EVALUATION OF AN ARTIFICIAL SERUM AS A SURROGATE MATRIX FOR CALIBRATION SAMPLES FOR A PREECLAMPSIA RISK PREDICTION TEST

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INTRODUCTION

A specific challenge of developing LC-MS assays for the quantification of endogenous compounds such as biomarkers, is the choice of a suitable matrix for the preparation of calibrators and QC samples. Ideally, these samples should consist of the authentic biological matrix containing an accurately known concentration of the analyte. For endogenous substances, care should be taken using the authentic biological matrix since it typically contains a baseline concentration of the analyte complicating the preparation of calibrators. Furthermore, the use of authentic matrix may lead to lot-to-lot variation affecting the manufacturability of calibrators and QC samples. In isotope-dilution mass spectrometry the use of a surrogate matrix, which mimics the authentic biological matrix as much as possible but is analyte-free, has been propose to overcome this challenge[1,2].

Here we evaluate the suitability of a protein-free substitute for plasma or serum[2,3], SeraSub™ (CST Technologies, New York, USA), as a surrogate matrix for the preparation of calibrators for PrePsia™ (Metabolomic Diagnostics, Cork, Ireland). PrePsia™ is a screening test to predict the risk of preterm preeclampsia in early pregnancy involving the analysis of specific metabolite biomarkers employing LC-MS/MS.

METHODS

- Stable Isotope Labeled Internal Standards (SIL-ISTD) for each metabolite.
- Sample preparation: addition of SIL-ISTDs, protein precipitation and supernatant transfer performed semi-automated using an Agilent BRAVO liquid handling system (turnaround for one 96-well plate <2 h).
- Multiplex LC-ESI-MS/MS assay with a run time of 5 min (Agilent Infinity HPLC coupled to Agilent 6460 Triple Quadrupole mass spectrometer, equipped with an Agilent Jet Stream ion source).
- Preparation of calibration curves (n=6) by spiking 7 calibrator levels per matrix: BSA 5% in PBS, SeraSub™ and human EDTA plasma as comparator.
- Validity of SeraSub™ as a surrogate matrix by assessing:
  - Parallelism of calibration curves in surrogate matrices with calibration curves in authentic matrix, human plasma (slope of a curve combining 6 calibrator sets in surrogate matrix within ±10% of slope obtained in plasma; different intercepts are acceptable, as plasma has endogenous levels).
  - Linearity: correlation coefficient for curve based on all 6 calibrator replicates (r^2>0.95).
  - Repeatability: coefficient of variation for calibrator replicates (CV≤20% (Cal-1); CV≤15% (Cal-2 to Cal-7).
  - Levels of metabolites in unspiked surrogate matrix samples.
  - Matrix effect (preliminary): comparison of absolute ISTD areas (no endogenous levels) in surrogate matrix against plasma.

RESULTS

- Calibration curves in SeraSub™ were visually parallel to the curves in plasma (authentic matrix) for all three metabolites (Figure 1). For the BSA based matrix, only the curve for Met A showed parallelism with the curve in plasma. Calibration curve parameters confirmed these results (Table 1): the slopes for SeraSub™ curves were within ±10% of the respective slopes in plasma (Met A: 0.16-0.2; Met B: 0.07-0.09; Met C: 0.27-0.33).
- Linearity criteria in the investigated calibration interval were met for both surrogate matrices (Table 1).
- Acceptable repeatability was obtained for all calibrators in the three matrices (CV≤20% for Cal-1 and CV≤15% for Cal-2 to Cal-7), as shown in Table 2.
- SeraSub™ showed no traces of the analytes of interest, whereas for BSA this was highly dependent on the procurement source as shown in Figure 2 for Met C. BSA Lot 3 was selected to evaluate the other parameters assessed in this study, based on its low levels of target metabolites.
- The preliminary matrix effect of SeraSub™ on the metabolites was close to plasma (Figure 3).

TABLE 1: Calibration curves parameters (n=6 per level)

	Slope			r^2		
	SeraSub™	BSA	Plasma	SeraSub™	BSA	Plasma
Met A	0.19	0.20	0.18	0.99	1.00	0.96
Met B	0.09	0.10	0.08	0.97	0.99	0.99
Met C	0.29	0.38	0.30	0.95	0.99	0.97

TABLE 2: CV for each calibrator level (n=6)

	Cal-1	Cal-2	Cal-3	Cal-4	Cal-5	Cal-6	Cal-7
Met A							
SeraSub™	4%	13%	6%	5%	4%	7%	5%
BSA	3%	4%	5%	3%	3%	3%	4%
Plasma	10%	6%	8%	7%	8%	9%	6%
Met B							
SeraSub™	16%	12%	3%	10%	5%	8%	9%
BSA	5%	7%	5%	6%	3%	7%	9%
Plasma	7%	8%	11%	10%	5%	5%	6%
Met C							
SeraSub™	12%	10%	11%	12%	13%	13%	13%
BSA	6%	5%	4%	4%	6%	4%	3%
Plasma	13%	10%	9%	14%	5%	8%	14%

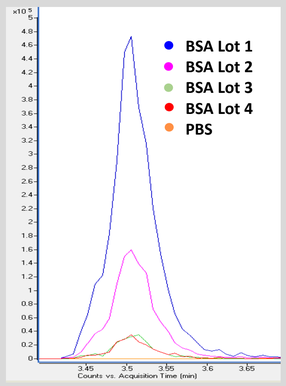

FIGURE 2. Overlay of baseline signals for Met C found in 4 different BSA lots. PBS (orange) was included for comparison.

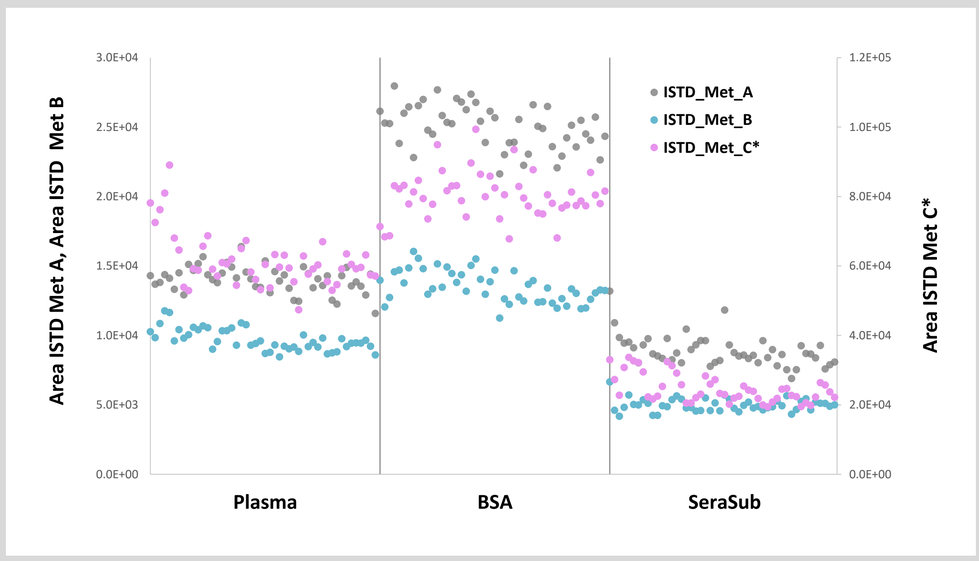

FIGURE 3.
Matrix effect comparison of the surrogate matrices (BSA, SeraSub™) against the authentic matrix (human plasma). *ISTD Met C is not 100% analogue to Met C.

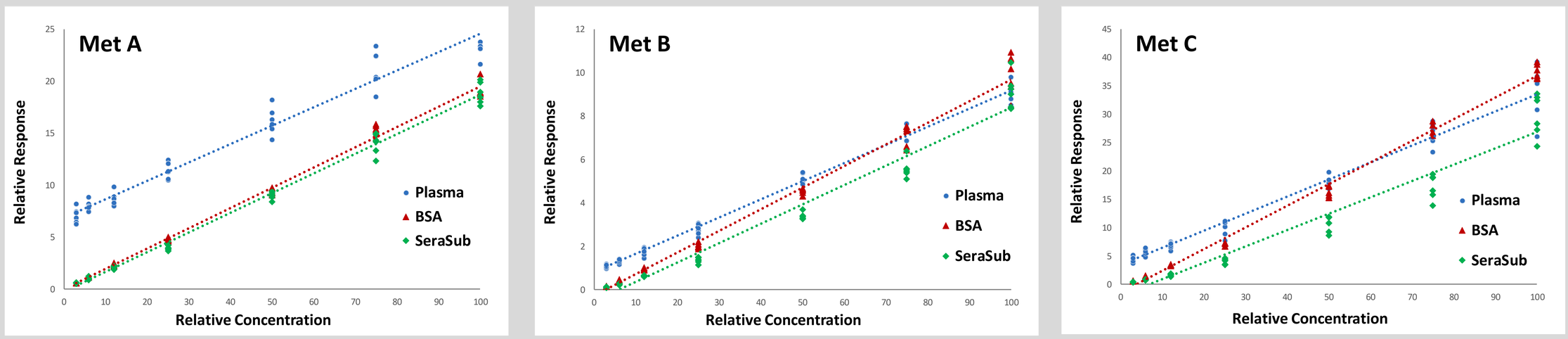

FIGURE 1. Calibration curves in plasma, BSA and SeraSub™ matrices for the 3 metabolites.

CONCLUSIONS

- ❑ Our results show that SeraSub™ is a promising possible alternative surrogate matrix to the well-established surrogate matrix BSA 5% in PBS for LC-MS assays.
- ❑ For the 3 metabolites tested in this evaluation study, SeraSub™ was superior to BSA in terms of parallelism of curves compared to the authentic matrix (human plasma). On the other hand, the performance of BSA, based on the linearity and repeatability, was better than Serasub™.
- ❑ For commercial assay development, the choice between SeraSub™ and other possible surrogate matrices will also consider manufacturability aspects such as price, availability, lot to lot variability and long-term stability.

[1]Thakare R et al. *J Pharmaceut Biomed* 2016, 128:426-37. [2]van de Merbel NC. *Trends Anal Chem* 2008, 27(10):924-33. [3]Hess C et al. *Forensic Sci Int* 2018, 283:150-55.

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