Agilent Technologies
Innovating the HP Way

Fast Methods Development Software for 1100 Series LC/MSD

Technical Note

1100 Series LC/MDS

May 1998

Introduction

One task in method development is to identify the optimum set of operating parameters for the analysis. The 1100 Series LC/MSD has incorporated software that significantly reduces the time to optimize the mass spectrometry parameters to develop a robust method. This capability allows even novice users to develop good methods.

Traditional approaches using an infusion pump and manual optimization of lens voltages and source operating conditions take considerable skill and mass spectrometry knowledge to get optimum results. The approach uses the HPLC system coupled with flow injection analysis (no column) to accomplish the same task in a fast, automated, and repeatable manner. Novice operators as well as more experienced mass spectrometry operators can both benefit from the reduced time to optimize their instrument conditions.

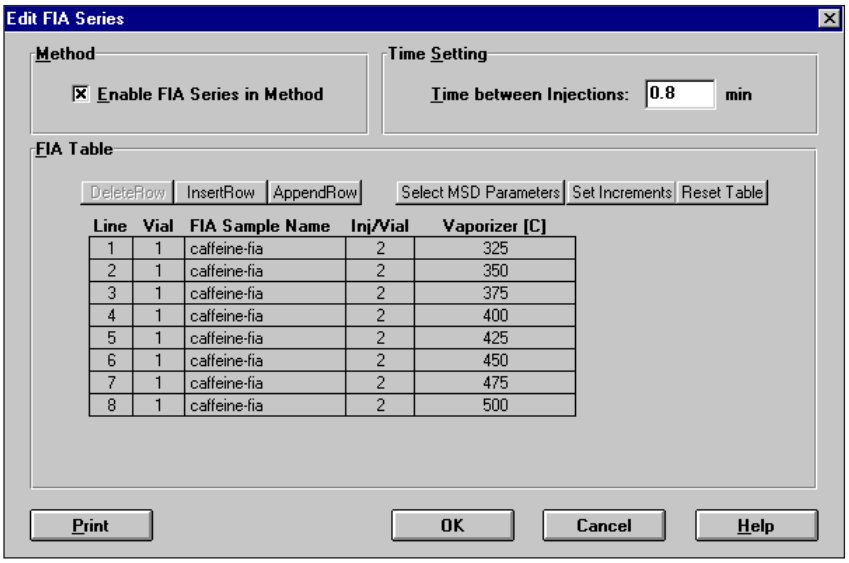

Figure 1. The Edit FIA Series table for specifying the FIA settings.

Design Approach

The 1100 LC/MSD system has been designed to greatly simplify the task of MS instrument parameter optimization. A cool, non-heated capillary de-couples the outer spray chamber region from the mass spectrometer ion optics. Unlike earlier generation LC/MS systems, this design allows the HPLC flow rate conditions to be set independently of the ion optics lens in the high vacuum region of the mass spectrometer. As a consequence, the ion optics does not have to be adjusted for changes in the HPLC flow rate conditions.

Infusion vs. FIA Modes of Operation

Infusion pumps have been used to optimize instrument performance. The major disadvantage is that it does not lend itself to source optimization because infusion is normally a low flow rate technique. Infusion typically requires teeing the infusion pump into a flowing HPLC stream.

The 1100 LC/MSD can be optimized using an infusion approach. However, the use of flow injection analysis (FIA) can achieve the same result quicker, with less sample, and without re-plumbing the system.

Optimization

Typically instrument sensitivity is optimized by three key parameter settings: fragmentor voltage, capillary voltage and (for APCI) vaporizer temperature. The other spray chamber settings (nebulizing gas pressure, drying gas temperature and flow rate) can be set to normal default conditions for an acceptable broad optimum performance. The APCI vaporizer temperature setting is a function of HPLC solvent and flow rate and requires optimization.

The first step is to go to Method and Run Control View and click on edit FIA series. When editing the FIA series, a FIA Series Table is provided to display and record the parameters that were optimized (Figure 1). The time between injections is typically set at 0.8 minutes for applications without a column.

The fragmentor voltage is the first parameter to optimize since it has the greatest impact on sensitivity and fragmentation. The objective is to find the optimum voltage which provides either a strong molecular ion or both a strong molecular ion and good relative abundance of fragment ions. At this point either "autofill" or "append row" features can be used to set the beginning and ending voltages coupled with the increment size.

The FIA data are then collected and stored in a single data file. Typically a single run takes about 10 minutes. The parameter being optimized is automatically annotated above each injection. Extracted ion chromatograms can be displayed so that the optimum setting can be determined for each ion of interest.

When a set voltage compromises the response for different groups of ions, a dynamic fragmentor ramp voltage can be used. In this mode, the fragmentor voltage changes as a function of the mass during scan or selected ion monitoring operation. In this way, different voltages can be applied to optimize response from both molecular and fragment ions.

Typical ranges for the parameter settings are given in Table 1. The fragmentor voltage and APCI vaporizer temperature are the two most frequently optimized parameters since they greatly affect system sensitivity. Next, the capillary voltage (Vcap) and discharge corona current are optimized. Smaller molecules generally optimize at lower Vcap, and larger molecules require higher Vcap. At higher corona current, greater signal is generated but this needs to be balanced with increased background noise at the higher corona current settings. If the HPLC is running at a flow rate below 50 l/min, the source conditions (nebulizing gas pressure and drying gas temperature and pressure) also need to be optimized.

Experimental

This procedure was followed to optimize the pseudo-molecular ion and two fragment ions. The total ion chromatogram and extracted ions (185, 111 m/z) are shown in Figure 2. The best fragmentor voltage setting is 40 volts for mitoguazone since this gives a strong signal for the pseudo-molecular ion (185 m/z) as well as a good response from the fragment ion (111 m/z). Setting the fragmentor at 60 volts, would result in a relatively weak parent ion signal while below 30 volts would result in weak signals for the fragment ions.

Table 1. MS Parameter Settings.

Operating parameter	Recommended Range	Incremental Step	Frequency of Optimization	Note
Fragmentor voltage	30–150 volts	10 volts	Frequent	1
APCI vaporizer temperature	300–500°C	25°C	Frequent	2
Capillary voltage	2500–5000 volts	500 volts	Infrequent	3
APCI corona current	2–10 microamps	1 microamp	Infrequent	4
Drying gas temperature	150–350°C	50°C	Flow rates below 50 µl/min	5
Drying gas flow rate	3–13 L/min	1 L/min	Flow rates below 50 µl/min	6
Nebulizing gas pressure	10–60 psig	5 psig	Flow rates below 50 µl/min	7

1. Fragmentor voltage is compound dependent. Rugged compounds require higher voltage settings.
2. APCI vaporizer temperature is dependent upon solvents and flow rates. Aqueous solvents and higher flow rates require more heat (greater temperature).
3. Normally broad optimum for most compounds. Sensitivity of lower molecular weight compounds may be optimized with lower Vcap settings. Sensitivity of higher molecular weight compounds may be optimized at higher Vcap settings.
4. Normally broad optimum for most compounds. Negative ion APCI consult on-line help for solution chemistry tips.
5. Preferred setting of 350°C. Lower temperature required for flow rates below 50 µl/min.
6. Default setting of 10 L/min. Less drying gas required for flow rates below 50 µl/min. See on-line help.
7. Default setting of 25 psi. Lower setting for flow rates below 50 µl/min. See on-line help.

Next, the same approach was employed for the capillary voltage. In Figure 3. capillary voltage is optimal at 4000 volts. Note that in this example, there is relatively little difference between 3000 and 6000 volts.

APCI optimization was also performed for caffeine. The HPLC conditions were 0.600 µl/min of 50/50 methanol/water. The APCI vaporizer temperature was started at 325°C and was increased in 25°C increments to a final value of 500°C. Double injections were done for each vaporizer temperature since there is a small lag time to stabilize the vaporizer temperature. The optimal APCI vaporizer temperature was 500°C (Figure 4). The overall signal response grew progressively stronger with increasing temperature since caffeine is a very thermally stable and volatile molecule, which benefit from higher vaporizer temperatures.

The APCI corona discharge current (Figure 5) was optimized starting at 1 µamp and increasing in 1 µamp steps to 10 µamps. The optimal corona current was 2 µamp. Note that by increasing the corona discharge current for caffeine, there was a slight decline in overall signal. Other compounds sometimes show a more dramatic response to changes in corona current.

FIA can be set up to use a standard column switching valve to switch between normal HPLC mode and FIA optimization mode without need for extra connections, system flushing, and HPLC hook up.

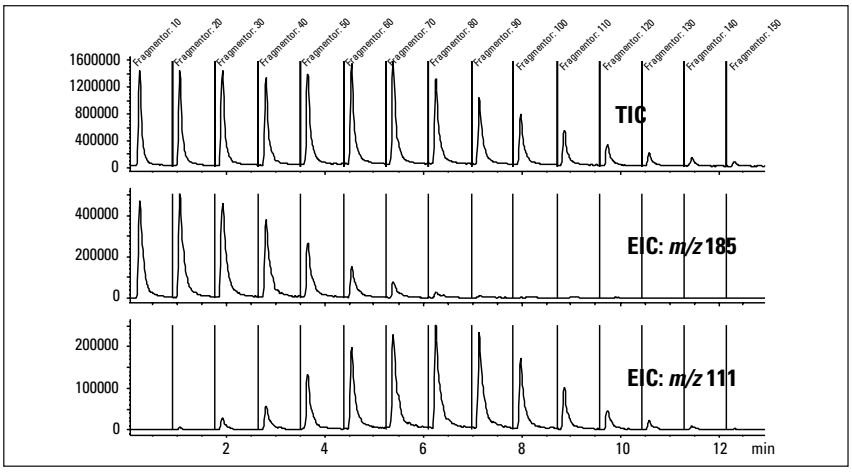

Figure 2. Optimization of fragmentor voltage for the pseudomolecular ion and a fragment ion of mitoguazone.

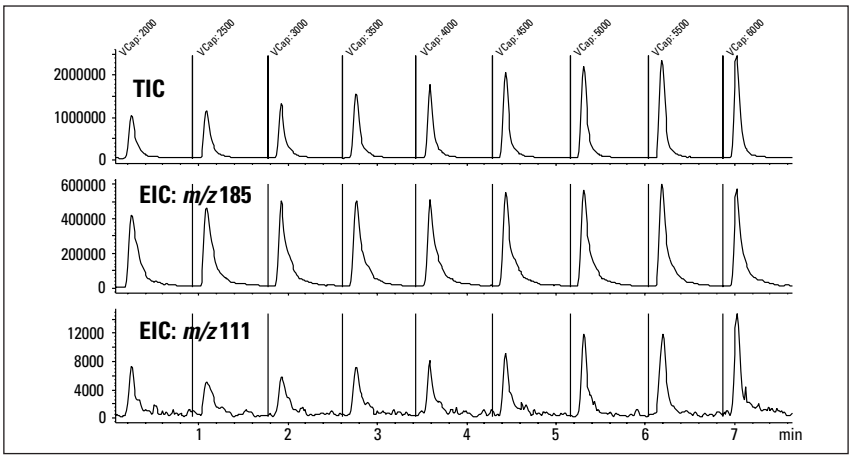

Figure 3. Optimization of capillary voltage for the pseudomolecular ion and a fragment ion of mitoguazone.

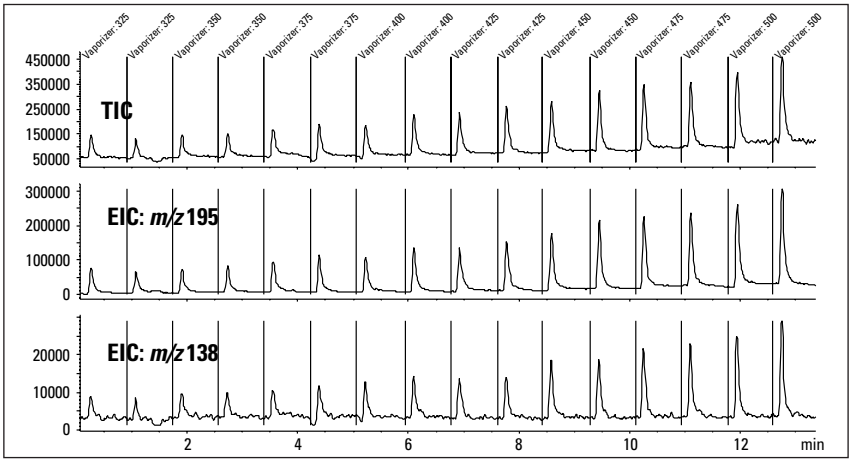

Figure 4. Optimization of vaporizer temperature for the pseudomolecular ion and a fragment ion of caffeine.

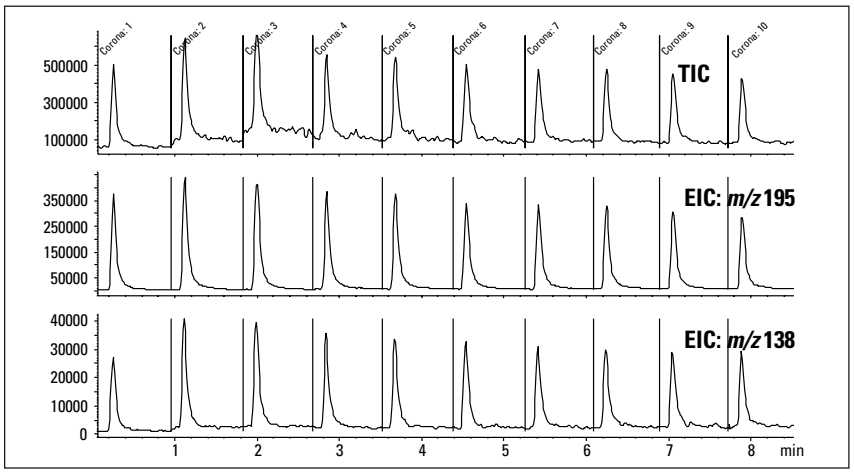

Figure 5. Optimization of corona current for the pseudomolecular ion and a fragment ion of caffeine.

Conclusion

FIA optimization is much faster than infusion since FIA provides automation of the optimization steps.

Single data files containing multiple injections are used to visually select optimum parameters. The single data file offers the convenience of tracking and archival of the parameter optimization.

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