Application of Triple Quadrupole MS with Acquisition Speed Improvements for Pesticide Analysis

Harald Oser, Michael Ugarov, Qingyu Song, Michael Konicek, Claudia Martins, Neloni Wijeratne, Thermo Fisher Scientific, San Jose, California, USA, 95134

ABSTRACT

Purpose: Minimizing the RF/DC settling time for the quadrupole mass filters should allow the user to either measure more transitions per unit time or be able to acquire more date points per unit time without compromising data quality. The optimized RF/DC settling time leads to the possibility to analyze more compounds in the same run, or to have better ion statistics leading to better quantitation.

A Thermo Scientific™ TSQ Quantis™ Triple Stage Quadrupole Mass Spectrometer has been upgraded with a non linear RF/DC settling time model in order to improve the scan speed. A new diagnostic routine was developed to maximize the improvement. The performance of the instrument has been evaluated in the screening of hundreds of pesticides. Data will be presented showing improvements in this domain compared to the standard linear RF/DC settling time model.

Methods: The prototype instrument was used to run a mixture of 19 pesticides.

Results: Gains in speed performance were measured and results from acquisition of up to 650 SRMs per second are reported below.

INTRODUCTION

Speed of stabilization of key voltages responsible for the operation of a mass spectrometer is critical for applications that target tens or hundreds of SRM transitions during a chromatographic run. Timely settling of RF/DC voltages on the rods of the quadrupole mass analyzer is particularly important to guarantee reproducible performance at very short dwell times. Both improved slew rate and stabilization been the target of modifications leading to the implementation of a non linear model for the RF/DC settling time.

MATERIALS AND METHODS

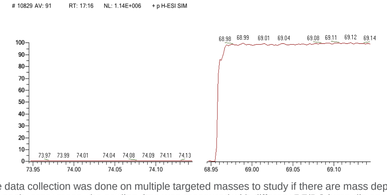
Hardware Setup

Standard production TSQ Quantis and TSQ Altis™ Triple Stage Quadrupole Mass Spectrometers have been used to characterize the ramp speed and settling time of the RF/DC voltages. A fully automated diagnostic was developed and applied that measures time until the ion signal is fully settled after a jump to a subsequent SRM transition occurs.

To measure the settling time, the raw signal detected by the electron multiplier was used. During the measurement, only the RF/DC voltage on the selected quads were jumped from a selected dummy transition to a targeted transition.

A typical ion flux after the voltage change can be seen in figure 1 from which the minimal required settling time can be measured.

Figure 1. Ion flux for two different RF/DC settings



The data collection was done on multiple targeted masses to study if there are mass dependencies. For each target mass, the settling time was measured with different RF/DC jump distance, both up and down.

Experiments were performed for both Q1 and Q3 mass filters, in absence and presence of collision gas. Figure 2 shows the settling time as a function of delta mass (mass jump distance) for the Q3 mass filter. From this type of data we can extract mass dependent settlings times in absence and in presence of collision gas as shown in figures 3a and 3b for masses 69 amu and 922 amu.

This experimental results suggest that a power function *settling time = a * mass ^ (power)* is a better function for calculation of the minimal settling time than the linear approximation. The settling time is also mass dependent. This is mainly due to the time of flight effect that at the same kinetic energy heavier ions took longer time to fly though the quadrupole.

MATERIALS AND METHODS (continued)

Figure 2. Mass dependent Settling Time for different mass jumps

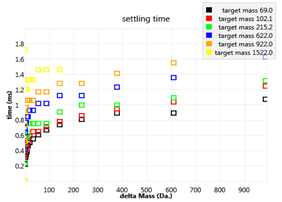


Figure 3a. Settling Time with and without Collision Gas for Mass 69 amu

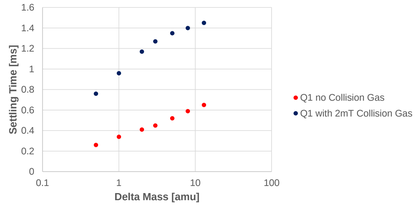
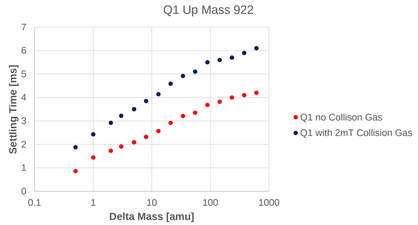


Figure 3b. Settling Time with and without Collision Gas for Mass 922 amu



Based on these findings, optimal inter-scan delay times can be chosen to be a function of key parameters, such as the target m/z and delta m/z for two transitions.

The model below was used for a nonlinear curve filter fitting to figure out the best formula for the settling time calculation.

Overall settling = A* (mass difference)^B + C * sqrt(target mass) where A is the scale of the power function, B is the exponent of the power function and C is the TOF coefficient.

A TSQ Quantis triple quadrupole mass spectrometer has been upgraded to the non linear settling time model and performance evaluated in the screening of hundreds of pesticides.

MATERIALS AND METHODS (continued)

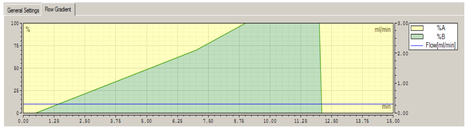
LC method: application setup

Because inappropriate application of a pesticide can result in serious health issues, determination of pesticide residues in foods and food products is an important part of routine food control.

The European Union (EU) legislation (European Regulation 396/2005 and Commission Directive 2006/125/EC), currently the strictest regulations, set maximum residue levels of pesticides in various products of plant and animal origin. These regulations present significant analytical challenges due to the low limits of quantification (LOQ) required in certain food matrices.

LC-MS/MS analysis was carried out using a Thermo Scientific Vanquish Flex™ Binary system coupled to the prototype triple quadrupole mass spectrometer. Thermo Scientific TraceFinder™ software was used for instrument control, analysis, data review and reporting. The LC conditions and gradient are shown bellow in Figure 4. A total run time of 15 minutes was used to separate the mixture of pesticides with good sensitivity (ng/L) and reproducibility, while maintaining good chromatographic separation.

Figure 4. LC method gradient and the injection amount



Phase A : 0.1% Formic Acid, 2% MeOH and 5 mM Ammonium formate in Water
Phase B : 0.1% Formic Acid, 2% Water and 5 mM Ammonium formate in MeOH

Column : Accucore C18 100x2.1 mm 2.6 u
Column Temperature : 25° C

Flow Rate : 300 µl/min
Injection Volume : 1µl

Concentrations ranging from 0.05 to 100 ppb with 3 replicates for each concentration level have been injected.

The list of compounds used is listed in table 1, this table also includes parent mass, product mass and collision energies used for this study.

All experiments where performed with 2mTorr Ar collision gas pressure.

Table 1. Used compounds, precursor mass, product mass and collision energies

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)
Aminocarb	209.074	136.929	23.69
Atrazine	216.074	103.917	28.27
Azoxystrobin	404.07	329	30.74
Benzoximate	364.05	198.988	10.23
Bifenazate	301.062	170.054	19.17
Carbaryl	202.04	145.071	10.23
Carbofuran	222.05	123	21.75
Clomazone	240.024	124.97	20.96
Cycloate	216.037	103.917	28.5
Cycluron	199.087	89.018	14.81
Cyflufenamid	413.012	223.042	22.02
Difenacoum	445.125	179	31.15
Dimethenamid	276.062	168.054	23.99
Dimoxystrobin	327.125	116	21.79
Fenpyroximat	422.162	215.083	25.62
Iprovalicarb	321.175	119.071	19.36
Isoprocarb	194.062	94.97	15.12
Trietazine	230.074	146	23.38
Trifloxystrobin	409.04	145	43.97

To characterize the overall acquisition speed of the instrument a method with 720 transitions was used. A 1.2s cycle time was applied to get 600 SRM transitions per second, which resulted in a dwell time of 0.438 ms for the linear settling time model.

MATERIALS AND METHODS (continued)

In the second set of experiments while keeping the cycle time constant at 1.2 s, we changed to the non linear model which changed the dwell time to 0.495 ms.

For the third set of experiments increased the number of SRM transitions to 650 by adding 60 dummy transitions to the method. A constant cycle time of 1.2 s resulted in a dwell time of 0.365 ms as shown bellow in table 2.

Table 2. Dwell time Comparison

SRM/Second	Number of added transitions	Total Number of Transitions	Dwell Time (mSec)
600 linear model	0	720	0.438
600 non linear model	0	720	0.495
650 non linear model	60	780	0.365

EXPERIMENTAL RESULTS

Linearity CV comparison for 600 linear model, 600 and 650 non linear model SRM per second

1 uL for 0.05 to 100 ppb levels of the samples have been injected to verify that instrument has a linear response. The results for all compounds are summarized in table 3 proof that with the non linear model for the RF/DC settling time of up to 650 SRM per second are achievable without compromising the quality of the results.

Table 3. Summary of Comparison between 600 linear model, 600 and 650 non linear model SRM per second

Compound	Precursor m/z	Product m/z	600 SRM/Second Linear model			600 SRM/Second Non linear model			650 SRM/Second Non linear model		
			LOQ	Average Area	%CV	LOQ	Average Area	%CV	LOQ	Average Area	%CV
Aminocarb	209.074	136.929	1	64307	10.9	0.5	33311	2.2	1	88888	1
Atrazine	216.074	103.917	5	84132	11.1	5	87972	1.1	1	20047	1.6
Azoxystrobin	404.07	329	5	229385	6.4	1	40616	9.4	1	40067	8
Benzoximate	364.05	198.988	1	66507	9	1	76922	3	0.5	32911	4.7
Bifenazate	301.062	170.054	5	56106	5.5	1	9586	7.7	5	74424	7
Carbaryl	202.04	145.071	5	146857	10.3	1	30328	5.8	5	143336	9
Carbofuran	222.05	123	0.5	59589	3	0.1	9895	2.7	0.1	11266	6.7
Clomazone	240.024	124.97	1	60114	8.8	1	62037	6.5	1	65408	3.9
Cycloate	216.037	103.917	5	72582	8.5	1	12934	11.4	5	65224	1.5
Cycluron	199.087	89.018	5	161995	5.6	1	33034	9.6	1	39206	9.8
Cyflufenamid	413.012	223.042	5	64219	5.7	5	119970	3.3	5	90988	4
Difenacoum	445.125	179	5	169956	14.6	5	159211	4.4	5	145569	5.5
Dimethenamid	276.062	168.054	1	21611	11.1	0.5	17087	8.6	1	34951	10.3
Dimoxystrobin	327.125	116	5	350340	6.9	1	88133	10	5	323501	5.1
Fenpyroximat	422.162	215.083	5	180636	3.3	1	17194	11.8	5	88634	10.7
Iprovalicarb	321.175	119.071	1	88370	1.2	0.5	39607	7	0.5	45131	4.4
Isoprocarb	194.062	94.97	5	181120	8.6	1	31129	10.4	1	24139	10.9
Trietazine	230.074	146	0.5	29699	10.3	0.5	57488	4.5	0.5	50657	6.6
Trifloxystrobin	409.04	145	1	68787	10.5	0.5	35760	8.5	1	55872	10.9

EXPERIMENTAL RESULTS (continued)

The results for Azoxystrobin collected with 600 linear model and 650 non linear model SRM per second are shown in figures 5a to 5b. All two measurements show an excellent linear response but improved LOQs and %CVs for the non linear model.

Figure 5a: 600 SRM per second linear model, LOQ = 5ppb

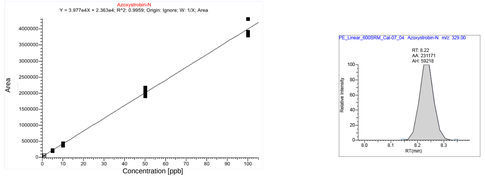
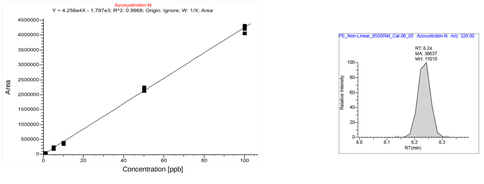


Figure 5c: 650 SRM per second non linear model, LOQ = 1ppb



CONCLUSIONS

- A new improved RF/DC settling time model was developed.
- This new RF/DC settling time model has been implemented and evaluated in a triple stage quadrupole Quantis™ mass spectrometer.
- The speed enhancement and performance of the developed model was evaluated with a 19 compound pesticide mixture.
- The results achieved with real world samples show that speeds of up to 650 SRM per second can be achieved without compromising quality of the results.
- As a result of the presented speed improvements it is possible to analyze more compounds in the same time window or better quantitation results with better ion statistics due to more scans across a chromatographic peak.
- Future work will include the continued evaluation of non linear models to further reduce settling time values in order to speed up the acquisition.

ACKNOWLEDGEMENTS

The authors would like to thank Pascual Cardenas and Daniel Valk for their tireless support and help during start up of the instruments.

TRADEMARKS/LICENSING

PO65531-EN0519S

ThermoFisher SCIENTIFIC