Mass Spectrometry in Drug Discovery: Investigating Ion Suppression across Screening Platforms

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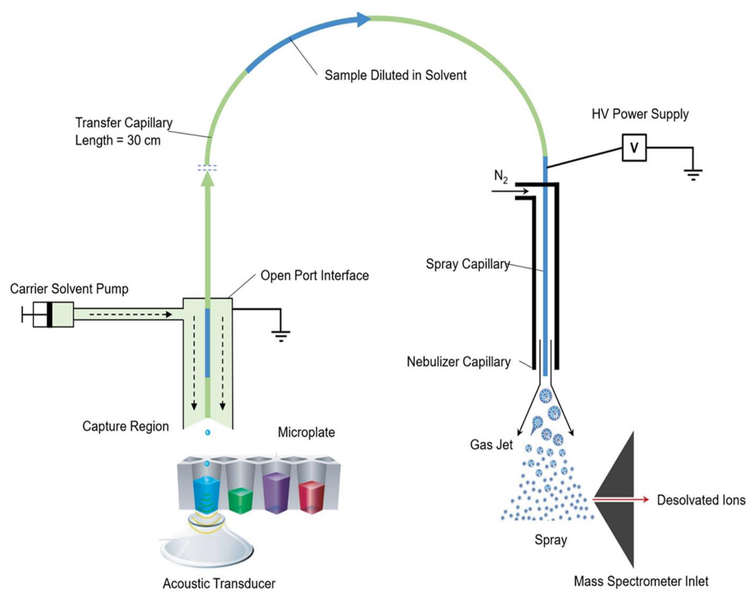
Background

- Over the past few decades, significant advancements in mass spectrometry (MS) have had remarkable impact on drug discovery and development.
- One of the most commonly employed MS-based platforms is the Agilent RapidFire®, a front-end sample introduction system that utilises on-line solid phase extraction (SPE) yield approximate cycle times of 7 to 10 seconds.[1,2]
- In 2020, SCIEX released their Echo® MS system; an instrument that utilises acoustic droplet ejection (ADE)-open port interface (OPI)-MS sampling. Able to deliver impressive sub-second cycle times,[3] the system ejects nano-litre (nL) droplets from a well plate directly into the MS source as illustrated in **Figure 1**.

Figure 1. ADE-OPI-MS Schematic [4]



- Whilst MS-based screening has proven invaluable in the drug discovery space, a major caveat to this technology is ion suppression. This is defined as an alteration of ionization efficiency of a target analyte due to co-eluting matrix components.[5]
- Ion suppression is analyte, matrix and instrument dependent, occurring in the ion source. The main consequences are increased limits of detection (LoDs), poor signal-to-noise (S/N) ratio and a reduced linear dynamic range.
- The most comprehensive ion suppression mitigation strategy is the removal of suppressive components from sample matrices. Among popular techniques are SPE and sample dilution, which are carried out on-line by the RapidFire® and Echo® prior to MS injection.

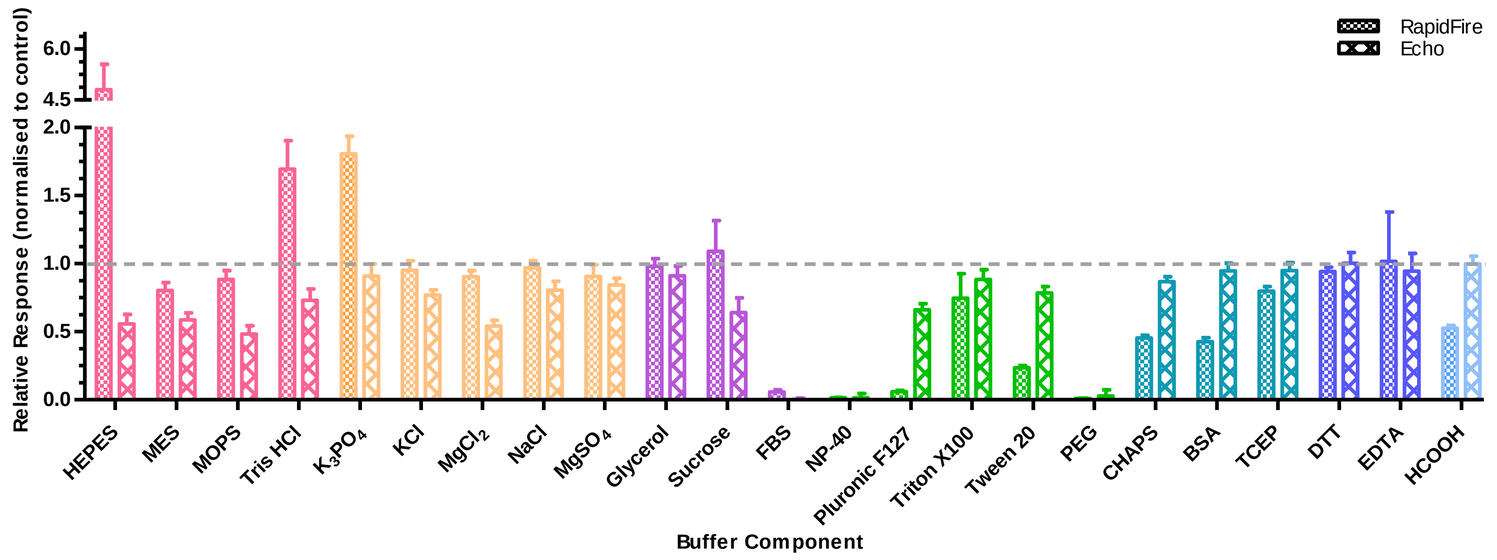
Objectives

- Evaluate the efficiency of the on-line sample preparation of both RapidFire® and Echo® tandem MS screening platforms with respect to ion suppression.
- Identify common assay buffer components that cause ion suppression.
- Assess impact of ion suppression on analyte limit of detection (LoD).

Methods

- All experiments were prepared in Echo® qualified 384-well clear flat bottom plates and were analysed on both screening platforms in succession.
- MS response of small molecule analytes choline and acetylcholine were monitored throughout this study, however only acetylcholine data has been shared in this publication.
- For identification of ion suppression effects, 45 µL aliquots of each buffer stock solution were added to the assay plate. 5 µL aliquots of a 25 µM solution of choline:acetylcholine (1:1) was then added to the plate.
- Serial dilutions of all assay buffer components were conducted in the assay plate. Briefly, 16-step, 1-in-2 serial dilutions were prepared in triplicate at a final volume of 45 µL before 5 µL aliquots of 25 µM choline:acetylcholine 1:1 were added to the full plate.

Figure 2. Relative response of 2.5 µM acetylcholine in aqueous samples containing typical concentrations of various screening assay buffer components.



Component classes separated by colour, from left to right: base buffers, salts, biological additives, non-ionic, zwitterionic and anionic detergents, quench.

- Ion suppression was quantitatively calculated for all experiments by dividing the analyte peak area in matrix by the corresponding water control. This also allowed for direct comparison between platforms.

Results

Impact of buffer on ionisation efficiency

- **Figure 2** illustrates the relative MS responses for acetylcholine on both screening platforms in the presence of buffer components at top concentrations.
- Major response discrepancies between platforms for HEPES, Tris HCl and K_3PO_4, where significant ion enhancement was observed on the RapidFire®.
- Most significant ion suppression observed in presence of non-ionic detergents.

Buffer effects on LoD

- Results from the RapidFire® delivered considerably higher LoDs for all buffer components in comparison to Echo®, with the exception of FBS (**Table 1**).
- Calculated r^2 values all above 0.9, indicating strong linear relationship.

Table 1. LoD and r^2 values for acetylcholine calibration curves in buffer and water

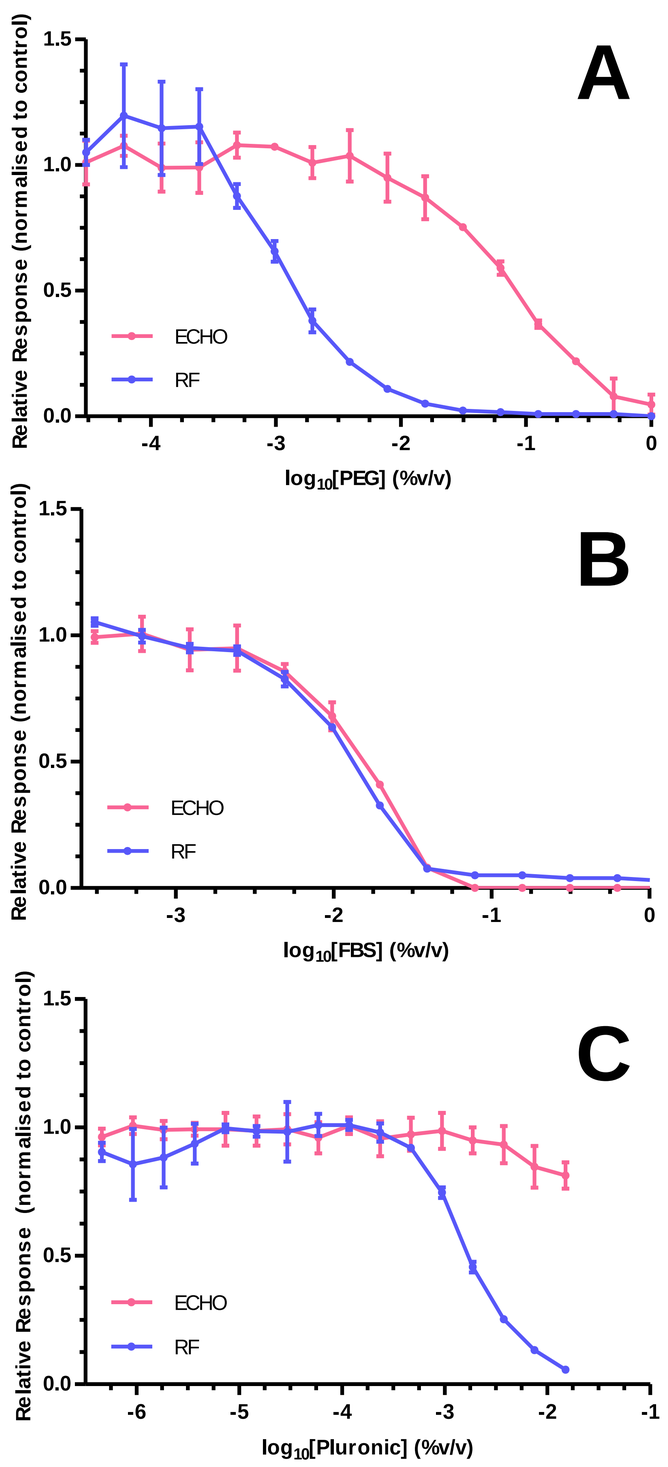
Buffer Component	LoD (µM)		r^2	
	RF	Echo	RF	Echo
PEG	12.50	0.10	0.98	0.98
NP-40	1.56	0.02	0.99	1.00
Pluronic F127	1.56	0.05	1.00	1.00
HEPES	0.78	0.05	1.00	0.99
FBS	>100	>100	N/A	N/A
Tween 20	0.39	0.05	0.99	1.00
Water	1.56	0.02	1.00	0.99

LoD = Mean + 3SD of blank sample

Impact of buffer concentration on LoD

- With respect to PEG and Pluronic F127, Echo® was more tolerant of higher assay buffer concentrations than RapidFire® (**Figure 3A and 3C**).
- Acetylcholine response with respect to FBS (**Figure 3B**) demonstrated identical results for both platforms.
- Inconsistency detected across platforms likely due to differences in modes of on-line separation.

Figure 3. Relative response of 2.5 µM acetylcholine in PEG, FBS and Pluronic F127.



Conclusions

- A comprehensive study of matrix dependent ion suppression effects across two MS-based platforms has been successfully undertaken.
- Results agree with the widely accepted conclusion that ion suppression is analyte, matrix and instrument dependent.
- A significant proportion of commonly used assay buffer components cause ion suppression.
- Echo® afforded remarkably lower LoDs in the presence of assay buffer components, improving analyte sensitivity greater than 100-fold in some cases.
- Echo® ADE-OPI-MS largely successful in mitigating ion suppression effects.

References

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