Evaluation of an Induction-Based Fluidics System for Delivery of Low Volume (nL) Samples

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INTRODUCTION

Chemical warfare agents (CWAs) are highly toxic compounds that induce toxic responses at very small doses when delivered thru percutaneous exposure. For studies employing small animals, manual digital pipettes and syringes have been shown to be neither accurate nor precise over the volume range required. A patented induction-based fluidics (IBF) sampling device (Figure 1) was evaluated as a method to accurately and precisely deliver nanoliter volumes of liquid to the epidermal surface of animals during percutaneous dosing studies. The device employs non-conductive electromagnetic fields to impel micro-droplets from a syringe (Figure 2, Sauter). To measure IBF system sample delivery accuracy and precision under experimental conditions, we developed a quantitative GC/MS method for methyl salicylate (MeS), a G-agent simulant; D_4-methyl salicylate (D_4-MeS) was used as internal standard.

METHODS

Sample Collection Method

- Sample volumes ranging from 5-500 nL were ejected from the IBF device into screwcap autosampler vials containing volumetric quantities of methanol.
- Accuracy and precision - 5 replicates at each volume were collected on 5 different days; 10 - 5 nL replicates were collected
- Accuracy was determined using the % recovery results; precision was determined using the %CV
- Samples were volumetrically diluted a second time to concentrations within the method dynamic range, internal standard was added in a 1:1 ratio with the sample, vials were securely capped and samples were analyzed.

Figure 1. Nanoliter device set-up with digital controller, 5 µL syringe and vial

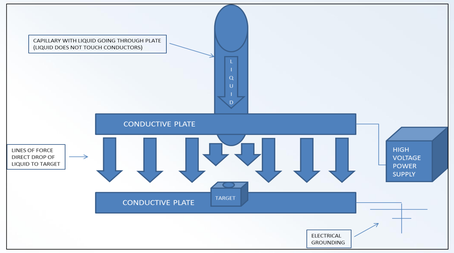

Figure 2. IBF diagram

Analytical Method

- Agilent 7890A GC/5970C MS with a ZB1-MS (0.25 mm ID x 250 µm film thickness x 15 m) column, a splitless GC method with the mass spectrometer operated in SIM mode was validated.
- Characteristic ions for MeS & D_4-MeS are depicted in the spectrum of Figure 3 and listed in Table 1.
- Calibration curve dynamic range was 40-8000 ng/mL (pg on-column).

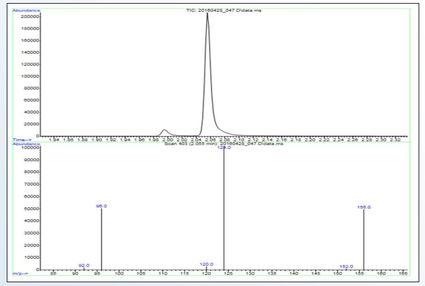

Figure 3. Methyl salicylate and D_4-MeS TIC (top) and spectrum (bottom).

Table 1. Compound properties

Compound	CAS#	M.W. (amu)	Formula	EI –MS Ions (M/Z)
Methyl salicylate (MeS)	119-36-8	152.15	$C_8H_8O_3$	120, 92, 152
D_4- Methyl salicylate (D_4-MeS)	1219802-12-6	156.17	$C_8H_4O_3D_4$	124, 96, 156

RESULTS

Analytical Method Results

- The MeS calibration curve fit a quadratic expression over the dynamic range with correlation coefficients routinely exceeding 0.995. A representative calibration curve is illustrated in Figure 4.
- To meet validation criteria, calibration curve points routinely quantified within ±20% of theoretical; positive controls at relative low and high concentrations were also analyzed with each sample batch and quantified within ±20% of theoretical.

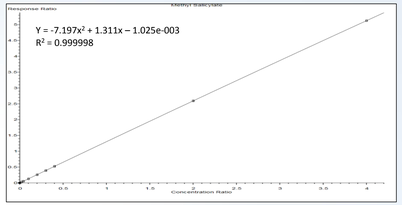

Figure 4. Calibration curve from 5th nanoliter device run; 40-8000 ng/mL MeS.

Sample Delivery Results

- A manual digital Hamilton syringe was evaluated with 10 replicates at each volume with variable results (Table 2).
  - % recoveries ranged from 38.2 – 85.9% with high %CVs (28.6 – 94.7%). These data agreed with previously unpublished data collected by Clarkson et.al., demonstrating the relative lack of accuracy and precision of this method.
- Mean recovery rates for the IBF sample delivery varied across volumes but were relatively accurate, ranging from 65.5 – 104.7% with mean %CV ranging from 1.9 – 60.6% (Table 3).
  - Not surprisingly, the most variable results were found at 5 nL, the lowest volume, where mean recovery rates ranged from 65.5 - 101.9% with mean %CVs between 21.0 – 60.6%.
  - At the 500 nL sample volume, mean recovery ranged from 85.8 - 104.4%, with %CVs between 1.9 – 6.2%, indicating that method accuracy and precision were relatively good at the higher volumes.

Table 2. Manual Syringe Delivery Accuracy & Precision

Manual Syringe Accuracy and Precision		Day 1		Day 2	
		% Recovery	% CV	% Recovery	% CV
Target Volume (nL)	10	44.0	66.9	42.5	54.2
	50	55.8	28.6	62.1	44.2
	100	NA	NA	38.2	94.7
	250	85.9	32.3	80.8	32.9
	500	NA	NA	84.9	37.4

Table 3. IBF Accuracy and Precision

IBF Accuracy and Precision		Day 1		Day 2		Day 3		Day 4		Day 5	
		% Recov	% CV	% Recov	% CV	% Recov	% CV	% Recov	% CV	% Recov	% CV
TARGET VOL (nL)	5	100.7	55.7	82.0	28.5	65.5	42.5	101.9	21.0	85.8	60.6
	10	86.8	12.4	93.4	22.0	89.3	27.0	91.5	10.3	99.5	16.0
	15	82.4	8.5	84.0	17.4	91.8	13.8	95.0	5.0	90.9	6.9
	25	83.2	5.1	92.0	14.0	91.5	8.4	103.8	6.4	84.7	14.7
	50	89.3	7.5	88.2	8.2	89.5	9.5	99.6	6.6	101.8	22.3
	100	88.7	10.1	89.0	4.3	101.6	5.1	99.9	4.2	102.2	5.3
	250	93.4	6.0	80.9	8.1	104.7	5.3	99.4	3.9	100.8	13.7
	500	90.2	3.2	85.8	6.2	96.6	3.7	104.4	1.9	101.2	3.7

DISCUSSION

Initial IBF recovery rates were low, 72.5 – 82.9%, leading to several changes in our method. First, the volume-to-count ratio was visually verified. This ensured that the correct volume was being dispensed. Also, the distance from syringe tip to liquid in the primary vial was minimized and controlled, as was the sample delivery interval, both reducing evaporative loss. After the second run of the study, the syringe tip was noted to be jagged, which could have affected sample delivery; it was therefore given a clean cut. Other confounding variables that could have contributed to lower recoveries and high %CVs included dilution error from the additional dilution steps required to bring sample concentrations within the assay dynamic range. To minimize this error, we aspirated some of the secondary vial's methanol before completely dispensing the primary sample. All of these adjustments resulted in relatively higher accuracy and precision, especially for the larger nanoliter volumes.

The system cannot be universally calibrated across liquids having diverse physical characteristics. A volume-to-count ratio would need to be established for each individual compound to ensure that the correct volume is being delivered. In the future, the method will be evaluated using a less volatile, more viscous liquid to simulate V agents.

CONCLUSIONS

- The IBF system could be a very useful method for delivering nL-quantity samples, since the data indicate that it is a relatively accurate and precise method.
- Further evaluations with compounds of different physical properties need to be conducted.

REFERENCES

1. Sauter, Andrew. nanoLiter MS Sample Prep manual, Nanoliter LLC, 2014.
2. Clarkson, Edward et al., Pipetting study data (Unpublished), November 2013.

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