1	C4F9	218.98508	218.9851	0.02	0.08	10	33352	316237	929462272
5	PFTBA264	1	2	C5F10N	263.986557	263.9866	0.04	0.14	0.1	33741	347200	297344000
6	PFTBA414	1	Check Mass	C8F16N	413.976977	413.9771	0.12	0.29	0.01	34419	434753	57538432
7	PFTBA502	1	2	C9F20N	501.970589	501.9705	-0.07	-0.14	0.1	34954	478720	42075776

Figure 5. Mass Calibration Table with all reported mass accuracy values for the calibrant and check masses well below 1 ppm.

Mass Calibration routine is performed on mass spectra which are summed over a period of time. The mass calibration coefficients are calculated from the averaged TOF data, assuming that TOF changes over that period are minimal. However, if for some reason the TOF of ions is changing significantly (e.g. TOF drift several nanoseconds due to electrical or dimensional changes in the mass analyzer) the standard mass calibration routine will not be enough to achieve satisfactory mass accuracy values (<1 ppm) in the data.

To address this problem, the Data Processing routine includes the **TOF Correction** option, which can be performed on the already collected data. In this routine, the lock mass correction is performed on each spectrum and calibration correction coefficients are saved with the data.

Automatic Drift Correction

The ChromaTOF software provides automatic TOF correction in real time during data acquisition – Automatic Drift Correction (ADC)^[1]. The routine relies on the presence of persistent ion peaks in spectra - background ions and/or ions from the PFTBA calibrant, which is typically co-introduced with the sample. The ADC is a very important feature for providing exceptional mass accuracy in the GC-HRT data (Figure 6). During the real time ADC application, the following steps are performed by ChromaTOF software:

- Generates an on-the-fly list of the detected m/z
- Removing m/z from the list of ion peaks which are considered unacceptable for use with ADC, for example saturated, too low intensity, too low m/z, etc.
- Evaluation of the signal stability of the qualified ion peaks over a certain time period (for example, 15 spectra), where m/z area and peak width variations should be within an acceptable range.
- Initial list of m/z is considered as a reference spectrum.
- After a set time period, a new spectrum is considered and the software looks for m/z's correction pairs. These are pairs of m/z's from the incoming spectrum & the reference spectrum.
- ADC picks the best pairs of ion peaks:
 - Ion peaks with high intensity, most stable areas, and stable widths are chosen preferentially.
 - ADC uses a maximum of 30 ion peak pairs for calculations.
- Each pair of ion peaks (reference and incoming) generates a drift correction slope and the robust weighted (by area) average of these slopes is computed.
- The resulting average is the drift correction.
- This drift correction is then multiplied by the TOFs in the original (unfiltered) spectrum to give the corrected TOFs

When the reference snapshot is taken, it is corrected using the reference snapshot from the previous acquisition. This corrects drift between acquisitions as well as within each acquisition.

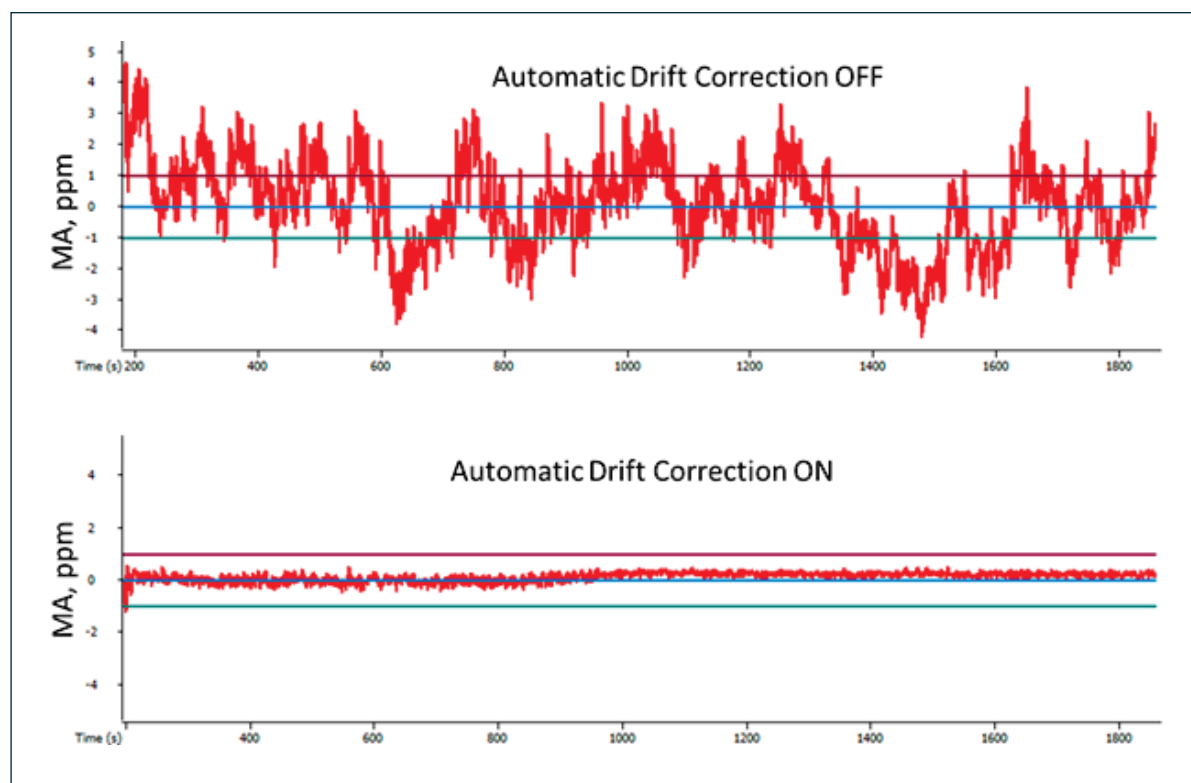


Figure 6. Mass Accuracy for m/z 218.98501 (PFTBA fragment ion, C₄F₉⁺) measured during 30 min acquisition with ADC OFF (top) and ADC ON (bottom).

Examples of the Mass Accuracy improvement while applied Automatic Drift Correction

The importance of the ADC implementations can be illustrated in two following examples.

Example 1. Figure 7 depicts the TIC and $m/z=276$ chromatogram plots of the standard mixture data. The Benzo[ghi]perylene ($C_{22}H_{12}$, $m/z=276.093352$) is the part of this mixture. The data were collected with the ADC ON and ADC OFF and the corresponding mass accuracy plots for the PFTBA fragment ion $C_4F_9^+$ with $m/z=218.985080$ (Figure 8). The PFTBA was co-introduced with the sample during the whole run. The automatic peak finding procedure of the ChromaTOF software has made correct analyte assignment with mass accuracy error 0.04 ppm when using data collected with ADC ON. The data processing of the sample, which was re-acquired with ADC OFF, is resulted in the wrong analyte assignment: the proposed analyte with the minimal mass error of 0.88 ppm was N4,N4-dimethyl-2-(phenylsulfonyl)-1,4-benzenediamine ($C_{14}H_{16}N_2O_2S$, $m/z=276.092700$), and the m/z of the correct analyte (Benzo[ghi]perylene) was off by -3.24 ppm (Figure 9). Thus, ADC implementation helped to reduce the mass accuracy error and allowed for correct analyte assignment.

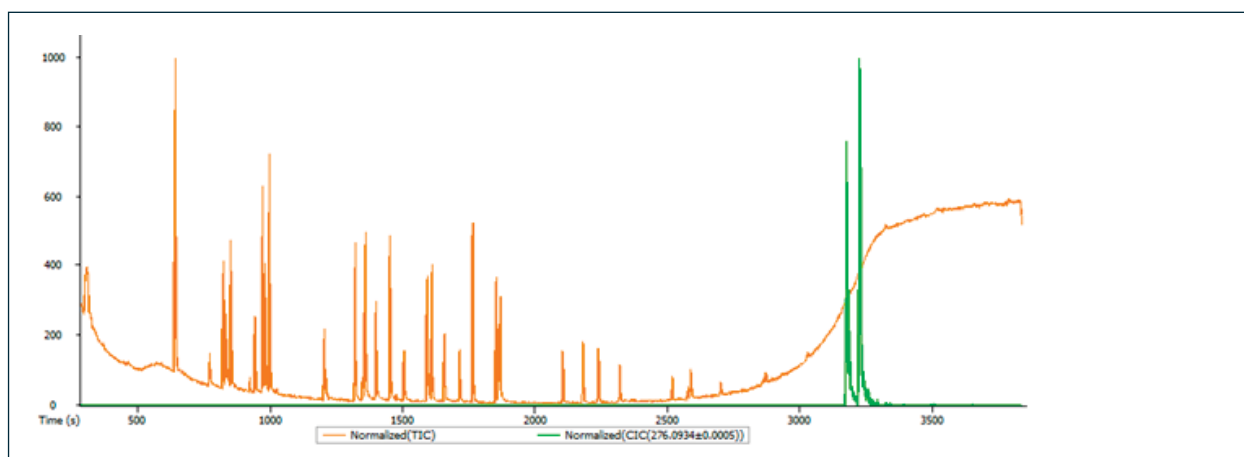


Figure 7. The test mixture chromatograms: TIC – red, $m/z=276.0934$ – green.

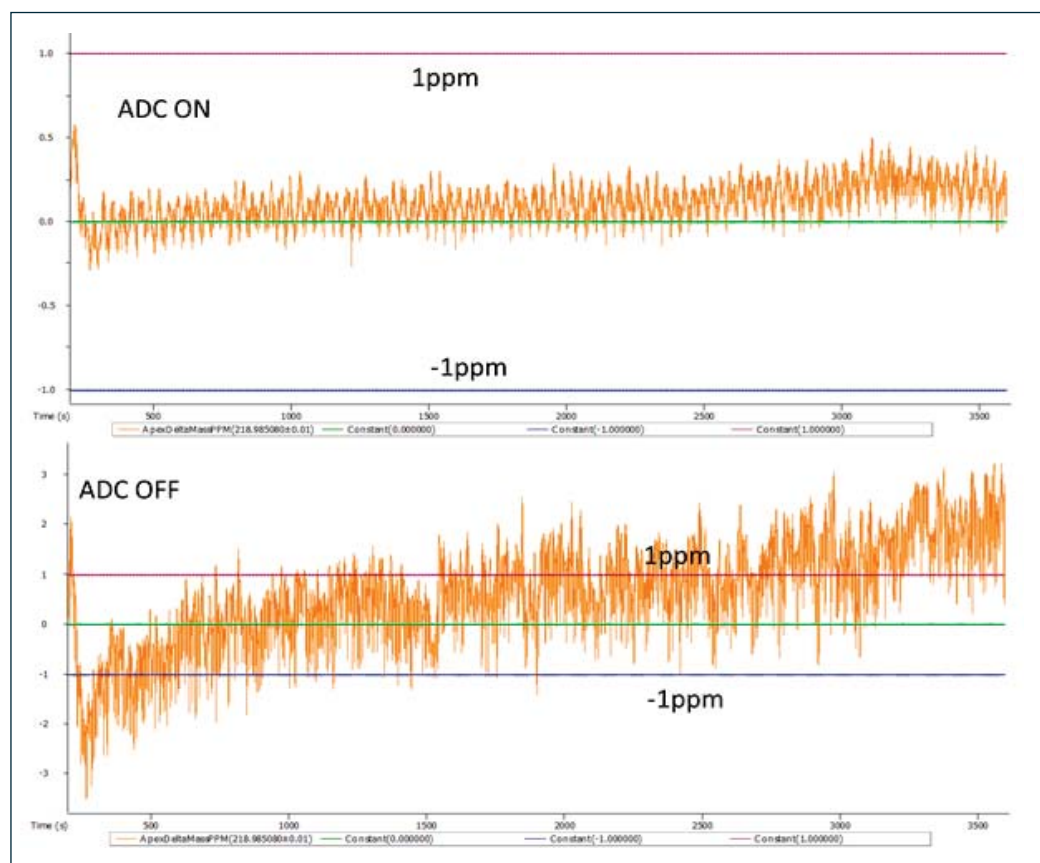


Figure 8. Mass accuracy plots for the PFTBA fragment ion $C_4F_9^+$, while analyzing test mixture with ADC ON (top) and ADC OFF (bottom).

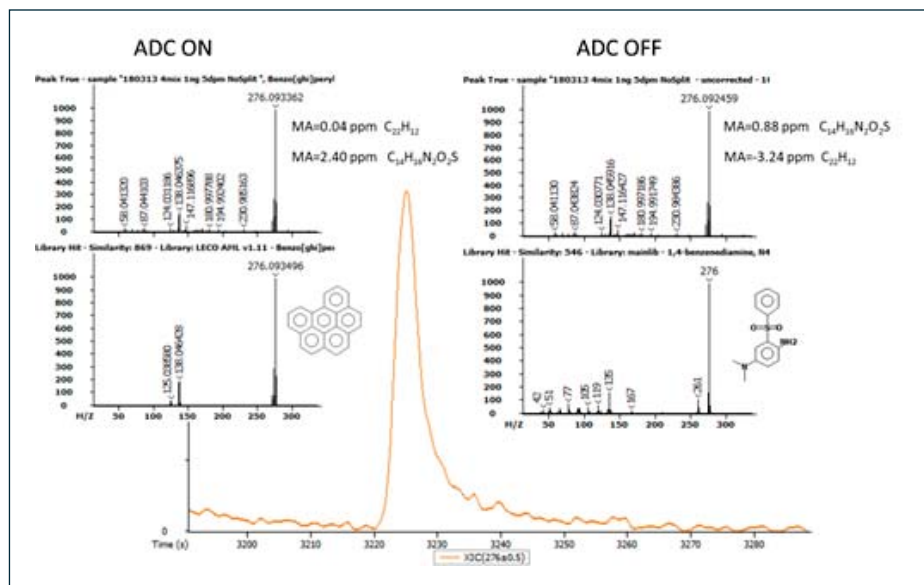


Figure 9. The results of the automatic analyte assignment based on accurate mass formula calculation: correct with ADC ON (left) and wrong with ADC OFF (right).

Example 2. The GC-HRT hardware can still provide in many cases mass accuracy even without ADC implementation, which can be considered good enough for other vendors' high resolution TOFMS. However, higher mass error will reduce user confidence in the automatically reported results. Figure 10 shows the test mixture chromatogram with overlaid $m/z=152.0620$ ion chromatogram, corresponding to the molecular ion of acenaphthylene ($C_{12}H_8$), which is a part of the mixture. The automatic analytes assigning results (Figure 11) are essentially the same with ADC ON or OFF – the acenaphthylene is correctly assigned in the both sets of data if the accepted mass error window is set to 5 ppm. However, if the mass error window is set to be narrower, for example 2 ppm as recommended for analysis of complex mixtures, the automatic peak assignment routine will not even include the correct analytes in the hit list while using accurate mass criteria. And the opportunity to apply high mass accuracy criteria for analyte assignment is the major advantage factor for analysis using high resolution mass spectrometry.

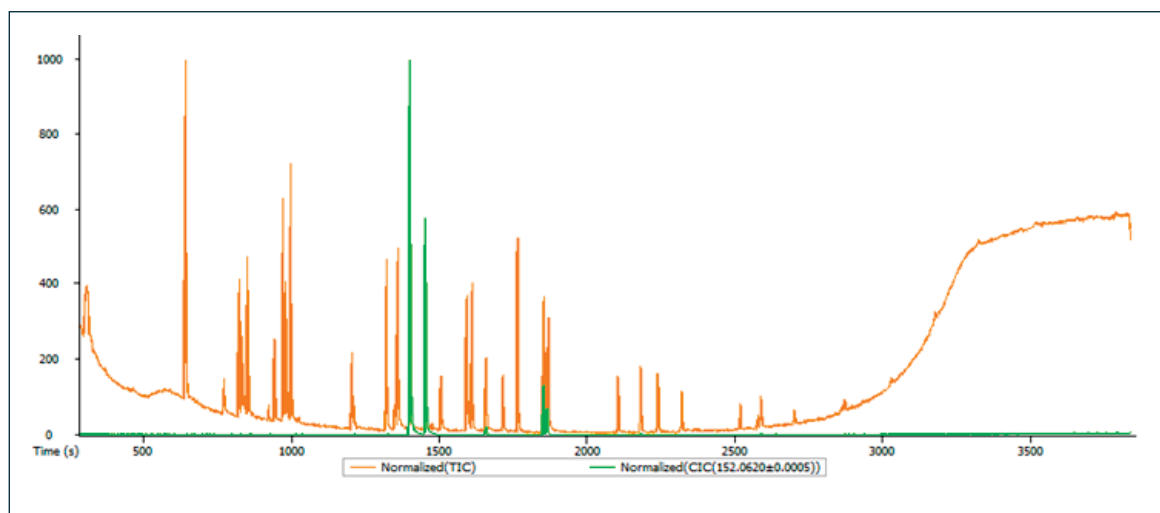


Figure 10. The test mixture chromatograms: TIC – red, $m/z=152.0620$ – green.

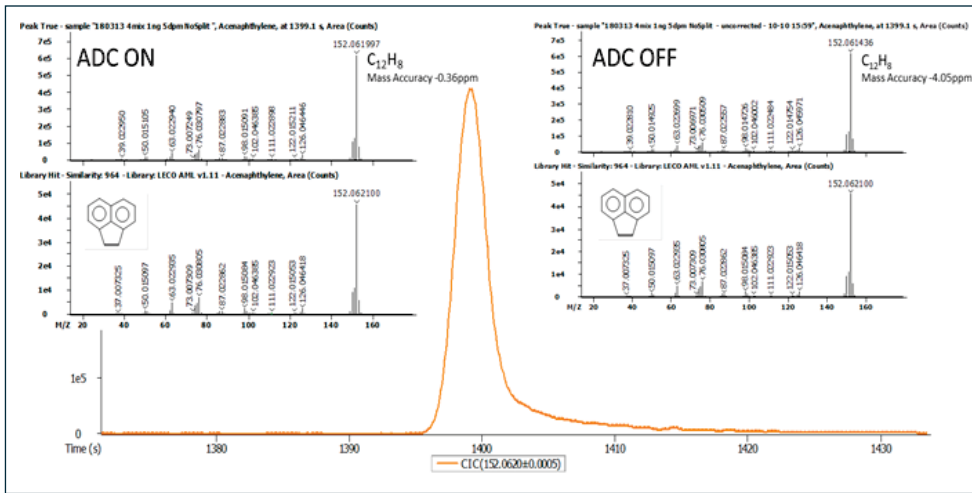


Figure 11. The results of the automatic analyte assignment based on accurate mass formula calculation: mass error -0.36 ppm with ADC ON (left) and mass error -4.05 ppm with ADC OFF (right).

Summary

The Automatic Drift Correction (ADC) routine is always applied in real time during GC-HRT operation. Its implementation allows significantly improved mass accuracy of the data and improved confidence of the analytes assignment using automatic data processing procedures.

References

[1] US Patent 9153424