Standard Addition: A Semi-Quantification Tool for Seized Drug Samples by ASAP-MS

Scott J. Campbell[1], John H. Moncur[1], Ben S. Campbell[1] and Nayan Mistry[2]

[1] SpectralWorks Ltd, Runcorn, UK and [2] Waters Corporation, Wilmslow, UK

Introduction

The increasing prevalence of illicit drug use represents a major public health concern, with global drug consumption rising by approximately 70 million people over the past decade[1]. In England and Wales, the number of drug seizures reached a record 269,321 between 2024 and 2025, marking a 24% increase compared with the previous year[2]. Illicit drugs are associated with a range of adverse health outcomes, including addiction, overdose, and long-term physical and psychological harm. Furthermore, the presence of cutting agents and adulterants can significantly increase the risks posed to users. Consequently, the reliable identification of compounds present within seized drug mixtures is essential for both harm assessment and forensic investigations.

Semi-quantification provides valuable insight into the relative concentrations of compounds within complex drug mixtures. Standard addition provides an accessible approach for triage workflows, allowing concentration estimates to be made while minimising matrix-induced variability associated with complex sample compositions. The technique involves spiking aliquots of the sample with known quantities of a target analyte and measuring the resulting increase in analytical response. A calibration curve is then generated, and extrapolation to the x-intercept allows the original concentration of the analyte in the sample to be determined. By compensating for matrix-induced signal suppression or enhancement, standard addition can improve the accuracy of concentration estimates in seized drug samples containing multiple active substances and cutting agents.

Experimental

Sample Preparation:

Three solutions were prepared containing increasing concentrations of alprazolam (50, 75, and 100 µg mL^{-1}), while the concentrations of lidocaine (25 µg mL^{-1}), caffeine (25 µg mL^{-1}), and paracetamol (50 µg mL^{-1}) were kept constant. The analytical response obtained for each solution was plotted against the added alprazolam concentration to generate a standard addition calibration line. For standard addition analysis, a 50 µg mL^{-1} alprazolam solution was used as the nominal unknown. Known quantities of alprazolam were then added to produce spiked samples, where a total concentration of 75 µg mL^{-1} corresponded to a 25 µg mL^{-1} addition.

Table 1. Composition of Simulated Drug Seizure Samples used For Standard Addition Analysis

Simulated Drug Seizure Sample (ug mL^{-1})	Alprazolam (ug mL^{-1})	Lidocaine (ug mL^{-1})	Caffeine (ug mL^{-1})	Paracetamol (ug mL^{-1}) (ISTD)
Unknown	50	25	25	50
Unknown + 25	75	25	25	50
Unknown + 50	100	25	25	50

Instrumental Method:

Samples were analysed on a Waters™ RADIAN™ ASAP mass detector using corona ionisation in positive ion mode. The MS method used a four-function scan experiment (m/z 50–450) with cone voltages of 15, 25, 35 and 50 V. Samples were loaded onto a cleaned (via bakeout) glass rod within the glass capillary inlet device before insertion into the ion source. Data were acquired and processed using SpectralWorks AnalyzerPro™ XD software. Acquired spectra were identified by library matching within AnalyzerPro XD against Waters' Seized Drug Reference Library, with candidate identifications accepted above a match score threshold of 85%.

Standard Addition:

Using the mixture prepared in Table 1, the standard addition method was employed to determine the unknown concentration of alprazolam within the sample matrix. Samples were analysed in triplicate, and AnalyzerPro XD was used to construct a calibration line, from which the unspiked concentration of alprazolam was subsequently determined via extrapolation.

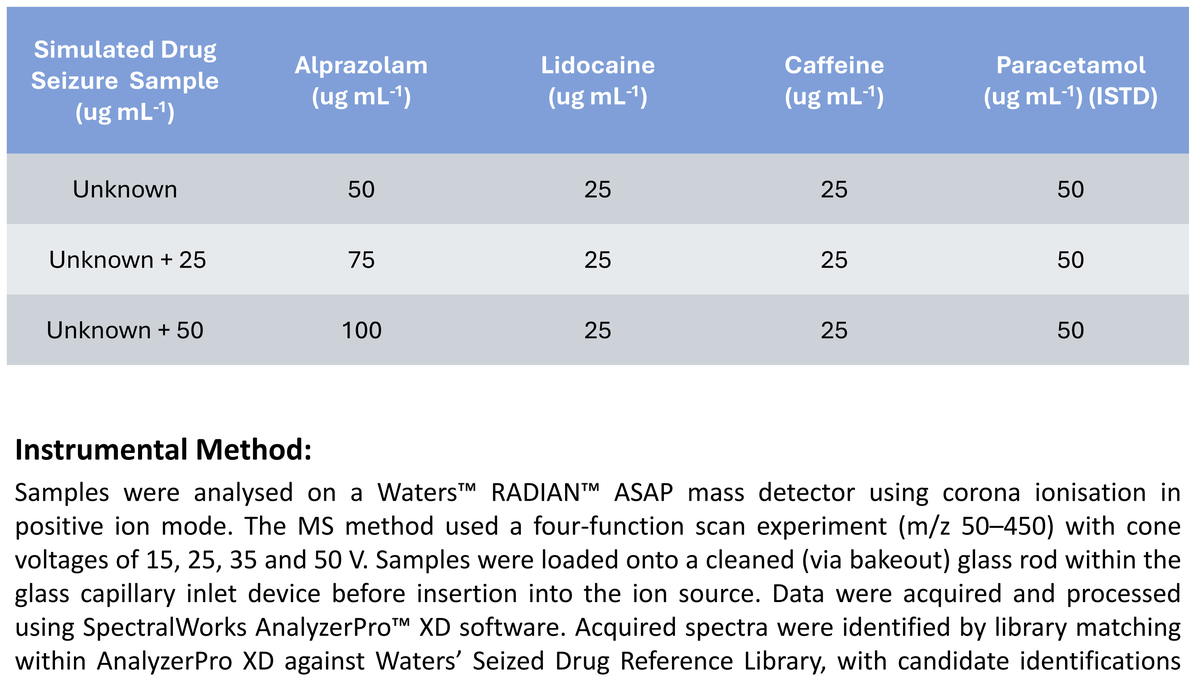

Figure 1. Targeted results from AnalyzerPro XD

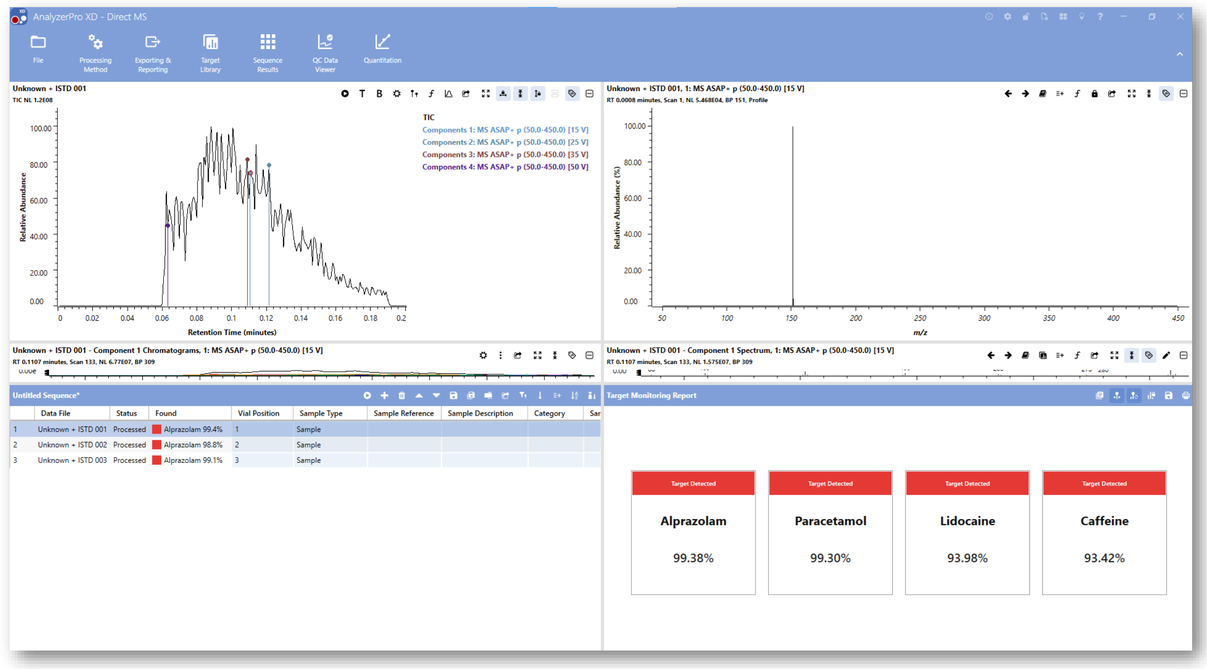

Figure 2. Targeted results from AnalyzerPro XD

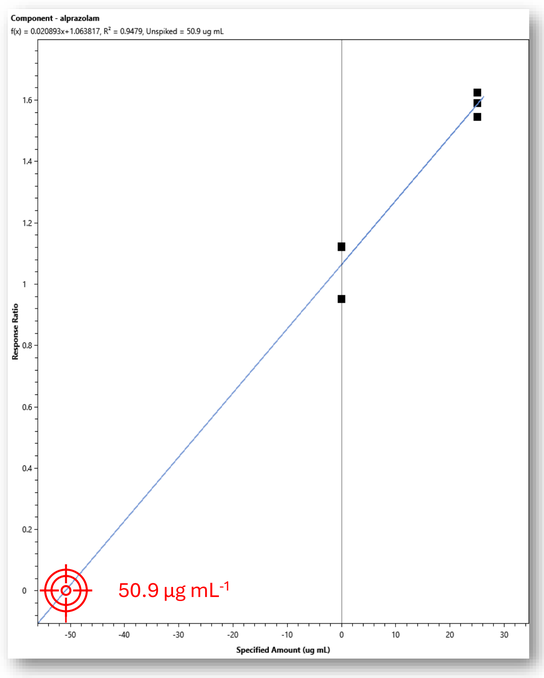

Figure 3. Standard Addition Calibration Line for Unknown and Spiked Samples (25 µg mL^{-1} Spike, 50 µg mL^{-1} IS)

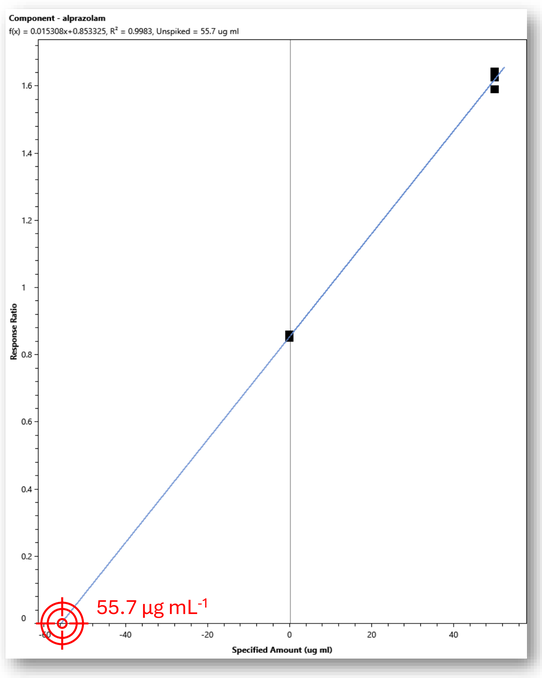

Figure 4. Standard Addition Calibration Line for Unknown and Spiked Samples (50 µg mL^{-1} Spike, 50 µg mL^{-1} IS)

Results

Figure 1 shows the results of initial testing, in which alprazolam, lidocaine, and caffeine were identified in the sample with high confidence scores. To assess the concentration of alprazolam, standard addition was performed using paracetamol as an internal standard at 50 µg mL^{-1}. An aliquot of the unknown sample was spiked with this internal standard and re-analysed; as illustrated in Figure 2, paracetamol was subsequently detected alongside the previously identified analytes.

Figure 3 was constructed using data obtained from the unknown sample containing an internal standard and from the same sample after the addition of a 25 µg mL^{-1} spike. The standard addition method produced an estimated unspiked concentration of 50.9 µg mL^{-1}.

Figure 4 was generated from the analysis of the unknown sample with an internal standard before and after the addition of a 50 µg mL^{-1} spike. The standard addition method determined an unspiked concentration of 55.7 µg mL^{-1}.

Conclusion

This study demonstrates that standard addition is an effective approach for estimating drug concentrations within complex seized-drug matrices. Initial screening using RADIAN™ ASAP mass detector and AnalyzerPro XD successfully identified alprazolam, lidocaine, caffeine, and paracetamol with high confidence. Applying standard addition to simulated seizure mixtures enabled reliable semi-quantification of alprazolam despite the presence of multiple cutting agents. Using two independent spike levels, the method produced unspiked concentration estimates of 50.9 µg mL^{-1} and 55.7 µg mL^{-1}, indicating good consistency and minimal matrix interference. Overall, these results highlight the suitability of rapid ASAP-MS screening combined with standard addition for supporting forensic drug analysis, providing both confident compound identification and robust semi-quantitative information.

References

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