



Optimizing robustness for high-throughput screening on the Echo[®] MS+ system

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Why is robustness important in high-throughput screening (HTS)? Insufficient robustness in analytical methods can lead to inconsistent data quality and downtime. This, in turn, results in prolonged project timelines, additional costs and an increased need for manual intervention.^{1,2} Components, including salts such NaCl or MgCl₂, or stabilizer proteins such as bovine serum albumin (BSA) and Prionex, are commonly used in sample matrices for HTS. Interactions with organic carrier solvents can cause these components precipitate out of solution and build up at the Open Port Interface (OPI). The gradual accumulation of matrix material can reduce instrument performance, leading to poor reproducibility and lowered sensitivity. To meet the robustness needs of HTS, the Echo[®] MS+ system has an OPI port wash. The washing cycle uses an aqueous solvent to facilitate the removal of these deposits and significantly enhance system robustness when analyzing large quantities of sample (Figure 1).

Key features of the Echo[®] MS+ system

- **Enhanced system robustness:** The washing cycle ensures the regular removal of dirty samples or sample matrices, maximizing instrument uptime and prolonging the lifetime of the OPI electrode assembly
- **≤3 min cycle time:** This 3-minute wash cycle can be seamlessly integrated into HTS in parallel with plate exchanges, without reducing throughput due to OPI port maintenance
- **Customizable wash protocol:** The washing procedure can be tailored by the user to accommodate varying needs, ensuring optimal conditions are met based on specific requirements

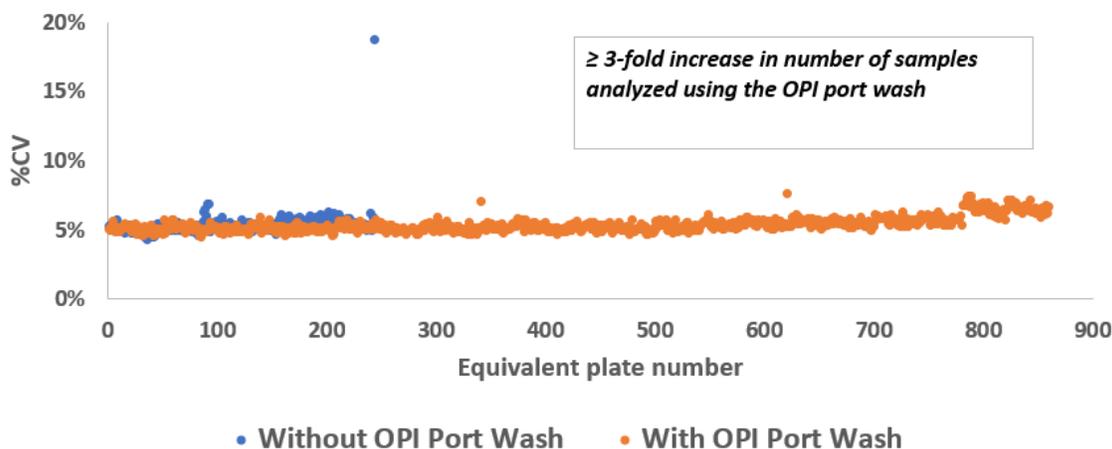


Figure 1. The comparison of AEMS system robustness. A comparison is shown for the %CV per plate equivalent for datasets without (blue) and with (orange) the OPI port wash. Without the OPI port wash, the sample run was limited to approximately 95,000 samples, equivalent to 247 plates. Beyond this point, the %CV increased from <10% to nearly 20%. Additionally, an overflow of carrier solvent occurred at the OPI, triggering the OPI leak sensor and rendering it impossible to resume the sample run. On the other hand, with the introduction of the OPI port wash, which consistently removes sample matrices, the sample run was extended to approximately 330,000 samples, equivalent to the 859 plates. Throughout this extended run, the %CV consistently remained <8%. The OPI port wash cycle was scheduled once every 384 ejections, simulating every 384-well plate.

Introduction

Here, we describe a proof-of-concept (PoC) study that demonstrates the value of integrating the OPI port wash function between batches. A comparative analysis was conducted between 2 scenarios: a baseline study without the OPI port wash and a study in which the OPI port wash was conducted after every 384 ejections.

The Echo® MS+ system includes an OPI port wash that facilitates the inline flushing of the OPI capture port and the OPI electrode assembly using a wash solvent as part of a user-defined routine analytical method.

In the Echo® MS+ system, the carrier solvent is drawn from the solvent bottle to the degasser using a gear pump to eliminate potential dissolved gases. The degasser outlet is directly connected to a pump equipped with a flowmeter for precise and accurate solvent delivery. The pump outlet is linked to a diverter valve, controlling the flow to the OPI for Acoustic Ejection Mass Spectrometry (AEMS) operations or to a waste container for pump purging. To conduct the OPI port wash, a second independently controlled pump system was introduced to deliver the wash solvent (Figure 2). This secondary system includes an additional solvent bottle, degasser and pump. Calibration of flow rates for water, methanol and acetonitrile combinations is performed to ensure precise control over a broad range of wash solvents. Two 6-port rotary valves are employed to achieve different flow conditions.

The OPI port wash performs a cycle consisting of 4 phases (Figure 3). The 2 phases of solvent exchange, including a wash solvent exchange and a carrier solvent exchange, ensure that the content of the OPI is emptied before the next solvent is introduced. Once the OPI is emptied, the cleaning and carrier solvent recovery phases are performed. The settings for the cleaning and recovery phases are adjustable.

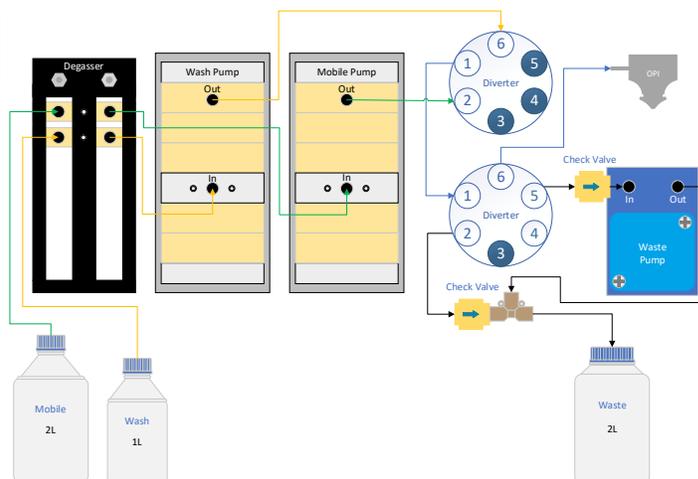


Figure 2. The fluidic delivery module of the system showing the connections between each component.

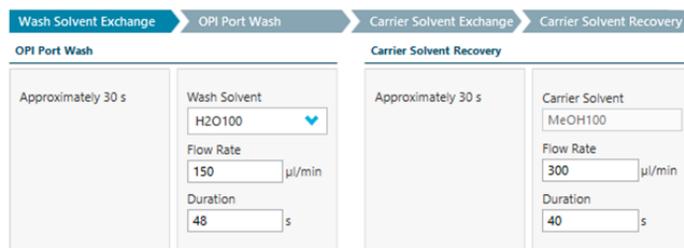


Figure 3. The OPI port wash performs a cycle consisting of 4 phases. The wash solvent exchange and carrier solvent exchange phases ensure that the content of the OPI is emptied before the next solvent is introduced. The 2 other phases include the OPI port wash and the carrier solvent recovery, for which settings can be defined by the user.

Methods

Sample preparation: Dextromethorphan Tris KCl (DXM), MgCl₂, dithiothreitol, BSA, Tween 20, ammonium acetate and citric acid were purchased from Sigma Aldrich. Acetonitrile was purchased from Thermo Fisher Scientific.

The 500nM DXM was prepared in buffer containing 10mM Tris (pH 7.5), 10mM KCl, 5mM MgCl₂, 1mM DTT, 0.01% BSA and 0.001% Tween 20.

Before analysis on the Echo[®] MS+ system, 50 µL of the sample was transferred to a well of an Echo[®] MS qualified 384-well polypropylene (PP) microplate (Beckman Coulter[®] Life Sciences, P/N: C74290). The plate was centrifuged at 1,530 g for 2 minutes with a long arm centrifuge in portrait mode.

Acoustic ejection: The Echo[®] MS+ system contains an additional bottle to contain wash solvent, wash solvent pump, a second diverter valve and an air/waste pump (Figure 2).

The carrier solvent was 50:50 (v/v), acetonitrile/methanol with 30mM ammonium acetate and 0.05mM citric acid (Table 1). In the study using the OPI port wash, 50:50 (v/v), methanol/water was used as the wash solvent. The wash solvent was introduced into the OPI for a scheduled 3-minute cleaning cycle after every 384 ejections.

Table 1. Acoustic ejection conditions.

Parameter	Value
Carrier solvent	50:50 (v/v), acetonitrile/methanol with 30mM ammonium acetate and 0.05mM citric acid
Carrier solvent flow rate	500 µL/min
Fluid class	AQ
Ejection volume	10 nL
Peak type	Standard

Mass spectrometry: The Echo[®] MS+ system with ZenoTOF 7600 system was controlled by SCIEX OS software (Table 2).

Table 2. Mass spectrometry method and source conditions.

Parameter	Value
Polarity	Positive
Ion source gas 1	90 psi
Ion source gas 2	45 psi
Curtain gas	30 psi
Source temperature	300°C
Ion spray voltage	5000 V
CAD gas	7
MS method	TOF MS
Mass range (m/z)	100–1000
Collision energy	10 V
Total scan time	0.1 s

Software: SCIEX OS software, version 3.1 was used for the optimization of the source parameters, automatic integration of the MS peaks and automatic creation of the results and associated *.txt files.

Baseline study without OPI port wash

A baseline study was first performed to determine how many plates the system can acquire before analytical performance deteriorates. The peak area and %CV of DXM were monitored. The study was designed to end if the OPI leak sensor was triggered or if the analyte peak shape deteriorated such that baseline separation was lost. It was not possible to re-establish the same carrier solvent flow rate without triggering the OPI leak sensor.

Once the baseline was established, the OPI was cleaned and the used electrode was replaced. A similar procedure was then performed, in which an OPI port wash step was added after every 384 ejections. In this procedure, triggering of the OPI leak sensor, analyte peak shape, peak area and %CV were monitored. The number of plates successfully acquired was determined and compared with that of the baseline study.

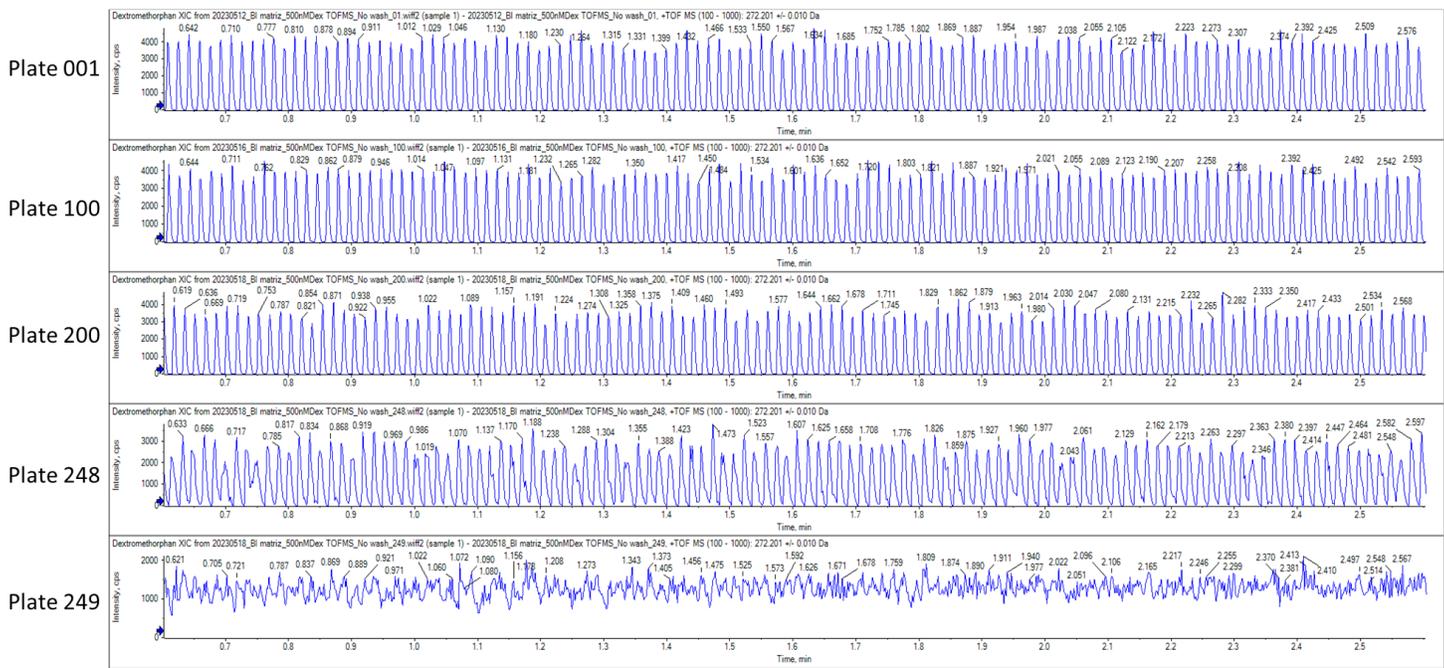
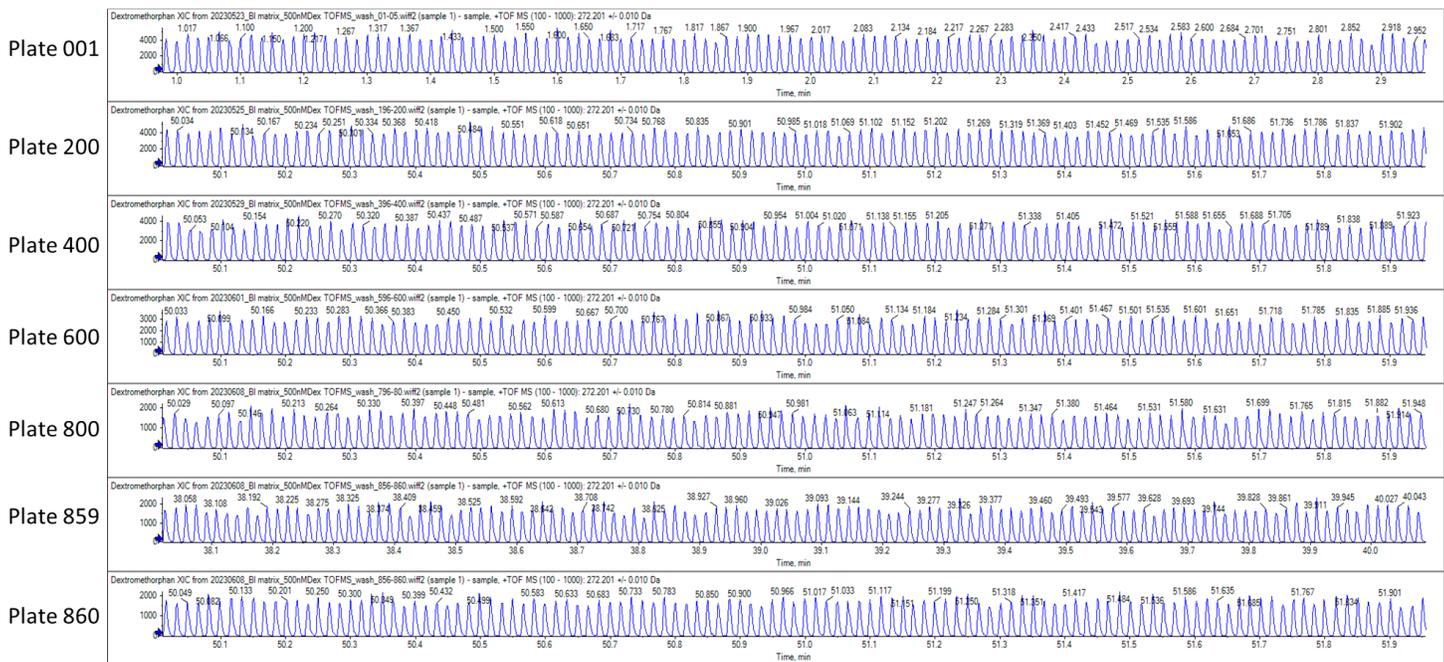
A**B**

Figure 4. The comparison of sonograms between the baseline study without OPI port wash and the study with OPI port wash after every 384 ejections. (A) In the baseline study, DXM exhibited optimal performance up to plate 248. The loss of all peak separation was observed when running plate 249. (B) When the OPI port wash cycle was scheduled after every 384 ejections, the peaks were still distinctive until plate 860.

In the baseline study, optimal performance was achieved through 247 plate equivalents. DXM peak separation was lost during the acquisition of plate 248. At this point, adjacent peaks started to merge and the peak-to-peak consistency could no

longer be maintained (Figure 4A). There was an overflow of carrier solvent at the OPI, which triggered the OPI leak sensor. Through plate 247, the %CV of DXM was maintained at <10%.

Once peak separation was lost, the %CV increased to nearly 20% (Figure 5A).

More than 3-fold system robustness improvement with OPI port wash

In contrast, when the OPI port wash cycle was scheduled once every 384 ejections, the %CV of DXM was maintained at <6% until plate 800 (Figure 5B). After that, the %CV of most plates continued to be <7%. This slight increase in %CV at plate 781 was attributed to the gradual decrease in peak area counts. The peaks continued to be distinctive until plate 860 (Figure 4B).

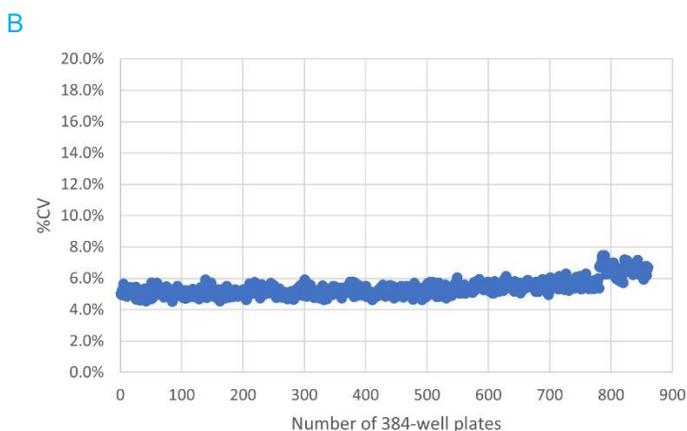
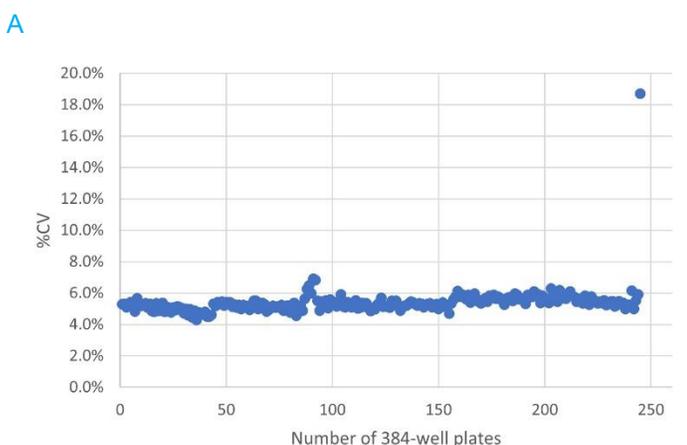


Figure 5. The comparison of %CVs between the baseline study without OPI port wash and the study with OPI port wash after every 384 ejections. (A) Until plate 247, the %CV of DXM remained consistently <10%, rising to almost 20% for plate 248 in the baseline study. (B) The %CV of DXM remained <6% through plate 800 and stayed <7% until plate 860 in the study with OPI port wash.

In both studies, images were captured of the OPI capture port after running plate 249 (Figure 6). In the baseline study, deposits were observed at the entrance of the transfer line within the OPI capture port (Figure 6A). In contrast, the entry into the transfer line within the OPI capture port was free from any observable

particles when a port wash was performed (Figure 6B). These results demonstrate that a scheduled OPI port cleaning is effective for online maintenance of the sample transfer path, while maintaining the optimal flow rate and minimizing instrument downtime. As each of the routine wash cycles lasts only 2–3 minutes, they can be scheduled to occur during plate exchanges. By doing so, the throughput of the screening campaign is minimally impacted.

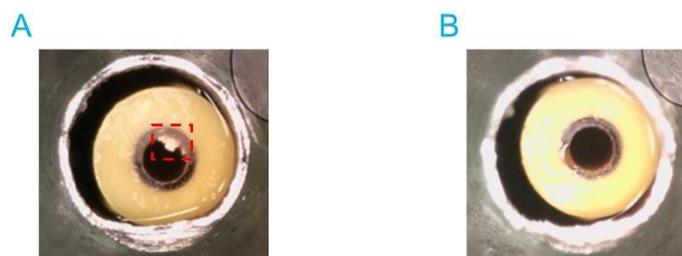


Figure 6. The bottom-up view of the OPI port after running plate 249. Images show the OPI from (A) the baseline study without the OPI port wash and (B) the study with OPI port wash performed after every 384 ejections. Both images were captured after running plate 249. The deposit inside the electrode assembly was clearly visible without the OPI port wash (red dashed rectangle), whereas no distinct foreign components were observed with the OPI port wash.

In this study, the carrier solvent was 50:50 (v/v), acetonitrile/methanol. This combination was used to simulate a worst-case scenario, as the highly organic solvent caused matrix precipitation in the OPI capture port. Moreover, the sample matrices were HTS-relevant and contained salts, detergents and proteins, all of which had the potential to precipitate in the organic carrier solvent. The wash solvent was 50:50 (v/v) methanol/water. The higher aqueous content of the wash solvent improved the maintenance efficiency. With just 50% aqueous wash solvent, we have reported a >3-fold increase in the running uptime of the transfer line.

Conclusions

- The OPI port wash was effective in increasing the acquisition uptime from 249 plates to more than 860 plates
- A >3-fold increase in the running uptime of the transfer line was achieved using challenging solvents
- The OPI port wash can be used as "end-of-day" maintenance or can be seamlessly integrated into automated HTS campaigns

References

1. Häbe TT, Liu C, Covey TR, et al. Ultrahigh-throughput ESI-MS: sampling pushed to six samples per second by acoustic ejection mass spectrometry. [Anal Chem. 2020, 92\(18\):12242–12249](#)
2. Simon RP, Häbe TT, Ries R, et al., Acoustic ejection mass spectrometry: a fully automatable technology for high-throughput screening in drug discovery. [SLAS Discov. 2021, 26\(8\): 961–973](#)

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