Run, MS, Run! Increasing System Robustness with Ion Control

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ABSTRACT

This presentation describes an approach used to reduce instrument contamination by controlling the formation of ions only when data collection is required; Scheduled-Ionspray (Scheduled-ISV). Even under accelerated contamination conditions, Scheduled-ISV enable sustained instrument operation over time period that were significantly longer (>5x) before cleaning was 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. Here we propose an alternative that could be used to achieve similar benefits with reduced complexity: ion formation control (IFC) with Scheduled-Ionspray (Scheduled-ISV).

MATERIALS AND METHODS

Reserpine (resp), rescinnamine (resc), clyndamicine (clyn) and verapamil (verp) were obtained from Sigma (St-Louis, MO) and stock solutions of 1mg/mL were prepared in methanol/water (50/50 (v/v)). The stock were combined and diluted to 75pg/uL (resp, resc, clyn) and 37.5pg/uL (verp) into water containing 0.1% formic acid. Urine samples were dilute 1:1 with water.

LC was performed using a Shimadzu Nexera UFLC system operated at a flow rate of 600uL/min with a Synergy Fusion RP (80A 2x50mm (4u)) column (Phenomenex). A 2 minute gradient of water and acetonitrile, both with 0.1% formic acid, was used for elution.

All samples were analyzed using a SCIEX QTRAP® 5500 system equipped with a Turbo V™ source. The following source and instrument conditions were used: G1=50psi, G2=70psi, 650oC, ISV=5500V, CUR=25. For each analyte, two MRM transitions were monitored for each compound using the conditions listed Table 1. A research version of Analyst® 1.6.2 software was used for acquisition. This version effectively turns off the ion source voltage when no data collection is required. The 'Delay Time' option was repurposed to control the time where the ion source voltage is turned off prior to data collection. The ion source voltage is also turned off once data collection is complete and remains off until the LC method is completed. In this version, the ion source voltage is also turned off between samples. This mode of operation, where the ionspray voltage is turned on for data collection only, is referred to as Scheduled-Ionspray (Scheduled-ISV) Using this approach, all conventional acquisition workflows (MRM, scheduled-MRM™ algorithm, IDA, SWATH® acquisition) can be supported with a common user interface.

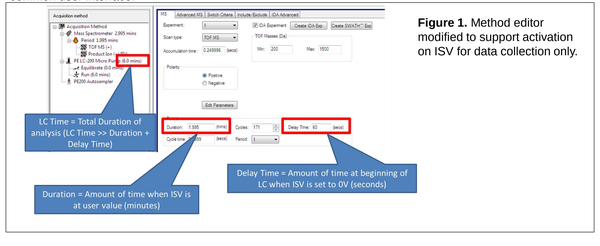

Figure 1. Method editor modified to support activation on ISV for data collection only.

RESULTS AND DISCUSSION

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 voltage on the source 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.

To evaluate reduction of contamination under typical LC analysis workflow, a test was developed to accelerate contamination and evaluate the effect of turning off the ion formation when data collection is not required. In order to perform a long term stability comparison, consideration for solution stability were addressed by injecting the analyte solution separately from the diluted urine (1:1 dilution with water), but the analysis was performed with the same gradient. To achieve this, the solution standard (2uL) was injected under isocratic conditions (95% aqueous). After 20 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 shows the injection sequence and the LC gradient conditions used. This figure also shows the region where 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.

Figures 3 and 4 show the fragment ion ration for reserpine and verapamil, respectively. When the ionspray voltage is kept on for the entire duration of the LC-MSMS analysis, a rapid decay in signal is observed between injection 500 and 700 (blue and purple data set). Under these conditions, accumulation of material on the orifice can be observed (Figure 5a). Cleaning of the orifice restored the bulk of signal (70-80% of the signal strength), but this step did required breaking the instrument vacuum.

In contrast, when the ion source voltage is active only for the data collection (time window 2 as per Fig.2), over 4200 injections of urine samples were performed and the ion ratio was maintained over that period. This represents a >8x improvements in terms of robustness of the system. Table 2 provides additional data regarding instrument performance in these 2 scenarios.

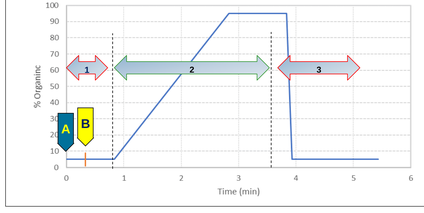

Figure 2. LC gradient conditions and injection sequence. Solution standard (2uL) is injected at A, and diluted urine (25uL) is injected at B. Source voltage was turned off for time window 1 and 3. Data collection was activated for time window 2, when the ion source voltage was activated).

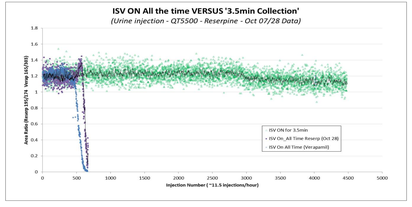

Figure 3. Reserpine MRM Ratio with/without ISV control

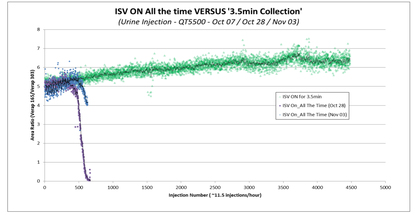

Figure 4. Verapamil MRM Ratio with/without ISV control

Figure 5 shows a photograph of the curtain plate as well as the orifice (front and back) after 700 injections of urine when the ionspray voltage was active all the time. Of note is the presence of residue located inside the orifice, extending towards the vacuum side of the orifice. By contrast, when over 4500 injections of urine were performed with Scheduled-ISV, the residue appeared to be located within the orifice thickness (Figure 6). Qualitatively, it appears like the amount of material deposited on the orifice is roughly equivalent at the end of these experiments, but one needs consider that nearly 8x more material was injected during the experiment where the ionspray potential was periodically decreased, and yet the MRM ratio was still maintained and no cleaning of the orifice was still required (no need to break vacuum). The curtain plate clearly had more material deposited after >4250 injections with Scheduled-ISV, but there is no need to break vacuum to clean this component.

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 Scheduled-ISV 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 2.3X 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.

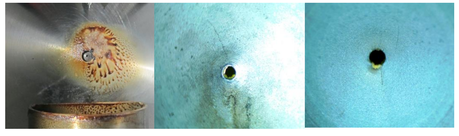

Figure 5. Scheduled ISV deactivated (on all the time) after 650 injections performed

Figure 6. Scheduled ISV activated with 4520 injections performed

Attribute	ISV ON All Time	ISV Control
Reserpine Signal Loss	>500	20
Verapamil Signal Loss	>500	18
Clyndamincine Signal Loss	>200	22
# Injections	620	4520
Hours of Operation	< 60	> 420
Urine Injected (mL)	6	45

Table 2. Performance of ISV control with LC-MSMS analysis

CONCLUSIONS

Controlling the ion formation can effectively reduce contamination of ion optics under analytical conditions. By scheduling the formation of ions (Scheduled-ISV), 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. However, based on the tested scenario (capture 80% of ion formation – entire eluting portion of the gradient), it is anticipated that the improvement in robustness could ensure sustained operation over a time periods that are 7x longer (or more).

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-ISV is the reduction on file size, especially when ToF detection is used in applications like SWATH® acquisition.

REFERENCES

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

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