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Reduced drying times enable rapid paper spray analysis of whole blood for clinical toxicology

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Acute recreational drug toxicity is a common reason for presentation to the emergency department, but confirmatory toxicological testing is rarely available. Instead, clinicians rely on patient self-reporting and managing the clinical features. Paper spray ionisation-mass spectrometry enables direct analysis of dried blood spots; however, commonly observed ≥60 minutes drying time restricts clinical utility. This study investigated the impact of reduced drying intervals on analytical performance. Ten analytes were fortified into drug-free whole blood spots as pseudo-unknown samples and dried for 10, 20, 40 or 60 minutes alongside calibration curves. Analyses were performed by a Thermo Fisher Scientific VeriSpray™ Paper Spray Ion Source coupled to a Thermo Fisher Scientific Altis™ Plus Triple Quadrupole Mass Spectrometer. Orthogonal regression compared calibration curves across drying intervals, while pseudo-unknowns were evaluated for bias (%) and precision (% CV). All drying times demonstrated statistical agreement, with 95% CI for slopes and intercepts including 1 and 0 respectively. Precision was excellent (≤1%CV) and bias ranged between −9% and 30%. Ion ratios met acceptance criteria, ensuring reliable identification. Additionally, instrument performance was unaffected by shorter drying times. Whole blood spots dried for as little as 10 minutes yield confirmatory results equivalent to 60 minutes protocols. Reducing sample turnaround time to within 15 minutes of sample collection, this study provides a strong rationale for further translational research to support toxicology workflows in the emergency department and inform management of patients presenting with acute recreational drug toxicity.

Introduction

Illicit drug use remains a substantial public health concern in England and Wales, with an estimated 3 million individuals aged 16 to 59 reporting use between April 2023 and March 2024.[1] In some cases, illicit drug use leads to hospitalisation, contextualised by the 16 994 hospital admissions in England for acute recreational drug toxicity (ARDT) between April 2019 and March 2020.[2] At present, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold standard confirmatory technique in toxicology due to its high sensitivity and selectivity. However, LC-MS/MS usually necessitates extensive sample preparation, extraction, and analysis, making it resource-intensive and time-consuming. Within the Emergency Department (ED), these limitations, compounded by scarcity of available instrumentation, mean that confirmatory analyses are rarely performed in acute toxicity presentations.[3,4] Currently, clinicians rely on self-reported drug histories and clinical features of drug toxicity to guide clinical management. However, this can be unreliable due to drug toxicity limiting the veracity of patient history (due to drowsiness or disorientation), lack of awareness of the drug composition, variable content of illicit drugs, and polydrug use clouding the characteristic clinical features of acute drug toxicity.[5,6] Consequently, there is an unmet need for rapid, analytically confirmed toxicological results to optimise patient management in the ED setting.

Ambient ionisation mass spectrometry (AIMS) enables the direct ionisation of samples under ambient conditions. Based on electrospray ionisation, paper spray ionisation (PSI) was introduced in 2010.[7] The method involves pipetting a small sample volume (≤10 μL) onto a triangular paper substrate and allowing it to dry. The dried spot is positioned near the mass spectrometer inlet, after which solvent is applied. The solvent both wets the paper and extracts analytes by capillary action. Application of a high voltage (3–5 kV) generates an electrospray at the paper tip, producing a so-called chronogram of analyte response within 2 minutes per sample.[8] The VeriSpray Paper

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Spray Ionisation Source coupled with a TSQ Altis Plus Triple Quadrupole Mass Spectrometer automates this workflow following spot drying, enabling rapid, unattended analysis. In comparison with LC-MS, the combination of PSI with dried matrix spots has the potential to deliver essential clinical toxicology data and eliminates chromatographic separation, reducing consumable use, turnaround time, and overall cost per analysis. However, a key limitation remains in that drying time is critical but inconsistently defined in PSI workflows. Reported drying intervals vary widely in the literature with the use of additional materials, ranging from immediate analyses with the addition of coagulants,[9] to 20 minutes using drying ovens.[10,11] In ambient conditions, whole blood has been left to dry for 90 minutes,[10] 120 minutes,[12] or as long as 24 hours;[13] oftentimes, exact drying times are not reported. As a result, there is no currently universally accepted drying time for whole blood spot analysis by PSI, a factor which is incompatible with urgent ED workflows. Despite various strategies having been proposed to accelerate drying, including the use of smaller-pore paper substrates,[14] surface pre-treatment,[9] and heating devices,[10] such interventions are impractical in acute hospital environments, where speed and simplicity are paramount. Furthermore, current literature lacks systematic evaluation of whole blood drying time under ambient conditions. In particular, the analytical impact of short, clinically realistic drying intervals on confirmatory performance in whole blood has not been established; addressing this gap is critical for the translation of PSI workflows into time-sensitive clinical toxicology settings.

This study therefore investigated whether semi-dry whole blood spots exhibit comparable analytical integrity to those that are fully dried. Whole blood spots dried for 60 minutes were used as a reference condition, against which spots dried for 10, 20, and 40 minutes were compared. By systematically evaluating the impact of reduced drying times on analytical performance, this study aims to provide practical evidence to inform drying time selection for time-critical clinical toxicology applications, rather than to define or replace an existing consensus. Additionally, while reduced drying intervals may impact bias and precision for targeted toxicological quantification, the primary objective was to establish whether shorter protocols could still provide reliable analyte confirmation as a baseline requirement. If achieved, this would represent a clinically valuable advance, offering more timely diagnostic support to clinicians managing suspected drug overdoses in the ED.

Methods

Standards and chemicals

Alprazolam, alprazolam-d5, benzoylecgonine (BZE), BZE-d3, cocaine, cocaine-d3, diazepam, diazepam-d5, gabapentin, methadone, methadone-d3, pregabalin, pregabalin-d6, zopiclone, zopiclone-d4 (1 mg mL^{-1} solution), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), EDDP-d3 (1 mg mL^{-1} as perchlorate solution), and ketamine-d4 (1 mg mL^{-1} as HCl salt solution) were purchased from Sigma-Aldrich (Gillingham, UK). Gabapentin-d4 (1 mg mL^{-1} solution) was purchased from LGC Standards (Teddington, UK). Ketamine (1 mg mL^{-1} as HCl salt solution) was donated from TICTAC Communications (London, UK). Stock solutions were diluted in methanol (MeOH) to 25–500 μg mL^{-1} for spiking and stored at −40 °C. LC-MS grade MeOH, acetonitrile (ACN), and formic acid were purchased from Fisher Scientific (Loughborough, UK). Ultrapure water (18.2 MΩ) was supplied by an ELGA PureLab® Chorus 1 system.

Paper spray-tandem mass spectrometry conditions

Samples were analysed using a VeriSpray Paper Spray Ion Source coupled to a TSQ Altis Plus Triple Quadrupole mass spectrometer. Each plate contained 24 triangular Whatman ET31 paper strips, capable of each being positioned 5 mm from the MS inlet. The spray solvent (ACN/H_2O/formic acid, 90 : 10 : 0.1 v/v/v) was dispensed in a programmed sequence: 10 μL with a 3 s delay, 10 μL s^{-1} for 4 seconds, 10 μL every 3 seconds for 9 seconds, 10 μL every 5 seconds for 15 seconds. 4.5 kV was applied to induce electrospray. Data were acquired in positive ionisation mode using selected reaction monitoring (SRM) with four transitions per analyte (Table S1), giving a 2 minutes runtime per sample. Key variables included Q1 and Q3 resolution of 0.7 Da FWHM, ion transfer tube temperature 325 °C, and CID gas pressure 1.5 mTorr. Data acquisition and processing used Thermo Fisher XCalibur™ 4.5 and TraceFinder™ Forensic 5.1, respectively.

Calibration and quality control (QC) sample preparation

Drug-free whole blood was pooled from 10 donors who gave written informed consent (King's College London Research Ethics Committee, RESCM-22/23-10507). For calibrators and quality control (QC) samples, 45 μL was spiked with 5 μL analyte/ISTD mix (Table 1), vortexed for 10 seconds, and 10 μL spotted in triplicate onto VeriSpray™ plates. Spots were dried under ambient conditions for 60 minutes.

Method validation

The method was validated with reference to ANSI/ASB 036 guidelines.[15] Five 8-point $1/x^2$ weighted calibration curves were prepared (Table 1) with regression statistics evaluated in Excel. The limit of detection (LOD) for each analyte was determined experimentally across five calibration runs.

LOD calculations from the standard deviation of the y-intercept (s_y) and the average slope (Avg_m) (eqn (1)).

$$LOD = (3.3 \times s_y)/Avg_m \quad (1)$$

Bias (%) and precision (% CV) were assessed from three QC levels over five runs, though conventional acceptance limits were not applied. Carryover was assessed using blank blood after the largest calibrator, while blank signals were determined from 10 donor spots. Interference was evaluated against 81 analytes (Table S2) and by analysing blank, analyte-fortified,

Table 1 Analytes in the study and their corresponding concentration ranges, QC concentrations, matched ISTDs and ISTD concentrations

Analyte	Calibration range (ng mL^{-1})	QC concentrations (ng mL^{-1})			ISTD	ISTD concentration (ng mL^{-1})
		Low	Med	High		
Alprazolam	50–1500	150	750	1200	Alprazolam-d5	750
BZE	150–3000	450	1500	2500	BZE-d3	1500
Cocaine	150–3000	450	1500	2500	Cocaine-d3	1500
Diazepam	150–3000	450	1500	2500	Diazepam-d5	1500
EDDP	25–500	75	250	400	EDDP-d3	250
Gabapentin	2000–20 000	6000	10 000	15 000	Gabapentin-d4	10 000
Ketamine	150–3000	450	1500	2500	Ketamine-d4	1500
Methadone	50–1500	150	750	1200	Methadone-d3	750
Pregabalin	2000–20 000	6000	10 000	15 000	Pregabalin-d6	10 000
Zopiclone	50–1500	150	750	1200	Zopiclone-d4	750

and ISTD-fortified blood; the method was considered interference-free when signals were <LLOQ and ion ratios inconsistent. Matrix effects (ME %) were assessed as described by Matuszewski *et al.* (2003)[16] by comparing chronogram areas of standard in fortified whole blood and methanol.

Study design

For drying time assessments, 5 μL of QC Med (Table 1) was spiked into 45 μL of whole blood to generate pseudo-unknown QCs. After vortex mixing, 10 μL aliquots were spotted in triplicate onto VeriSpray plates and dried under ambient laboratory conditions for 10, 20, 40 and 60 minutes. Drying was conducted at 23 °C under still-air conditions without covers, desiccants, or forced airflow to reflect the environment expected in an ED setting. Additional calibration curves and QCs were prepared at each interval. Overall, each drying interval (10, 20, 40, and 60 minutes) was assessed across three independent experimental runs alongside freshly prepared calibration curves and QCs, with pseudo-unknown samples prepared and analysed in triplicate at each interval to ensure reproducibility. Orthogonal regression (error variance ratio 0.95) and least squares fits compared 60 minutes curves with shorter drying times; regressions were considered equivalent if 95% confidence intervals (CIs) for slope and intercept included 1 and 0 respectively (Minitab Statistical Software, Version 21.4.3.0). Pseudo-unknown QCs were further assessed for potential systematic bias using Bland-Altman plots (Minitab Statistical Software, Version 21.4.3.0) and for bias (%) and precision (% CV). Conventional acceptance limits were not enforced, as the primary requirement was reliable confirmation. Ion ratios required to meet World Anti-Doping Agency (WADA) criteria,[17] with tolerance windows of ±10 (absolute) for ions >50–100%, ±20% for >25–50%, and ±5 (absolute) for 1–25% relative to the base peak. All analyses were performed for research use only to support the development of clinical toxicology workflows.

System suitability tests (SSTs)

To evaluate instrument performance, SSTs were conducted in between each pseudo-unknown sequence using 5 μL propranolol (100 ng mL^{-1} in MeOH, n = 3) under the paper spray-tandem mass spectrometry conditions outlined previously. The mean analyte area for propranolol was assessed at two SRM transitions (m/z 260.1 → 116.0 and m/z 260.1 → 183.0) and compared against baseline (day zero) reference values obtained from routine analyses to monitor long-term instrument performance. Semi-dry whole blood spots were considered not to impact instrument sensitivity or ionisation efficiency when the average propranolol signal did not fall below 80% of the established reference area. The semi-dry whole blood spots and the MS inlet were visually inspected following each drying interval to assess potential matrix buildup.

Results

Method validation

The regression of the calibration model exhibited strong linearity across all analytes, with the majority of R^2 values exceeding 0.98, large F-statistics, and small residual errors indicating robust model precision and reliable coefficient estimates. Bias was between −5% and 28% across all analytes, while within-run and between-run % CV were within 6% and 4% respectively. Analyte-dependent LODs ranged from 5.7 to 518 ng mL^{-1} in line with individual calibration ranges (Table S3). No interference to the assay was observed from blank, analyte-fortified, and ISTD-fortified whole blood, or interference QCs (Table S2). No carryover was observed after the largest calibrator. ME % were significant in the form of ion suppression, ranging from −80% to −56%, with all analytes showing bias and precision within ±20%, indicating that ion suppression did not negatively impact the quantification of analytes in this study.

Whole blood drying time study

Orthogonal regression was used to compare semi-dry (10, 20, 40 minutes) and fully dried (60 minutes) whole blood spots for each of the 10 analytes. For each comparison, the 95% CI requirements were met with the slope and intercept including 1 and 0. These results indicate that no proportional or constant bias was detected across analytes or drying times. Additionally, the least squares regression lines closely overlapped with that of the orthogonal regression fits, confirming a strong linear association and further supporting agreement

between drying times. For each analyte assessment, the regression equations showed negligible differences in slope or intercept across all drying times, providing further confidence that analyte calibration is unaffected by drying time. As the orthogonal regression fits produced similar results for all analytes and drying times, representative plots for EDDP are shown in Fig. 1, with consistent findings observed across the study. Orthogonal regression assessments therefore confirm that semi-dry conditions at 10, 20, or 40 minutes yield quantitative results statistically indistinguishable from those at 60 minutes. This suggests that the whole blood drying step may be shortened substantially when constructing calibration curves without introducing systematic error into the calibration itself or subsequent quantification.

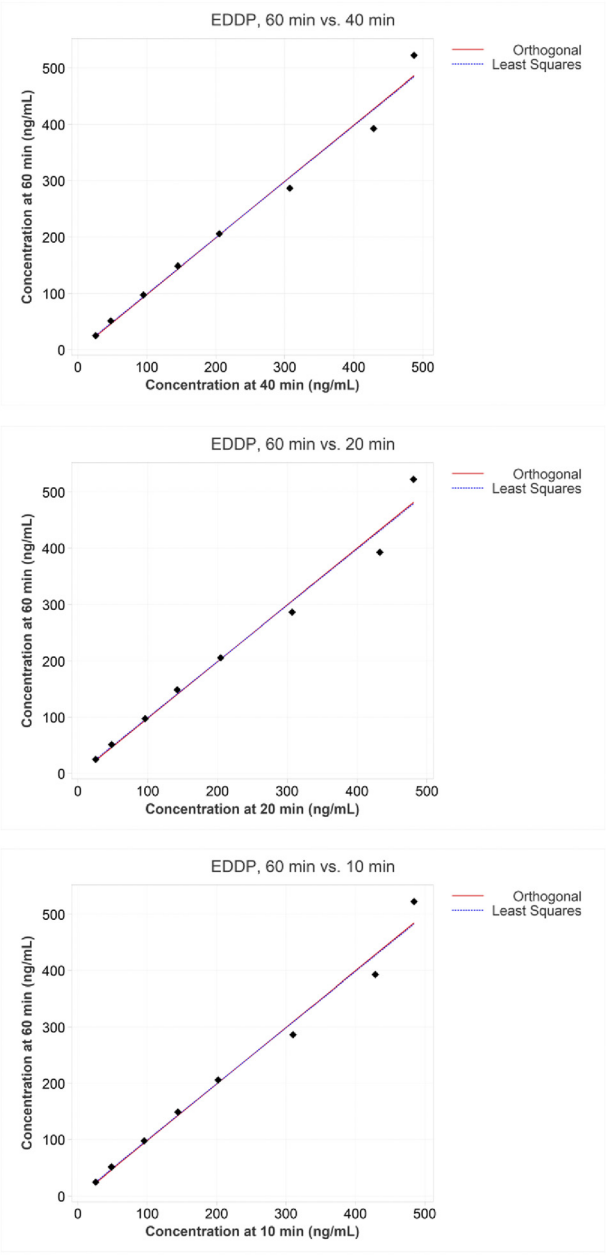

Fig. 1 Representative orthogonal regression plots for EDDP comparing 60 minutes with 40, 20, and 10 minutes drying times. Each plot displays the orthogonal and least squares regression fits. Generated using Minitab Statistical Software (version 21.4.3.0).

To evaluate potential systematic bias not captured by orthogonal regression, Bland-Altman analyses were performed for all analytes across 10, 20, and 40 minutes drying intervals relative to the 60 minutes reference. Representative plots for BZE are shown in Fig. 2a, displaying small mean differences, a finding consistent across all analytes except gabapentin, which fell within the predefined acceptable bias window. This indicates no evidence of proportional or concentration-dependent error and supports the conclusion that reduced drying times do not introduce systematic methodological deviation. Gabapentin showed distinct Bland-Altman plot patterns (Fig. 2b). At 20 and 40 minutes, the mean difference exhibited a pronounced reproducible negative bias relative to 60 minutes, while corresponding 10 minutes comparisons showed a substantially smaller and more clinically acceptable bias.

Bias (%) and precision (% CV) for the triplicate pseudo-unknown QCs across each drying time are represented in Table S4. Across all analytes and drying times, replicate precision was excellent, with % CV consistently ≤1%. This indicates minimal variability between replicates despite shortened drying. Mean bias values for the majority of analytes were between −9% and 13% for all analyte-time point combinations relative to the nominal value, even at 10 minutes drying times, where larger variability was expected. As above, gabapentin was an exception, exhibiting 30% bias when dried for 20 and 40 minutes. The deviations observed for gabapentin at these drying times were consistent across all runs, as evidenced by the low % CV values (≤1%); in Bland-Altman plots, the narrow spread of replicates was also seen, overall indicating that the effect was reproducible and systematic rather than stochastic or experimental variation.

Fig. 3 illustrates the distribution of bias across drying times for all analytes, with no systematic shift at shorter drying intervals. Furthermore, ion ratios across all analytes and drying times were within WADA tolerances, demonstrating that analyte identification was not compromised at shorter drying times. No significant differences in spray onset time, spray stability, or chronogram profile were observed between drying intervals, indicating that reduced drying times did not impact spray formation or duration. Additionally, visual inspection of the paper strips and MS inlet following each drying interval showed no visible residue buildup on the inlet or the paper tips (Fig. 4). One of the three paper strips prepared at the 10 minutes drying interval exhibited partial wicking of whole blood across the paper surface, although this migration did not extend to the paper tip or affect spray initiation or duration. SSTs using propranolol (500 pg) demonstrated consistent signal responses, with mean analyte areas remaining above 80% of established reference values (Table S5). Together, these findings indicate that reduced drying times and repeated whole-blood analyses did not produce any mea-

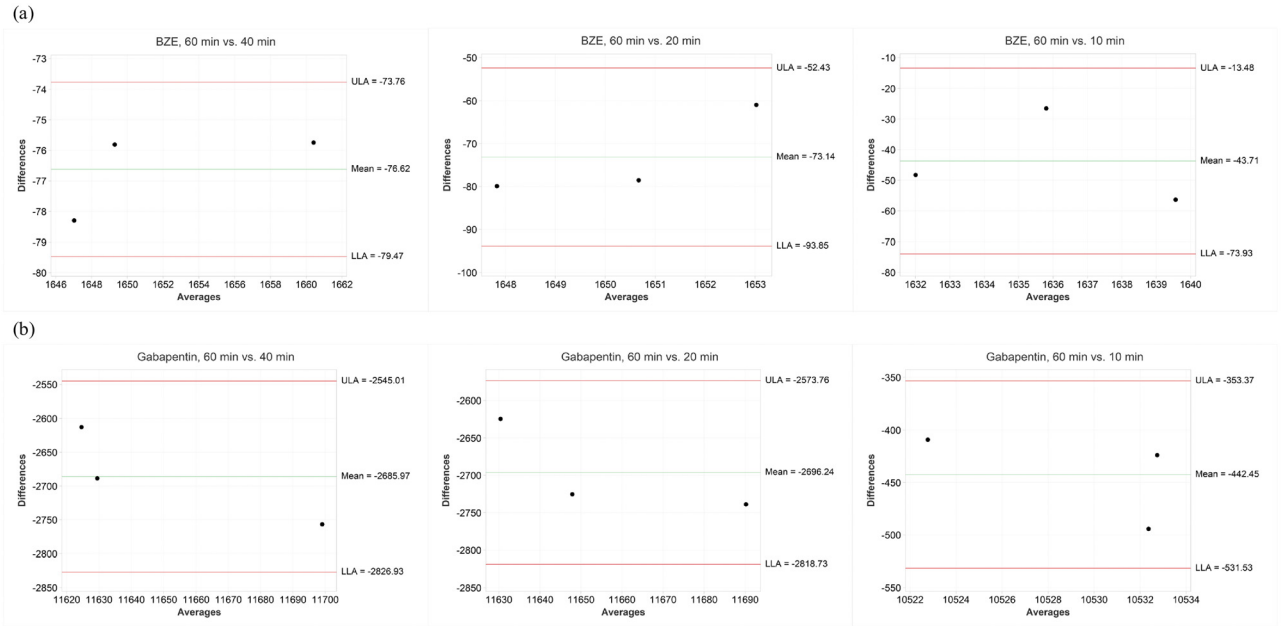

Fig. 2 Representative Bland-Altman plots for BZE (a) and gabapentin (b) comparing 60 minutes with 40, 20, and 10 minutes drying times. Generated using Minitab Statistical Software (version 21.4.3.0).

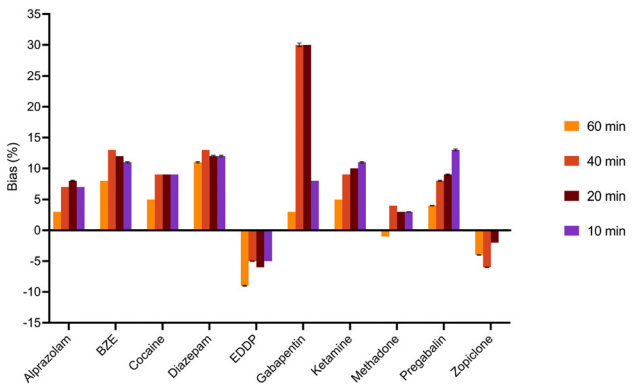

Fig. 3 Distribution of bias (%) across drying times (10, 20, 40, 60 minutes) for representative analytes. Bars show mean values from triplicate measurements; error bars represent the % CV. Generated using GraphPad Prism (version 10.6.1).

surable loss of sensitivity or change in ionisation efficiency over the duration of the study.

The combined regression and pseudo-unknown QC analyses demonstrate that semi-dry (10, 20, 40 minutes) and fully dried (60 minutes) blood spots are effectively interchangeable for the quantification of the 10 drugs of abuse observed in this study when analysed by PSI-MS. Orthogonal regression confirmed that slopes and intercepts were statistically indistinguishable from the line of identity, ruling out systematic bias. Evaluation of pseudo-unknown QCs reinforced these findings, with replicate precision consistently ≤1% and bias ≤30% across all conditions. Importantly, even at the shortest 10 minutes drying time, results were robust across all analytes and concentrations. Together, these data support the feasibility of shortening the drying step without compromising quantitative accuracy, precision, repeatability or ionisation efficiency, enabling faster turnaround of toxicology results in acute clinical settings.

Discussion

This study investigated whether 10 drugs of abuse in semi-dry whole blood spots could provide reliable confirmation compared to the conventional 60 minutes protocol. Typically, drying times ≥60 minutes are employed for whole blood to ensure spot homogeneity and stability on the paper substrate,[18] although efforts have been made to shorten the extensive recommended drying times. Wang *et al.* (2013)[14] investigated the use of ACN applied to wet blood spots on print paper to stabilise the wet matrix without drying the spot. Espy *et al.* (2012)[9] similarly used potassium aluminium sulphate to clot freshly spotted whole blood, enabling immediate analysis; by contrast, dried blood spots required a 2 hours drying time. Shi *et al.* (2015)[10] placed whole blood spots into a cartridge dryer or oven at 40 °C, reducing drying times from 90 minutes in ambient conditions to 20 minutes at warmer temperatures. Nevertheless, we were unable to locate studies which directly investigated the outcome of reduced drying intervals to accelerate clinical turnaround. Additionally, while published studies are beneficial for wider applications, they introduce a myriad of additional consumables and equipment, which would impact cost per analysis and sample turnaround time in a clinical setting.

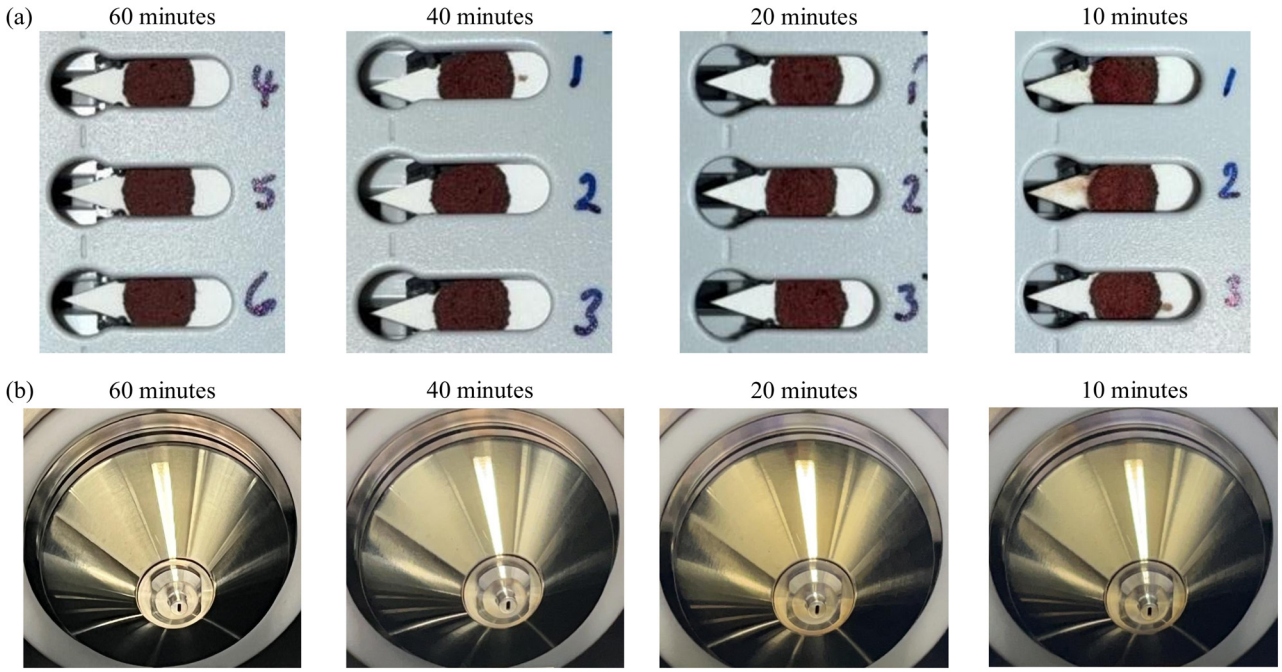

Fig. 4 Photographs of (a) triplicate paper strips at each drying interval and (b) the MS inlet after analysis of each drying time sequence. Whole blood spots did not wick to the paper tip, except minor wicking for replicate 2 at 10 minutes, which had no negative impact on the study. No inlet contamination was observed with the naked eye, and routine fortnightly cleaning was sufficient throughout the study, indicating the absence of increased maintenance requirements.

LLOQ concentrations were unaffected by varying drying times, indicating that sensitivity was not compromised throughout the study. Despite the baseline requirement of reliable and fast analyte confirmation rather than quantification, all analytes displayed exceptional precision (≤ 1% CV) and bias within −9% and 30%. Bland-Altman analyses further demonstrate that shortened drying does not introduce unacceptable systematic error for the analyte panel. Interpretive bioanalytical guidance cut-offs in toxicology broadly adhere to within ±20% limits for bias, and 20% CV limits for precision.[15,19,20] However, these are often adopted for broad LC-MS applications such as toxicokinetic, bioavailability, and forensic toxicology studies, and as such, are not necessary to maintain clinical utility with PSI investigations. Rather, stringency should be placed onto ion ratios to ensure adequate analyte identification, as reflected in WADA ion ratio limits.[17] Furthermore, clinically relevant thresholds differentiating therapeutic and toxic levels of xenobiotics often interweave and span several concentration levels,[21] at which point analytical deviations of ±30% are unlikely to alter risk stratification or patient management. System robustness is critical when introducing analytical workflows to a clinical setting. Consistent spray onset and chronogram profiles across all drying intervals further support that semi-dry whole blood spots did not alter ionisation dynamics, including when minor wicking was observed. No visible residue or signal deterioration was observed, and routine fortnightly cleaning of the ion source and ion transfer tube remained sufficient. Additionally, the consistency of SST results and minor whole blood wicking across all drying intervals supports that neither reduced drying times nor repeated whole blood analyses contribute to matrix-induced signal suppression. Although non-visible matrix components cannot be completely excluded, the stable SST performance and unchanged cleaning frequency suggest that any residual deposition did not adversely affect instrument robustness in this context. These results reinforce the suitability of PSI-MS for short turnaround toxicological testing without routine instrument recalibration or source cleaning between runs.

By nature, PSI does not require sample extraction, unlike LC-MS applications, by use of solid-phase and liquid-phase extraction procedures. As a result, unextracted whole blood analyses pose greater analytical challenges than unextracted plasma or serum, in part due to its high protein content influencing analyte distribution and desorption efficiency.[22,23] Additionally, analytes monitored in this study display variability in their binding to plasma proteins or partitioning into erythrocytes. For example, diazepam[24] and methadone[25] are strongly protein-bound (≥95%), while gabapentin[26] and pregabalin[27] display negligible protein binding (≤5%). Collectively, the variation in bias across the analyte panel reflects the broad physicochemical diversity of compounds analysed using a single workflow on cellulose-based paper, underscoring that variability is inevitable in ARDT settings where multiple drugs are likely to be detected. Nevertheless, the use of whole blood sampling eliminates the need for additional centrifugation

and preparation steps required for plasma analyses, further supporting the rapid analysis offered by PSI.

Despite the overall positive outcome of the study, a unique finding was the non-monotonic bias and larger Bland-Altman negative bias observed for gabapentin at 20 and 40 minutes, but not at 10 or 60 minutes. This is unlikely to be attributed to protein-binding due to its minimal affinity.[26] While gabapentin's polar, zwitterionic structure may render it sensitive to such biases, the precise mechanism remains unclear. Nevertheless, compound-dependent heterogeneities were observed in dried blood spots. For example, at full dryness, analyte redistribution may stabilise into a homogenous pattern, whereas after just 10 minutes, the spot remains fluid, albeit penetrating the paper substrate. Conversely, at intermediate 20 and 40 minutes drying times, as evaporation occurs, capillary-driven outward flow can concentrate solutes at the periphery, termed the "coffee-ring effect", therefore creating heterogenous deposition of the analyte.[28,29] These findings reinforce that while most analytes tolerate shortened drying, compound-specific effects remain an important consideration in method optimisation.

From a translational standpoint, it is encouraging that semi-dry whole blood spots dried for only 10 minutes provided confirmatory results with no significant loss of sensitivity, precision, or identity compared to fully dried samples. The absence of a consensus drying time in the literature underscores the need for systematic evaluation, and the present findings provide practical benchmarks for balancing analytical robustness with clinical turnaround requirements. Unlike previous studies that relied on chemical clotting agents, pretreatment, or elevated temperatures to accelerate drying, the present work demonstrates that reliable confirmatory results can be achieved using reduced drying times alone, without modification of the substrate or workflow. In an ED setting, this could reduce analytical turnaround from over an hour to less than 15 minutes, including both drying and analysis. PSI-MS, therefore, represents near real-time confirmatory analysis with minimal sample preparation. Implementing this workflow could reduce laboratory burden and enable earlier clinical decision-making in cases of ARDT by providing results to directly inform clinical patient management.

While further optimisation could enhance robustness, the primary objective was to establish a rapid and pragmatic workflow for clinical toxicology. Although this study demonstrates that whole blood spots dried for 10 minutes provide equivalent confirmatory performance to a fully dried 60 minutes protocol, the rapid workflow is intended to function as a complementary triage tool rather than a replacement for full quantitative LC-MS/MS analysis. Accordingly, the method is best suited as a binary tool to confirm the presence or absence of drugs within clinically actionable timeframes, rather than to achieve exhaustive quantification within remits of traditional bioanalytical guidelines. Defining the method as a binary tool therefore reflects its intended scope: a fast, confirmatory triage technique that augments, rather than replaces, fully dried whole blood spot workflows in clinical toxicology. Future research should focus on expanding the analyte panel to include a wider range of clinically relevant substances and evaluating capillary blood sampling for routine ED practice. In parallel, developments in modified paper substrates and on-spot hydrolysis and derivatisation techniques should be explored, as these approaches have shown potential to enhance analyte recovery, improve sensitivity, and reduce drying requirements.[30–32] Integrating such advances could further optimise this workflow for rapid confirmatory testing in acute clinical settings.

Conclusion

This study confirmed the robustness of PSI-MS for the rapid quantification and confirmation of semi-dry whole blood spots in toxicological applications. Across 10 clinically relevant analytes, orthogonal regression showed no statistically significant differences between all drying times, and the precision of pseudo-unknown QCs remained excellent throughout. Although bias varied, ion ratios were consistent with stringent WADA criteria, ensuring reliable analyte confirmation. By eliminating the need for prolonged drying, extraction, or chromatography, this study has the potential to support and accelerate clinical decision-making in cases of ARDT. SSTs and MS inlet inspections confirmed that reduced drying times did not impair ionisation or instrument performance. While compound-specific effects were observed, the method's overall robustness highlights its promise as a point-of-care solution in the ED. Ultimately, drying whole blood spots for as little as 10 minutes does not compromise analytical integrity; this has the potential to provide considerable clinical value to support more rapid and accurate diagnosis and management of ARDT presentations to the ED. Further, by demonstrating equivalence between 10 and 60 minutes drying protocols, this study reduces a major temporal bottleneck that may have limited the clinical relevance of PSI of whole blood. Importantly, this improvement is achieved without additional hardware, reagents, or sample handling steps, supporting feasibility in routine hospital laboratory settings.

Author contributions

All authors contributed to the study conceptualisation and design. Material preparation, data collection and analysis were performed by SB. The original draft of the manuscript was written by SB and all authors contributed to review and editing of previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article, including experimental details, method validation results, and system suitability assessments, have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5an01148e.

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