Benefits of API Temperature Programmable Drying Gas in LC/MS and LC/MS/MS Analysis

Inspiring Excellence

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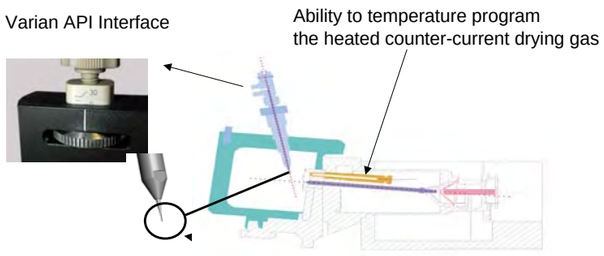
Introduction

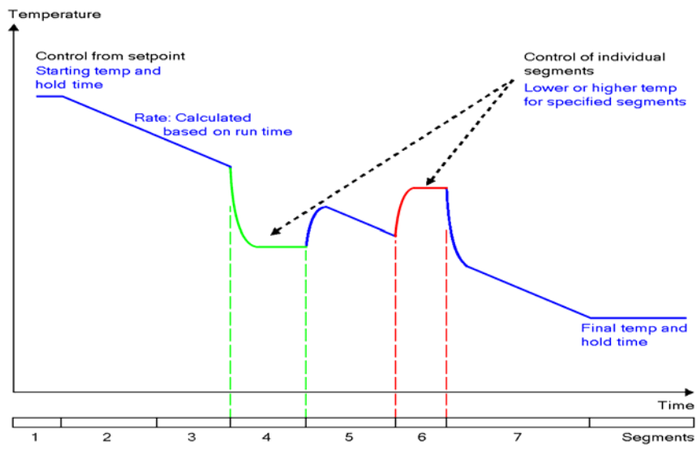
LC/MS and LC/MS/MS techniques require careful optimization of all API source conditions in order to obtain the best sensitivity and increase the robustness of LC/MS methods. Typical parameters that are adjusted in the electrospray ionization technique (ESI) include the flow rate and temperature of the drying gas used to evaporate the HPLC effluent. Traditionally, an operator is only able to set the drying gas temperature isothermally to minimize sample contamination and carryover and insure that the electrospray droplets are desolvated effectively.

A new feature added exclusively to Varian mass spectrometer products is SelecTemp™, the ability to temperature program the API drying gas during a gradient HPLC separation. Most separations start out highly aqueous. As the SelecTemp program progresses, more organic phase is mixed in, thus the need for a high drying gas temperature should decrease throughout the gradient program. Constant elevated temperatures may result in excess heating of the electrospray droplets and will cause destruction of thermally labile compounds. SelecTemp also allows the operator to specify a drying gas temperature for an individual mass analysis segment in an analytical run.

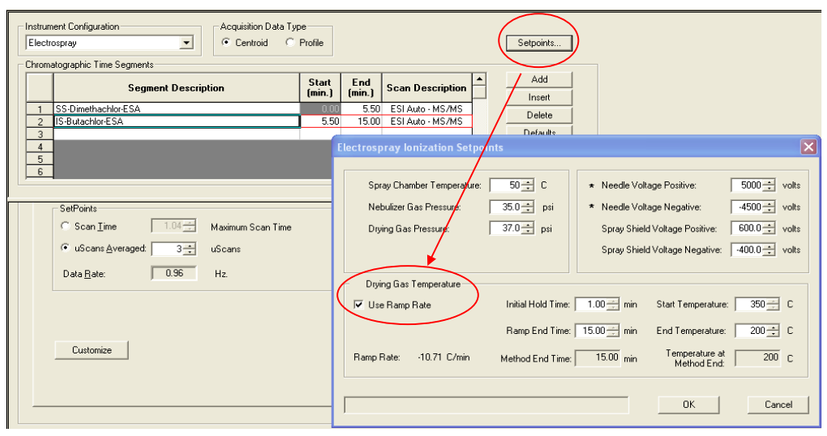


Adjustment of the inner capillary needle
to maximize nebulization efficiency

As seen from the profile below, SelecTemp can be used to gradually reduce the temperature of the drying gas throughout the entire gradient program and/or control individual segments. The rate at which the ramping takes place is calculated based upon the run time of the analysis.



Operator programs the segments easily using the Method Editor program in the software



Results and Discussion

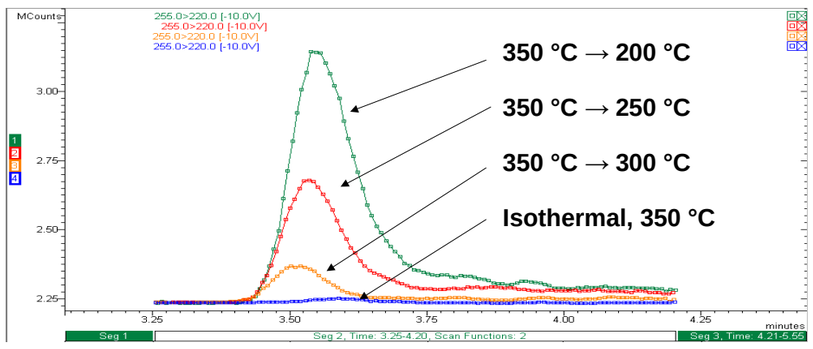


Figure 1: 3-Hydroxycarbofuran ammonium adduct using SelecTemp™.

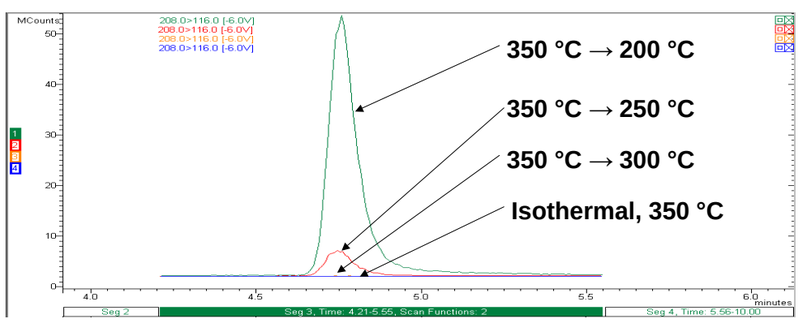


Figure 2: Aldicarb ammonium adduct using SelecTemp™.

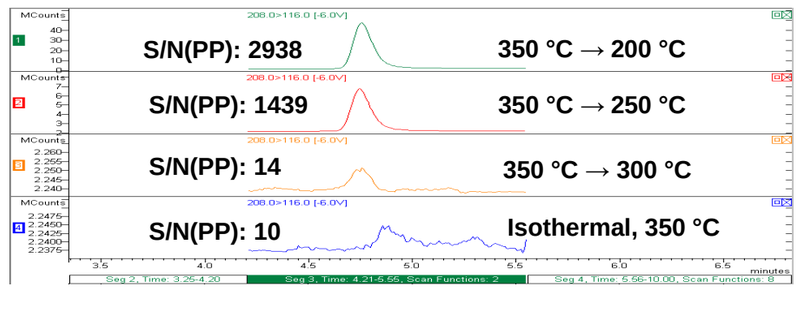
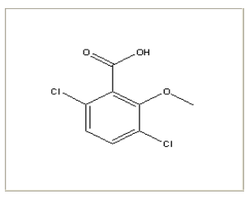


Figure 3: Aldicarb ammonium adduct sensitivity with SelecTemp™.

Dicamba, a chlorinated acid herbicide, was also studied. Preliminary data demonstrate that it performs much better at lower drying gas temperatures such as 150 °C. A series of flow injections (Figure 4) were made using the same ESI API on a Varian 500-MS Ion Trap MS. Figure 5 shows an MS chromatogram of vegetable extract spiked with dicamba analyzed at two different drying gas temperatures, 300 °C and 150 °C. Under last optimized conditions, an intense peak is detected with a S/N ratio of 330:1 while the peak is almost absent at 330 °C.

Varian 500-MS



Dicamba
benzoic acid herbicide (MW 220u)

Experimental

Several carbamate pesticides readily form ammonium adducts in the presence of ammonium acetate. Table 1 lists the general HPLC conditions used and Table 2 shows the carbamates studied. Precursor and product ion pairs listed in red were found to provide the best sensitivity and were subsequently used for quantitation.

Table 1: HPLC conditions

Column:	Pursuit C18, 3 µm, 100 x 2 mm @ 25 °C
Mixer:	50 µL static mixer
Solvent A:	0.1% formic acid: 2mM ammonium acetate in water, v/v
Solvent B:	acetonitrile

Time (min:sec)	%A	%B	Flow (mL/min)
0:00	80	20	0.2
2:00	60	40	0.2
9:00	30	70	0.2
9:03	5	95	0.2
10:00	5	95	0.2
10:01	80	20	0.2
16:00	80	20	0.2

Table 2: Precursor and product ions for carbamate pesticides

Analyte	M+H+	Product Ion(s)	M+NH4+	Product Ion(s)
Aldicarb sulfone	223	166	240	86, 76
Methomyl	163	88, 106	180	NA
3-Hydroxycarbofuran	238	163	255	220, 163
Dioxacarb	224	167, 123	241	167
Aldicarb	191	115	208	89, 116
Propoxur	210	111, 168	227	170
Carbofuran	222	123, 165	239	NA
Carbaryl	202	145	219	145, 127
Methiocarb	226	121, 169	243	169
Promecarb	208	109, 151	225	151
IS (Carbaryl -13C6)	208	NA	225	151

The table below is a summary of the LC/MS API and acquisition conditions for analysis with the Varian 320-MS LC Triple Quadrupole MS.

Ionization Mode	ESI Positive
Collision Gas	2.0 mTorr Argon
API Drying Gas	39 psi
API Drying Gas Temp	350 °C - 200 °C (prog.)
API Nebulizing gas	50 psi
Scan Time	1 sec
SIM Width	0.7 Da
Needle	5000 V
Shield	600 V
Capillary	variable
Detector	1900 V

Varian 320-MS LC Triple Quasrupole

The ability to program the drying gas temperature for the carbamate analysis provides the user with the opportunity to maintain sensitivity for key thermally labile compounds in the run. Compounds such as aldicarb and 3-hydroxycarbofuran form intense ammonium adduct ions that are ideal precursors for MS/MS analysis. However, these compounds are thermally labile and show rapid decomposition at API drying gas temperatures above 200 °C.

For these experiments, the initial temperature of the drying gas was set at 350 °C, then ramped down to 200 °C in the appropriate analytical segment. Figures 1, 2 and 3 show an improvement of up to 300X in S/N for select carbamates as the temperature is ramped down to various temperatures within each segment. The SelecTemp feature allows the operator to keep the temperature of the gas hot at times when the gradient is highly aqueous, thus providing less chance of source contamination with large, undesolvated droplets. The higher temperature of the drying gas during the equilibration time will also provide the benefit of cleaning out the source prior to the next injection, improving overall robustness of the analytical procedure. The API will stay cleaner if it is operated most of the time at a higher drying gas temperature, minimizing the need for source maintenance.

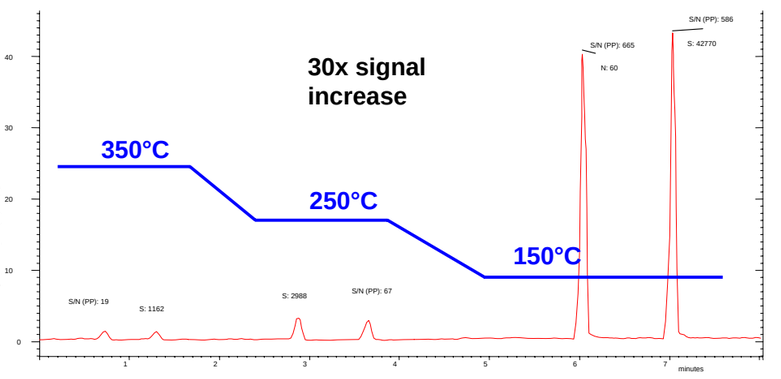


Figure 4: Dicamba flow injections (x2) on Varian 500-MS LC Ion Trap.

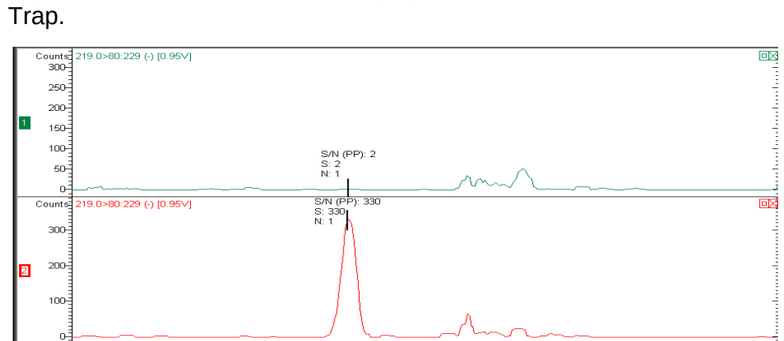


Figure 5: Dicamba MS/MS analysis in vegetable extract on Varian 500-MS LC Ion Trap.

Conclusions

Typical reversed-phase gradient HPLC separations start with:

- High aqueous content with high drying gas temperature
- Organic phase increases overtime (reversed phase separation)
- Too much heat (temperature) that degrades thermally labile compounds

SelecTemp allows API drying gas programming for method development.

- Software controlled temperature programmable drying gas ramp for the method with additional programming at selected time points throughout a complex LC/MS analysis
- Enables optimized analysis of thermally labile compounds
- Enables lower limits of detection for difficult compounds
- Enhances productivity by allowing more compounds to be included in a singleLC/MS run
- Improves method robustness and sample throughput