Technology

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Increasing System Robustness with Ion Formation Control

Maximize length of time between front end cleanings with one click.

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Overview

Here we describe an approach used to reduce instrument contamination by controlling the formation of ions only when data collection is required. Even under accelerated contamination conditions, this feature, called **Scheduled Ionization**, enables sustained instrument operation over time periods that are significantly longer before cleaning is required.

Introduction

As MS instruments move into more routine and automated environments, where far less operator interventions are needed, sustained performance over long periods is key. To ensure long term robustness, assays and systems are typically setup to minimize both front end and vacuum optics contamination. To mitigate front end contamination, divert valves are frequently employed as part of the overall system. These are typically under software control and traditionally used to divert the solvent front, which contains a higher level of salts, away from the ion source region.Though effective at contamination reduction, divert valves do complicate the fluidics of the overall system and introduce an additional mechanical component that could fail, become contaminated or clogged. Here we propose an alternative that could be used to achieve similar benefits with reduced complexity: ion formation control feature called Scheduled Ionization.

In a previous study, it was shown that ions generated at the source can lead to instrument contamination in various locations of the ion optics ranging from the front end (orifice) to other components located in vacuum[1]. A significant reduction in contamination was achieved when the ion flux was blocked by using a low-resolution differential mobility interface. This approach provides control of ion sampling even during data collection, as one could discriminate different ion populations during analysis.

In a similar fashion, many LC-MS users have relied on a divert valve to ensure that system cleanliness is maintained over long periods of time[2]. In these set-ups, the LC flow is mechanically diverted from the source when no data is required. This approach adds additional components to the system, could lead to additional broadening of the LC peak, and is prone to wear-and-tear. An alternative approach is to **control the ion formation** during the analysis, that is, simply to activate the ionization voltage on the source and acquire data only when ion formation is required. Since relevant MS information is typically collected over a limited portion of the LC analysis, it is proposed that ion formation can be stopped when data collection is not required, and re-activated for MS data collection. By **reducing the number of ions sampled by the instrument**, a significant reduction in contamination is expected, which would reduce the need for instrument cleaning.

Materials and Methods

A standard mix solution containing Clenbuterol, Verapamil, Reserpine and Rescinnamine was prepared in mobile phase A (2% acetonitrile aqueous solution with 0.1% formic acid) at concentrations such that the height of the peak resulting from a 5µL injection is between 1×10^5 and 1×10^6 counts per second in MRM mode. The preparation of the standard solution was not done quantitatively as the goal was specific signal intensities, and concentration values are not applied in the processing of the data. Liquid chromatography was performed using an Agilent 1200 HPLC system operated at a flow rate of 600uL/min with a Synergy Fusion RP (80A 2x50mm, 4µ) column with SecurityGuard guard column (Phenomenex). A 5-minute gradient of water and acetonitrile, both with 0.1% formic acid, was used for elution.
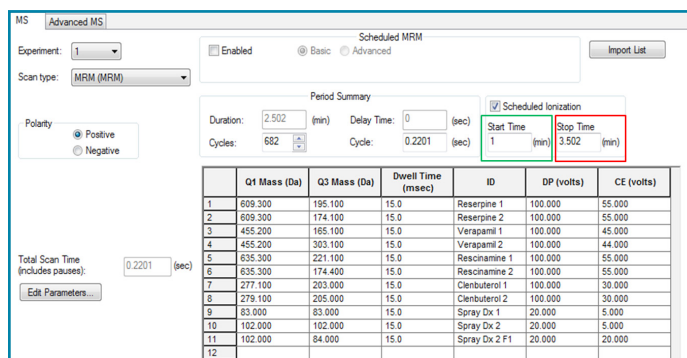

Figure 1. Method editor modified to support Scheduled Ionization.

All samples were analyzed using a SCIEX QTRAP® 4500 system equipped with a Turbo V™ source. The following source and instrument conditions were used: GS1=50psi, GS2=70psi, 650°C, ISV=5000V, **CUR=10**. For each analyte, two MRM transitions were monitored for each compound. A pre-commercialization version of Analyst® software 1.7 was used for acquisition. This version turns off the ionization voltage when no data collection is required (Figure 1). The ionization voltage is also turned off once data collection is complete and remains off until the LC method is completed, and turned off between samples. This mode of operation, where the ionization voltage is turned on for data collection only, is referred to as Scheduled Ionization. Using this approach, all conventional acquisition workflows (MRM, scheduled-MRM™ algorithm, IDA, SWATH® acquisition) can be supported with a common user interface. Scheduled Ionization can also be used with the Heated Nebulizer source, or APCI (atmospheric pressure chemical ionization).

To evaluate reduction of contamination under typical LC analysis workflow, a test was developed to **accelerate contamination** (CUR = 10) and evaluate the effect of turning off the ion formation when data collection is not required. To perform a long-term stability comparison, consideration for solution stability was addressed by injecting the analyte solution separately from the diluted urine (1:1 dilution with water), using a two-step injection sequence. To achieve this, the solution standard (2uL) was injected under isocratic conditions (95% aqueous). After 18 seconds, an injection of diluted urine (25uL) was performed. The isocratic LC conditions were maintained for an additional 15 seconds before performing gradient to 95% organic over 2 minutes.
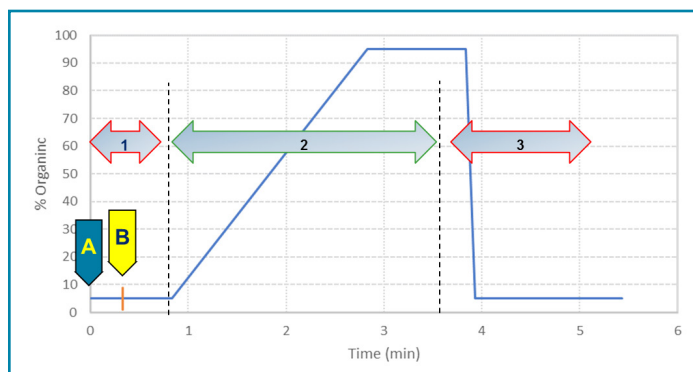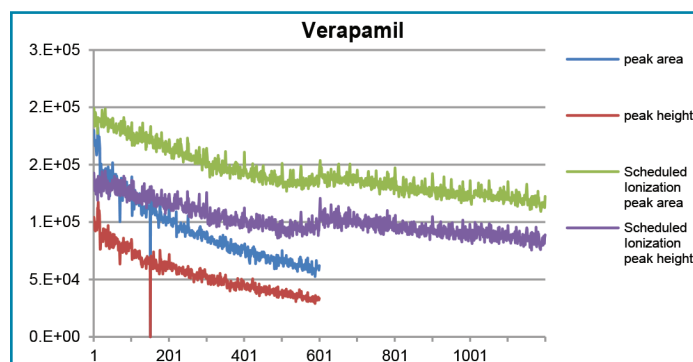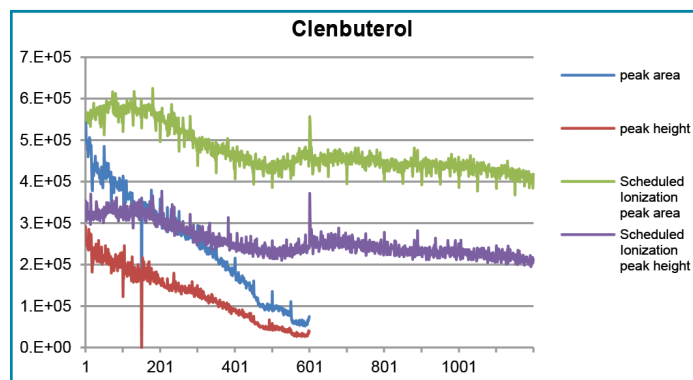

Figure 2. LC gradient conditions and injection sequence. Solution standard (2uL) is injected at A, and diluted urine (25uL) is injected at B. Source voltage was turned off for time window 1 and 3. Data collection was activated for time window 2, when the ionization voltage was activated.

Figure 2 shows the injection sequence and the LC gradient conditions used. This figure also shows the region where the ionization voltage is turned on and data is collected (region 2). The region of data collection was set to cover the bulk of the LC gradient which represents a case with maximum data collection and minimum reduction in ion flux that can lead to contamination.
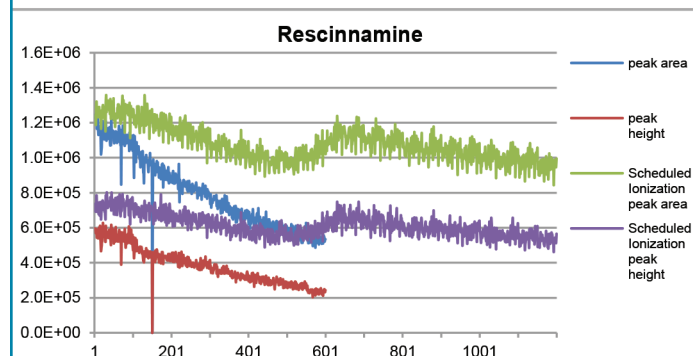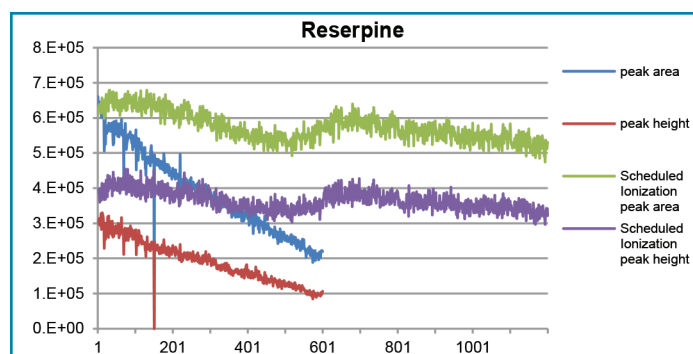
Results and Discussion

Normal mode of operation was run first, where the ionization voltage is on for the entire duration of the run, for a total of 600 injections. Scheduled Ionization mode of operation was run for a total of 1200 injections. The data was processed; peak areas and heights were plotted for comparison. Figures 3 and 4 show plots of the peak areas and heights for all analytes for both modes of operation.

Figures 3. Peak areas and heights for Clenbuterol and Verapamil, with and without Scheduled Ionization.

When the ionization voltage is kept on for the entire duration of the LC-MS/MS analysis, a rapid decay of signal is observed between injection 1 and 600. Under these conditions, physical accumulation of material on the orifice can be observed.

In contrast, when the ionization voltage is active only for the data collection (time window 2 as per Figure 2), 1200 injections of urine samples were performed and the peak heights and areas were maintained over that period. This represents approximately 2x improvement in terms of robustness of the system. Table 1 provides additional data regarding instrument performance in these 2 scenarios.

Figures 4. Peak areas and heights for Reserpine and Rescinnamine, with and without Scheduled Ionization.

The extent of contamination reduction will vary greatly with the acquisition workflow selected by the user. The conditions used in the current work were aimed at testing minimal reduction in data collection, and therefore 'maximizing' contamination even with the Scheduled Ionization feature activated. To estimate the amount of contamination reduction that could be achieved, the following formula could be used:

$$\% \ Reduction \ Contamination = 100 \ X \ \frac{LC \ Time + Injection \ Time - Data \ Time}{LC \ Time \ + Injection \ Time}$$

Under the conditions used for the current experiment, with an LC time of 5.5 min, an injection time of 25 sec, and data collection of 3.5 min, the expected reduction in contamination would be ~43%.

If we correlate the reduction in contamination to time between instrument cleanings, we would expect 2.3 times longer between cleanings. The experimental data suggest that the instrument can show little sign of contamination well beyond this estimated time, even when large portions of the chromatogram have data collected. The elimination of sampling the void volume (salts and materials unretained on the column) at the front of the gradient may contribute to this. Further reducing the data collection region with respect to the LC analysis time could lead to even larger effective reduction in contamination.

Conclusion

Controlling the ion formation can effectively reduce contamination of ion optics under analytical conditions. By using the Scheduled Ionization feature, it is possible to extend the use of the instrument over prolonged duration due to reduction of contamination of the front end (orifice region). The reduction of contamination will vary based on the region of data collection selected by the user and will therefore vary between scenarios.

The proposed approach offers benefits that are similar to using a divert valve, but removes the complexity of additional hardware. The simplification of this setup also provides added benefits that the electrode is never operated dry and is constantly rinsed with solvent, which could reduce risk of carry-over and improve electrode lifetime. An additional side benefit of the use of Scheduled Ionization is the reduction on file size, especially when ToF detection is used in applications like SWATH® acquisition.

References

1. Kang, Y.; Schneider, B.B., Covey, T.R., "Improved Mass Spectrometry Robustness with DMS Pre-Filter", 62nd ASMS Conference on Mass Spectrometry and Allied Topics 2014. 15–19 June 2014. Baltimore.

2. Krstulovic, A.M. et al., "Application of LC-MS Methodology in the Development of Pharmaceuticals" LC-GC Europe (2002) p.2-9.

Table 1. Peak areas of 5 injections (~ 30 minutes of run time) were averaged and compared at selected number of injections for both modes of operation to show the decline in sensitivity. Ratios were calculated against the average of the first 5 injections in the data set.

Area loss after x injections	Clenbuterol		Verapamil		Reserpine		Rescinnamine	
	Normal mode	Scheduled-ISV	Normal mode	Scheduled-ISV	Normal mode	Scheduled-ISV	Normal mode	Scheduled-ISV
50	0.80	1.00	0.82	0.95	0.89	1.04	0.93	1.04
100	0.76	1.02	0.74	0.91	0.84	1.03	0.86	1.02
300	0.51	0.88	0.52	0.78	0.61	0.96	0.64	0.91
600	0.31	0.82	0.36	0.70	0.34	0.92	0.44	0.87
1200	-	0.71	-	0.60	-	0.84	-	0.78