Agilent Technologies

How Autotune vs. Standard Spectra Tune Can Affect Analyte Ion Ratios using Agilent 5973 Mass Spectrometers

There have been many questions over the years, with the introduction of the 5973 MSD, about the differences between Autotune and the other tunes available in the Agilent Technologies GCMSD SW package. These differences have caused some confusion between older technology and the current system being shipped. Lets first look at what questions may be asked when trying to compare the tunes.

1. The autotune now gives higher mass 219 ratio than older equipment.
2. The 5973 used to tune with mass ratio of 219 at 70%, now it is 102%. (Only an example)
3. Why does my autotune method give mass spectra different from the library and standard spectra tune matches better to the library?

These are just some examples of the questions that have arisen over the last years of introduction.

Lets first look how the macros drive the tune for the different types in the GCMS SW.

Autotune sets the ratios for there maximum abundance across the entire mass range of the tune. Standard Spectra tune has ratio targets for it to achieve for the specific ions that it is looking for. This means that the Autotune has a minimum target but no maximum where as the Standard Spectra Tune has a minimum and a maximum for the ions it is looking for. Let's look at the Tune Wizard for each tune and see what can be set in the software. These are shown in Figure 1through 6 on the next page.

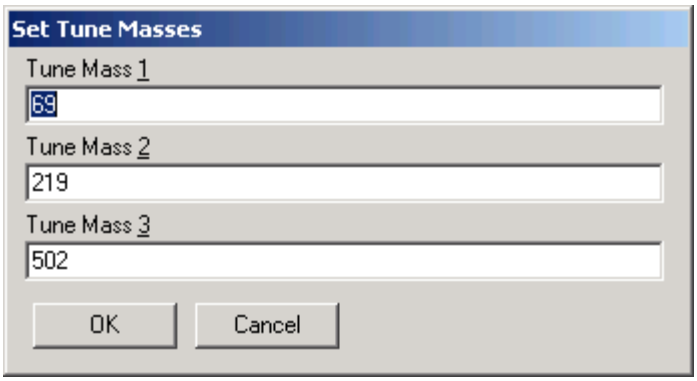

Figure 1 Autotune

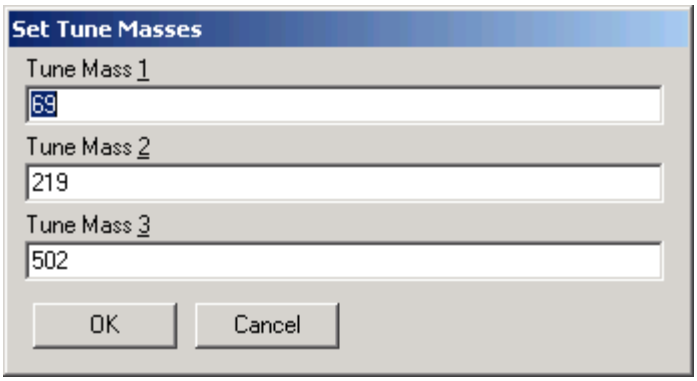

Figure 2 Standard Spectra Tune

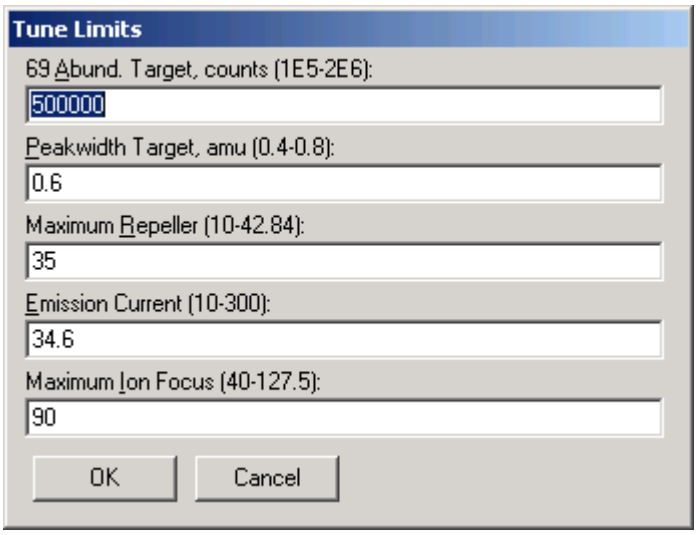

Figure 3 Autotune

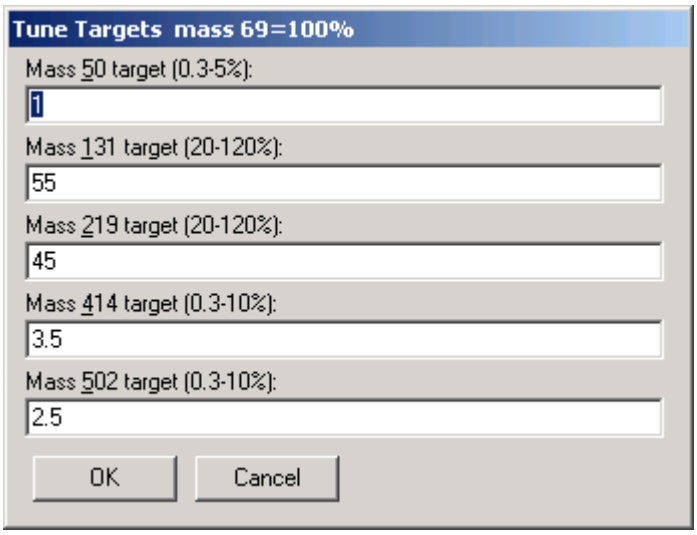

Figure 4 Standard Spectra Tune

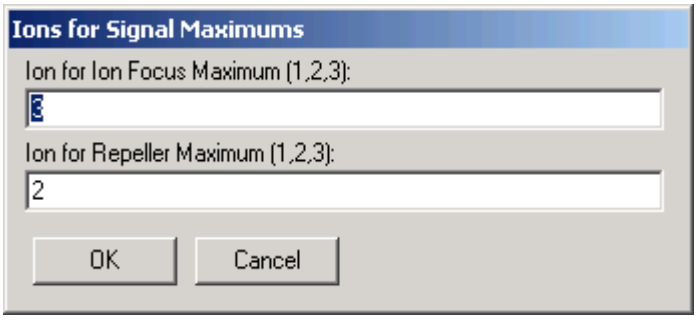

Figure 5 Both Tunes

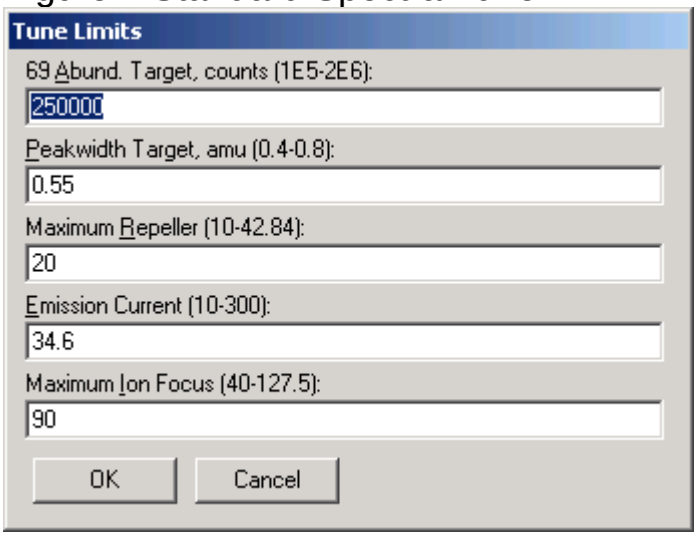

Figure 6 Standard Spectra Tune

As we can see the Tune Wizard's first option (Figure 1 and Figure 2) are exactly the same. They both allow you to set the 3 major tune masses. Typically it will always be 69, 219, and mass 502. Now if we compare the next panel that comes up, you will see that Figure 3 differs from Figure 4 on what it allows you to set. Figure 3 does have the same options as Figure 6 so what does this mean. Figure 4 allows the user to set a target abundance for masses 50, 131, 219, 414, and 502. This then creates an upper and lower limit scenario where as the autotune's macro is hard coded only with lower limits not upper limits. Now Figure 3 and Figure 6 allows the user to set raw abundance of mass 69, peak width, maximum repeller voltage, emission current voltage, and maximum ion focus. As for Figure 5 both tunes have this option. With the extra window in Standard Spectra Tune (Figure 4) we can maintain from day to day or week to week the ratio's of the ions by giving them a target to achieve which in turn gives the software an upper and lower limit to look at.

Now how does this affect the ion ratios of the analytes that the method is acquiring? Let's use same examples of autotune and how it may affect the analysis of the compounds on interest.

Monday Day X

Autotune Results	Analyte Ratios	
69 100%	303 20%	
219 75%	182 70%	(In this example these masses are being
502 5.2%		compared to a target mass of 82)

Monday Day Y

Autotune Results	Analyte Ratios	
69 100%	303 25%	
219 102%	182 120%	(In this example these masses are being
502 9.0%		compared to a target mass of 82)

Now we notice that the ratio of mass 182 compared to mass 82 of the analyte has drastically changed however when we examine the Autotune ratios they are in Agilent specifications. This increase in sensitivity for mass 182 is directly proportional to the gain in abundance for the ratio of mass 219 in the Autotune from Monday Day Y. The

thing to remember with Autotune is there is no upper limit to the tune but a lower limit. Now let's compare the same analyte using Standard Spectra Tune. In this tune we have specified ion ratio targets as listed in Figure 4.

Monday Day X

Standard Spectra Tune Results	Analyte Ratios
69 100%	303 12%
219 47%	182 50% (In this example these masses
502 2.2%	are being compared to a target mass of 82)

Monday Day Y

Standard Spectra Tune Results	Analyte Ratios
69 100%	303 10%
219 44%	182 47% (In this example these masses
502 2.6%	are being compared to a target mass of 82)

Now we can see the data from 2 separate days, and tuning each day, the ratios are closer in relation to each other when comparing subsequent data from different days. This does not mean that the GCMS is having a problem with the autotune, it means that we can control easier what the tune is doing by giving it a target to achieve, and by doing this the macros then can establish an upper and lower limit to work with. This type of tuning criteria should be established by the laboratory, however if the laboratory intends to look at historical data over time, it will be easier for the laboratory to cross correlate data from day to day and instrument to instrument.

This document is intended to give the laboratory personnel the background on how the tuning algorithm works and to determine what tune will be best for the needs of the laboratory.