Journal of Mass Spectrometry and Advances in the Clinical Lab 32 (2024) 41–46

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

Research Article

Therapeutic drug monitoring of clozapine in human serum by high-throughput paper spray mass spectrometry

A. Saatchi [a,b], T.M. Zarkovic [a,b], S.A. Borden [a,b], J. Palaty [c], C.G. Gill [a,b,d,e,f,*]

[a] Applied Environmental Research Laboratories, Department of Chemistry, Vancouver Island University, Nanaimo, BC, Canada
[b] Department of Chemistry, University of Victoria, Victoria, BC, Canada
[c] Fraser Health Authority, Vancouver, BC, Canada
[d] Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada
[e] Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA
[f] Canadian Institute for Substance Use Research (CISUR), University of Victoria, Victoria, BC, Canada

ARTICLE INFO

Keywords:
Clozapine and norclozapine
Therapeutic drug monitoring
Paper spray mass spectrometry
Tandem mass spectrometry
High-throughput

ABSTRACT

Introduction: Monitoring the atypical antipsychotic drug clozapine is crucial to ensure patient safety. This article showcases a high-throughput analytical method for measuring clozapine and its primary metabolite norclozapine (N-desmethylclozapine) in serum using paper spray mass spectrometry (PS-MS).
Objectives: This study aimed to assess the viability of a PS-MS method for the rapid measurement of clozapine and norclozapine in human serum samples as an alternative to liquid chromatography mass spectrometry (LC-MS).
Methods: Serum samples were processed by protein precipitation followed by deposition of the supernatant containing labelled internal standards onto paper spray substrates mounted in cartridges. Analytes were then analyzed using a triple quadrupole mass spectrometer equipped with a commercial paper spray ionization source. The results obtained from the patient samples were compared to those from a validated LC-MS assay.
Results: PS-MS calibrations for clozapine and norclozapine were linear ($R^2 > 0.99$) over five days. Between-run precision was below 8 %, and within-run precision did not exceed 10 %. When compared to a validated LC-MS method, the mean bias for 39 patient samples was −9% for clozapine and −1% for norclozapine, with no outliers. Mass spectrometry ion ratio comparisons indicated no interference for patient samples above the lower limit of quantification. There was less than 7 % change in the measured concentrations of both analytes over five days for samples dried on paper substrates. Notably, virtually no maintenance of the MS source was required during this study.
Conclusion: This study illustrates the potential of PS-MS for serum drug monitoring in the clinical laboratory.

Introduction

Therapeutic drug monitoring (TDM) involves regularly measuring drug concentration in patients, and it is essential for ensuring safe and effective dosing. Clozapine, an atypical antipsychotic with a history of evolving recommendations and dosage guidelines, requires TDM to ensure patient safety [1]. Clozapine is only prescribed for treatment-resistant schizophrenia due to serious dose-independent side-effects, which include agranulocytosis and neutropenia [2]. TDM of clozapine is required for preventing dose-dependent side effects such as tonic-clonic seizures, tachycardia, cardiac arrest, and respiratory depression [2,3]. The most clinically significant metabolite of clozapine, norclozapine (N-desmethylclozapine), is measured simultaneously because of its potential to exacerbate side-effects [4]. Serum concentrations of clozapine above 750 ng/mL reportedly cause a five-fold higher rate of tonic-clonic seizures, while concentrations above 600 ng/mL do not correlate with improved treatment outcomes [4,5]. Serum clozapine levels can vary significantly between individuals following a given dose. Several factors influence these levels, including tobacco use, pharmacogenetics, and drug-drug interactions [6–8]. Due to these factors, frequent monitoring

Abbreviations: ESI, Electrospray ionization; LC-MS, Liquid chromatography mass spectrometry; LLOQ, Lower limit of quantitation; LOD, Limit of detection; MRM, Multiple reaction monitoring; MS/MS, Tandem mass spectrometry; PS-MS, Paper spray mass spectrometry; QC, Quality control; TDM, Therapeutic drug monitoring.

* Corresponding author at: Applied Environmental Research Laboratories (AERL), Department of Chemistry, Vancouver Island University, 900 Fifth Street, Nanaimo, BC V9R 5S5, Canada.
E-mail address: Chris.Gill@viu.ca (C.G. Gill).

https://doi.org/10.1016/j.jmsacl.2024.02.003
Received 30 June 2023; Received in revised form 2 February 2024; Accepted 12 February 2024
Available online 15 February 2024

is necessary to ensure the safety and effectiveness of clozapine treatment.

Clinical laboratories routinely measure clozapine and norclozapine in patient serum by liquid chromatography mass spectrometry (LC-MS), the gold standard for quantitation of most drugs in biofluids. This method provides unparalleled selectivity and precision, but requires significant sample preparation, maintenance, and consumables [9,10]. This has prompted a search for simplified TDM for clozapine: for example, recent LC-MS methods describe blood samples dried on paper, extracted with organic solvent, and introduced into a liquid-chromatograph [11,12]. These methods make use of paper as a convenient sampling tool. In this study, paper is utilized as both a sampling substrate and an ionization platform. By applying voltage to samples deposited on pointed paper strips, the charged analytes are emitted directly into a mass spectrometer. This ionization technique is referred to as paper spray, which is a "soft ionization" method for analytes conducted under atmospheric conditions similar to electrospray ionization.

Paper spray was first described in 2010 as an ambient ionization technique coupled to mass spectrometry (PS-MS) [13]. Direct sampling and analyses of illicit drugs, pharmaceuticals, biofluids, explosives, and environmental pollutants have been described in the literature [10,14–22]. PS-MS, like many direct mass spectrometry techniques, offers rapid sample turnaround and minimal sample preparation. PS-MS often foregoes any sample clean-up and pre-concentration steps by leveraging the paper substrate upon which sample is deposited as a crude but effective chromatographic medium to reduce sample matrix interferences [17,23]. Moreover, sample-to-sample carryover is minimized by the use of a new paper strip for each analysis.

In PS-MS analysis, a small volume of sample (typically $\leq$ 10 μL) is deposited onto the paper strip and left to dry. The dried sample spot and paper strip are then moistened with a suitable solvent immediately before applying the high voltage necessary to generate ions. For quantitative measurements, the PS-MS signals for each analyte in a sample are normalized to co-deposited internal standards. This is achieved by utilizing pre-determined response factors or calibration curves to directly quantify the analytes [14,15,24,16–23]. Using this approach, the quantitative accuracy of PS-MS measurements is comparable to that of LC-MS techniques. Here, we present a method for analyzing serum clozapine and norclozapine in human serum samples using PS-MS. Our method has been evaluated based on the criteria established by the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices [25]. The strategy employed in this study is well-suited for high-throughput applications, made possible by the availability of robotic auto-samplers capable of handling the multiplexed paper strip cartridge plates used, which feature a 96-well sample plate geometry.

Materials and Methods

Solvents and Reference Materials.

LC-MS grade acetonitrile, methanol, formic acid, and water were purchased from Fisher Scientific (Ottawa, ON, Canada). Analytical reference standards of clozapine and norclozapine, as well as their stable-isotope labelled standards (IS) clozapine-d_4 and norclozapine-d_8 were purchased from Cerilliant Corporation (Round Rock, TX, USA). Blank human serum was obtained from MilliporeSigma (Burlington, MA, USA). Anonymized human serum samples were provided by LifeLabs Medical Laboratory Services. As only fully anonymized patient samples were used that were not obtained specifically for use in this study through an interaction or intervention with living individuals, neither informed consent nor IRB review were required.

Standard and Sample Preparation.

Analytical reference standards were added to blank human serum to produce six non-zero calibration standards (5, 10, 50, 100, 500, 1000 ng/mL) for both analytes. Patient samples, standards, and QC (100 μL) were mixed with 150 μL of acetonitrile containing both IS at 100 ng/mL in a 1.5:1 vol ratio to effect a simple protein precipitation. The mixture was briefly vortexed and centrifuged with a mini-centrifuge (Sprout® Plus, Heathrow Scientific, Vernon Hills, IL) for one minute to pellet precipitated materials. Aliquots (10 μL) of the supernatant were spotted on individual, single-use PS-MS sample strips for analysis. Unless otherwise indicated, all measurements were performed in triplicate. Fig. 1 presents the sample workflow used for this study.

PS-MS sample strip plates containing 24 individual paper strips (VeriSpray™ Sample Plates, Thermo Fisher Scientific, San Jose, CA) were used for this study. Sample spots were air dried under ambient conditions for about 5 min prior to analysis.

Mass spectrometry.

Tandem mass spectrometry (MS/MS) measurements were performed with a TSQ-Fortis™ Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific) in conjunction with the PS-MS ionization source (VeriSpray™ Paper Spray Ionization Source, Thermo Fisher Scientific). For all measurements, a 90/9.9/0.1 (% v/v) methanol, water, and formic acid solution was used for both the sample spot rewet and spray solvents. Direct infusion of 100 ng/mL standards with electrospray ionization (ESI) was used to optimize MS/MS parameters for the measurements (ESI source parameters are given in Table S1). MS/MS transitions and instrument-specific parameters are provided in the supplementary information (Table S2, S3, S4) and typical ion signal chronograms are given in Fig. S2. Data analysis was performed using the vendor software (TraceFinder™ 5.1, Thermo Fisher Scientific).

PS-MS Measurements.

PS-MS calibration of clozapine and norclozapine was performed with freshly made solutions daily for five days. Three levels of QC were prepared by spiking pooled blank human serum samples previously shown by LC-MS to be free of the target analytes. Each level was run once (i.e., triplicate measurement) over four days.

The limit of detection (LOD) for each compound was determined as 3.3 times the standard deviation of the five y-intercepts divided by the average slope of the five calibrations [25]. The lowest calibrator was assessed based on the criteria of bias and imprecision, with the acceptability threshold set at less than 20 % for both parameters Accuracy was assessed by comparing the results of 39 patient samples with those from a validated LC-MS method. Additionally, processed sample stability studies were conducted by measuring a patient sample pool at 24-hour intervals over a span of five days, with five measurements taken each day.

Results and discussion

Method Calibration.

Clozapine and norclozapine gave $R^2 > 0.99$ for all PS-MS calibrations over the range 5 – 1000 ng/mL using linear regression with 1/x weighting. Over a five-day period, the relative standard deviations of the calibration slopes were 1.4 % and 4.0 % for clozapine and norclozapine respectively, demonstrating consistent inter-day measurement response. Key results are summarised in Tables 1 and 2, and bias plots for a typical daily calibration are given in Fig. 2. We note that four of five norclozapine calibrations met the requirements for a LLOQ of 5 ng/mL. With a LLOQ of 10 ng/mL for norclozapine, five calibrators were utilized for these studies. The absence of significant bias across the evaluated range indicates that the linear regression model is appropriate.

Method Accuracy.

Anonymous patient samples (n = 39) previously analyzed over several days by a validated comparator LC-MS method in a clinical laboratory (singleton measurements, refer to Supporting Information) were measured in triplicate on one day by PS-MS (Table S7). Discrepancies between PS-MS and LC-MS results for individual samples (Fig. 3) were between −17 % and + 8 % for clozapine and −17 % and + 20 % for norclozapine. No significant outliers were observed. The mean bias for all samples was -9 ± 6 % for clozapine and -1 ± 8 % for norclozapine. These results compare favourably with a proposed allowed performance

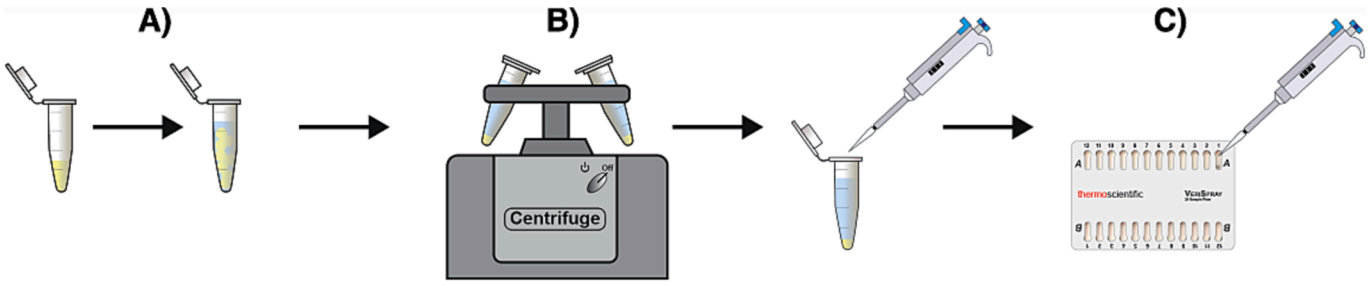

Fig. 1. Schematic workflow for clozapine and norclozapine analysis by PS-MS. A) Serum protein is precipitated with 1.5:1 vol addition of MeCN containing 100 ng/mL internal standard. B) Low-speed benchtop centrifugation for 1 min. C) 10 µL aliquot of supernatant is spotted on a paper strip in a sample plate.

Table 1
Daily calibration data for clozapine and norclozapine by PS-MS.

	Clozapine			Norclozapine		
Day	Slope	Y-intercept	R2	Slope	Y-intercept	R2
1	0.0086	−0.0029	0.997	0.011	0.0054	0.995
2	0.0088	0.0028	0.999	0.012	0.036	0.997
3	0.0088	0.0035	0.999	0.011	0.025	0.995
4	0.0087	0.0045	0.998	0.012	0.010	0.992
5	0.0086	−0.0028	0.997	0.011	0.006	0.995
Average	0.0087	0.0010	0.998	0.012	0.014	0.995

Table 2
Detection Limits for clozapine and norclozapine by PS-MS.

Analyte	LOD (ng/mL)	LLOQ (ng/mL)	Evaluated range (ng/mL)
Clozapine	1.4	5	5–1000
Norclozapine	4.1	10	5–1000

limit of 12 % for values above 100 ng/mL [26]. All samples measured by PS-MS were within the calibration levels of the study, with daily calibrations used for all measurements. Although a sample dilution study was not conducted in the current work, based upon our experience with PS-MS and the agreement with (singleton) LC-MS results, we anticipate that reducing the sample matrix concentrations should not have a negative impact upon PS-MS analytical performance.

Method Precision.

Precision for QC samples at levels measured by the LC-MS comparator method is summarized in Table 3 and graphically presented in the Supporting Information (Fig. S1). While SWGTOX guidelines specify five days for this evaluation, the instrument used for this study became unavailable for QC measurements on the fifth day as it was moved off-site for a dedicated project [27]. None the less, the observed precision for clozapine is acceptable when judged against between-run CV values being less than one-third of the 12 % allowed performance limit [26]. As each QC was only run once each day (as triplicate measurement), the maximum CV of the four triplicate measurements is provided.

As an additional test of specificity, PS-MS qualifier/quantifier ion ratios were evaluated for patient samples (n = 39) against a target of the average ion ratios for calibration standards well above the LLOQ (i.e., levels 3 to 6). All clozapine and norclozapine measurements returned ion ratios within ± 2.5 % of the target for clozapine and ± 6.2 % for norclozapine (Fig. 4). Despite the lack of chromatography, rapid analysis times and minimized sample preparation, the PS-MS analysis of serum samples demonstrated no obvious interference and consistent ion ratio values. Given the wide range of patient clozapine concentrations, it is reasonable to assume a broad range of metabolite ratios as well: the consistent clozapine and norclozapine ion ratios suggest that neither experienced significant interference from other metabolites. Given that SWGTOX guidelines specify ± 20 % for ion ratio agreement [25], this suggests these results illustrate acceptable method specificity.

Processed Sample Stability.

The stability of processed samples stored as dried sera on PS-MS paper strips was investigated using another pool of patient sera. The levels of clozapine and norclozapine were determined by LC-MS (Fig. 5). It was observed that there was no significant change in the measured concentrations of clozapine or norclozapine using PS-MS for dried sera samples over a period of five days, compared to the initial measurement values. The maximum % bias relative to day zero was −6.2 % (Table S8).

A high-throughput method should ideally require minimal instrument maintenance. Paper spray, similar to direct infusion by ESI, does not divert the sample matrix to waste. However, since the MS inlet is potentially exposed to all material deposited on the paper strips, any blockage in the inlet could lead to signal failure and hinder ion transmission. Nevertheless, in this study, the MS inlet maintained its signal strength and reproducibility even after two days of daily calibration runs. In terms of maintenance, only a simple wipe of the MS inlet with a clean tissue was required. Consistent with findings by others, reproducible results can be obtained for at least 240 consecutive samples without any maintenance of the inlet [28]. In general, PS-MS methods leverage the paper sampling substrate as a crude but effective chromatographic medium, reducing sample matrix interferences by retaining them on the paper [17,23]. Although the sample size in our study is

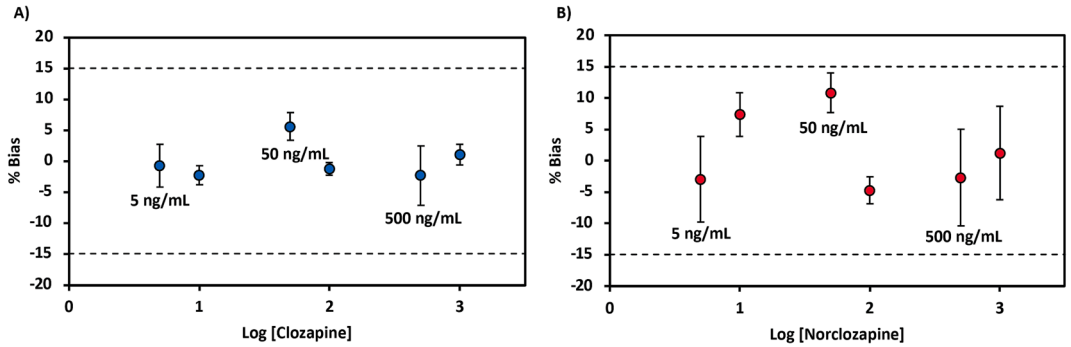

Fig. 2. Bias plots for clozapine and norclozapine (day 3 calibration data; error bars are +/- the standard deviation).

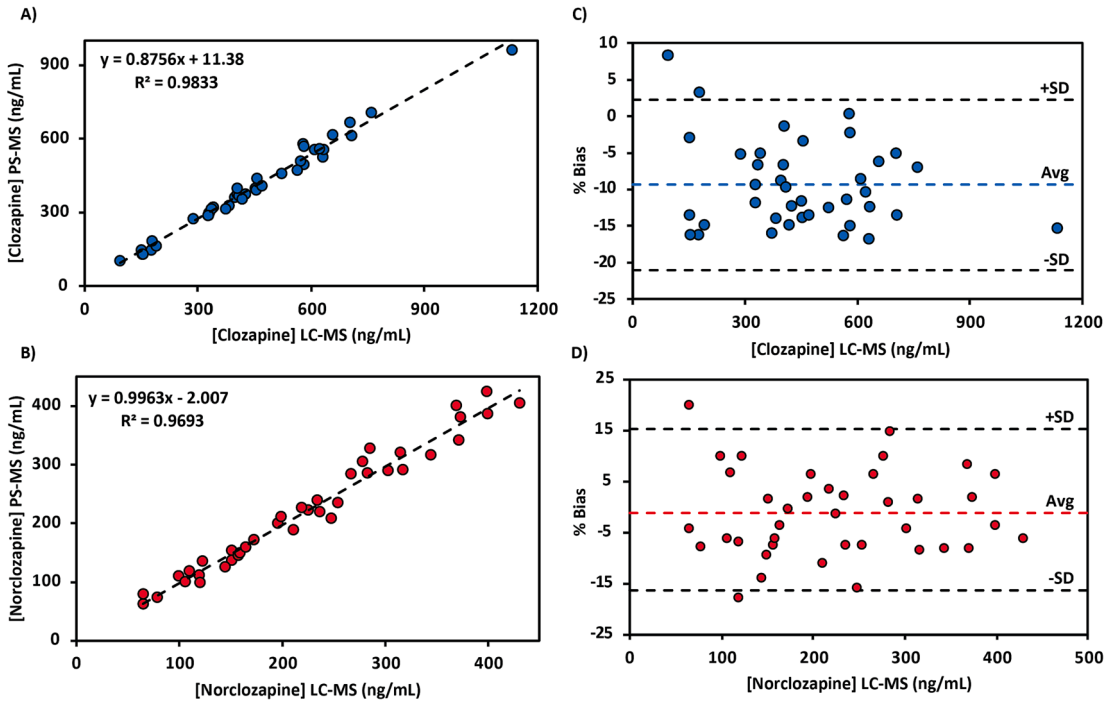

Fig. 3. Comparison of PS-MS and LC-MS analyses of patient samples: A) Clozapine; B) Norclozapine; C) Bias plot for clozapine; D) Bias plot for norclozapine. Least squares linear regression was utilized for panels A and B.

Table 3
PS-MS precision for clozapine and norclozapine QC.

Clozapine	**QC-Low [134 ng/mL]**	**QC-Med [398 ng/mL]**	**QC-High [664 ng/mL]**
Max %CV of Individual Measurements	2.8	1.5	1.5
Between Run %CV	2.9	2.2	2.3
Norclozapine	**QC-Low [92 ng/mL]**	**QC-Med [224 ng/mL]**	**QC-High [309 ng/mL]**
Max %CV of Individual Measurements	5.7	8.1	3.0
Between Run %CV	6.7	9.7	6.1

Ethics doc file deleted.

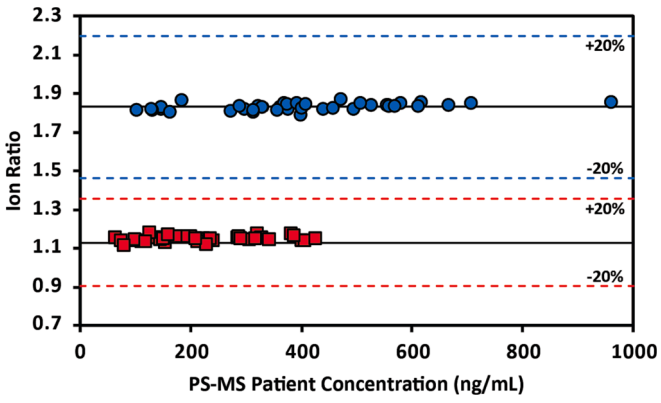

Fig. 4. Qualifier/Quantifier ion ratios for the PS-MS measurement of clozapine (blue circles) and norclozapine (red squares) in patient serum samples. The solid lines represent average ion ratios for clozapine (1.83) and norclozapine (1.13) obtained from calibration standards (Tables S5 and S6).

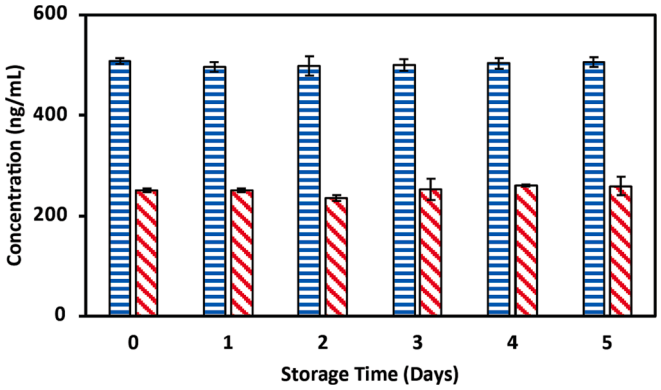

Fig. 5. Stability of serum clozapine [494 ng/mL] (horizontal blue hatches) and norclozapine [256 ng/mL] (diagonal red hatches) for samples dried on PS-MS paper strips and stored under ambient conditions. All processed sample stability measurements were performed 5 times: error bars are +/- the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relatively small compared to the workflows commonly encountered in clinical laboratories, we are encouraged by the simplicity and robustness demonstrated with PS-MS in these investigations. Various techniques to increase MS assay throughput are available, such as isotope tagging or multiplexing HPLCs [29], but they are accompanied by dramatically increased hardware costs and operational complexity that make them unsuitable for any but the largest laboratories. Minimal analysis time is another key requirement of a high-throughput method. This PS-MS method requires less than 10 min for sample preparation (max. 5 min of handling and 5 min of drying time) and only 1.25 min of MS analysis per sample, yielding a total analysis time of less than 11.5 min per sample. Given that 24 sample strips are available on each PS-MS sample plate, and the system plate loader can accommodate up to 10 plates, this approach is amenable to high-throughput, automated measurements. Our group has previously shown that simultaneous, direct PS-MS

analysis of 49 target drugs and their respective labelled internal standards [16] can be achieved in a 1.5 min MS measurement. This illustrates the potential ease of expanding the presented method to other antipsychotics (*e.g.* risperidone). Although not demonstrated in this study, all the preparation steps for PS-MS measurements can be further simplified with an automated workflow using a liquid handler, since the VeriSpray™ sample plate cartridges also employ a 96-well plate geometry.

Conclusion

This study introduces a rapid and robust method for the measurement of serum clozapine and norclozapine using paper spray mass spectrometry. The correlations observed between PS-MS and LC-MS measurements of clozapine and norclozapine in patient samples meet clinical requirements without any outliers. The selectivity of the method was verified in patient samples with concentrations above LLOQ by ion ratios within a ± 7 % tolerance. Furthermore, serum samples dried on PS-MS sample strips yield reproducible measurement results for at least 5 days of storage under ambient conditions. Considering the combination of a rapid analysis time, negligible MS inlet contamination, and the suitability of the presented method for laboratory automation, PS-MS is a viable option for the analysis of serum clozapine and norclozapine.

Ethics statement

Anonymous serum clozapine specimens for Paper Spray Mass Spectrometry drug testing academic research were provided free of charge by LifeLabs via an approved specimen request agreement dated September 7, 2021. No patient information or identification was provided with the samples, only quantitative TDM data obtained by LC-MS. As only fully anonymized patient samples were used that were not obtained specifically for use in this study through an interaction or intervention with living individuals, neither informed consent nor IRB review were required.

Funding information

This work was supported by the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant Program funding (RGPIN-2021-02981).

CRediT authorship contribution statement

A. Saatchi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **T.M. Zarkovic:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **S. A. Borden:** Methodology, Writing – review & editing. **J. Palaty:** Resources, Investigation, Writing – review & editing. **C.G. Gill:** Conceptualization, Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the University of Victoria and Vancouver Island University for their ongoing support of our research and students. We thank Thermo Fisher Scientific for providing the instrument used in this study and for ongoing technical support, and LifeLabs Medical Laboratories for their research collaborations and for providing the anonymous patient samples that enabled this study. The authors are publishing this work in memory of the lead author Armin Saatchi, who passed away suddenly during the preparation of this manuscript. All who knew him will miss his tireless enthusiasm and impeccable research ethics. We dedicate this work to his memory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2024.02.003.

References

[1] J. de Leon, Personalizing Dosing of Risperidone, Paliperidone and Clozapine Using Therapeutic Drug Monitoring and Pharmacogenetics, Neuropharmacology 2020 (168) (May 2019) 107656, https://doi.org/10.1016/j.neuropharm.2019.05.033.
[2] E. Molden, Therapeutic Drug Monitoring of Clozapine in Adults with Schizophrenia: A Review of Challenges and Strategies, Expert Opin. Drug Metab. Toxicol. 17 (10) (2021) 1211–1221, https://doi.org/10.1080/17425255.2021.1974400.
[3] A. Stark, J. Scott, A Review of the Use of Clozapine Levels to Guide Treatment and Determine Cause of Death, Aust. n. z. J. Psychiatry 46 (9) (2012) 816–825, https://doi.org/10.1177/0004867412438871.
[4] M.S.A. Tan, F. Honarparvar, J.R. Falconer, H.S. Parekh, P. Pandey, D.J. Siskind, A Systematic Review and Meta-Analysis of the Association between Clozapine and Norclozapine Serum Levels and Peripheral Adverse Drug Reactions, Psychopharmacology (berl). 238 (3) (2021) 615–637, https://doi.org/10.1007/s00213-020-05746-y.
[5] A.P. Rajkumar, B. Poonkuzhali, A. Kuruvilla, M. Jacob, K.S. Jacob, Clinical Predictors of Serum Clozapine Levels in Patients with Treatment-Resistant Schizophrenia, Int. Clin. Psychopharmacol. 28 (1) (2013) 50–56, https://doi.org/10.1097/YIC.0b013e32835ac9da.
[6] J. Kirchheiner, K. Nickchen, M. Bauer, M.L. Wong, J. Licinio, I. Roots, J. Brockmöller, Pharmacogenetics of Antidepressants and Antipsychotics: The Contribution of Allelic Variations to the Phenotype of Drug Response, Mol. Psychiatry 9 (5) (2004) 442–473, https://doi.org/10.1038/sj.mp.4001494.
[7] T. Haslemo, P.H. Eikeseth, L. Tanum, E. Molden, H. Refsum, The Effect of Variable Cigarette Consumption on the Interaction with Clozapine and Olanzapine, Eur. J. Clin. Pharmacol. 62 (12) (2006) 1049–1053, https://doi.org/10.1007/s00228-006-0209-9.
[8] E. Spina, M.A. Barbieri, G. Cicala, J. de Leon, Clinically Relevant Interactions between Atypical Antipsychotics and Anti-Infective Agents, Pharmaceuticals 13 (12) (2020) 1–33, https://doi.org/10.3390/ph13120439.
[9] S.M. Dong, J. Heathcote, Therapeutic Drug Monitoring of Clozapine and Norclozapine in Human Serum Using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry, J. Anal. Toxicol. 33 (4) (2009) 198–203, https://doi.org/10.1093/jat/33.4.198.
[10] C. Costa, C. Frampas, K.A. Longman, V. Palitsin, M. Ismail, P. Sears, R. Nilforooshan, M.J. Bailey, Paper Spray Screening and Liquid Chromatography/Mass Spectrometry Confirmation for Medication Adherence Testing: A Two-Step Process, Rapid Commun. Mass Spectrom. 35 (S2) (2021) 1–7, https://doi.org/10.1002/rcm.8553.
[11] E. Carniel, K.A. dos Santos, L. de Andrade de Lima, R. Kohlrausch, A. Linden, M. V. Antunes, Determination of Clozapine and Norclozapine in Dried Plasma Spot and Dried Blood Spot by Liquid Chromatography-Tandem Mass Spectrometry, J. Pharm. Biomed. Anal. 210 (2022) 114591, https://doi.org/10.1016/j.jpba.2022.114591.
[12] I. Möller, K. Held, D. Klimpel, T. Nadulski, B. Dufaux, Development and Validation of an LC–MS/MS Method for Relevant Drugs in Epilepsy Patients Using Dried Blood Spots, Biomed. Chromatogr. 35 (9) (2021), https://doi.org/10.1002/bmc.5130.
[13] J. Liu, H. Wang, N.E. Manicke, J.-M. Lin, R.G. Cooks, Z. Ouyang, Development, Characterization, and Application of Paper Spray Ionization, Anal. Chem. 82 (6) (2010) 2463–2471, https://doi.org/10.1021/ac902854g.
[14] G.W. Vandergrift, C.G. Gill, Paper Spray Mass Spectrometry: A New Drug Checking Tool for Harm Reduction in the Opioid Overdose Crisis, J. Mass Spectrom. 54 (9) (2019) 729–737, https://doi.org/10.1002/jms.4431.
[15] G.W. Vandergrift, A.J. Hessels, J. Palaty, E.T. Krogh, C.G. Gill, Paper Spray Mass Spectrometry for the Direct, Semi-Quantitative Measurement of Fentanyl and Norfentanyl in Complex Matrices, Clin. Biochem. 54 (February) (2018) 106–111, https://doi.org/10.1016/j.clinbiochem.2018.02.005.
[16] S.A. Borden, A. Saatchi, G.W. Vandergrift, J. Palaty, M. Lysyshyn, C.G. Gill, A New Quantitative Drug Checking Technology for Harm Reduction: Pilot Study in Vancouver, Canada Using Paper Spray Mass Spectrometry, Drug Alcohol Rev. 41 (2) (2022) 410–418, https://doi.org/10.1111/dar.13370.
[17] S.A. Borden, J. Palaty, V. Termopoli, G. Famiglini, A. Cappiello, C.G. Gill, P. Palma, Mass Spectrometry Analysis of Drugs of Abuse: Challenges and Emerging Strategies, Mass Spectrom. Rev. 39 (5–6) (2020) 703–744, https://doi.org/10.1002/mas.21624.
[18] J. Liu, X. He, Y. He, M. Yu, L. Jiang, B. Chen, Development and Application of Paper Spray Ionization Mass Spectrometry, Prog. Chem. 29 (6) (2017) 659–666, https://doi.org/10.7536/PC170352.

[19] J. McKenna, R. Jett, K. Shanks, N.E. Manicke, Toxicological Drug Screening Using Paper Spray High-Resolution Tandem Mass Spectrometry (HR-MS/MS), J. Anal. Toxicol. 42 (5) (2018) 300–310, https://doi.org/10.1093/jat/bky001.
[20] R.Z. Shi, E.T.M. El Gierari, N.E. Manicke, J.D. Faix, Rapid Measurement of Tacrolimus in Whole Blood by Paper Spray-Tandem Mass Spectrometry (PS-MS/MS), Clin. Chim. Acta 441 (2015) 99–104, https://doi.org/10.1016/j.cca.2014.12.022.
[21] J.E. Keating, J.T. Minges, S.H. Randell, G.L. Glish, Paper Spray Mass Spectrometry for High-Throughput Quantification of Nicotine and Cotinine, Anal. Methods 10 (1) (2018) 46–50, https://doi.org/10.1039/c7ay02204b.
[22] Y. Su, H. Wang, J. Liu, P. Wei, R.G. Cooks, Z. Ouyang, Quantitative Paper Spray Mass Spectrometry Analysis of Drugs of Abuse, Analyst 138 (16) (2013) 4443–4447, https://doi.org/10.1039/c3an00934c.
[23] S.A. Borden, A. Saatchi, E.T. Krogh, C.G. Gill, Rapid and Quantitative Determination of Fentanyls and Pharmaceuticals from Powdered Drug Samples by Paper Spray Mass Spectrometry, Anal. Sci. Adv. 1 (2) (2020) 97–108, https://doi.org/10.1002/ansa.202000083.
[24] J.H. Kennedy, J. Palaty, C.G. Gill, J.M. Wiseman, Rapid Analysis of Fentanyls and Other Novel Psychoactive Substances in Substance Use Disorder Patient Urine Using Paper Spray Mass Spectrometry, Rapid Commun. Mass Spectrom. 32 (15) (2018) 1280–1286, https://doi.org/10.1002/rcm.8164.
[25] Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. *J. Anal. Toxicol.* **2013**, *37* (7), 452–474. Doi: 10.1093/jat/bkt054.
[26] Chemical Pathology Analytical Performance Specifications. *The Royal College of Pathologists of Australasia*. 2022.
[27] A. Larnder, A. Saatchi, S.A. Borden, B. Moa, C.G. Gill, B. Wallace, D. Hore, Variability in the Unregulated Opioid Market in the Context of Extreme Rates of Overdose, Drug Alcohol Depend. 235 (2022) 109427, https://doi.org/10.1016/J.DRUGALCDEP.2022.109427.
[28] Samra, S.; Boeser, C.; Walker, K. L.; Wijeratne, N. R. Robustness Characterization of a Quantitative Whole Blood Assay Using PaperSpray Technology for Clinical Research. *Thermo Sci.* **2019**, Technichal Note 65426.
[29] Netzel, B. C.; Cradic, K. W.; Bro, E. T.; Girtman, A. B.; Cyr, R. C.; Singh, R. J.; Grebe, S. K. G. Increasing Liquid Chromatography-Tandem Mass Spectrometry Throughput by Mass Tagging: A Sample-Multiplexed High-Throughput Assay for 25-Hydroxyvitamin D 2 and D 3. **2010**. Doi: 10.1373/clinchem.2010.157115.