Development of Procedures for Casework Specimen Collection and Processing for Organic Gunshot Residue Analysis

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A Thesis submitted in fulfilment of the requirements for the award

of the degree

Doctor of Philosophy

from

University of Technology Sydney

by

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CERTIFICATE OF AUTHORSHIP AND ORIGINALITY

I, Regina Verena Taudte, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

Regina Verena Taudte

01.03.2016

LIST OF PUBLICATIONS

The chapters presented in this thesis have been published, accepted for publication or prepared for submission to journals as follows:

Chapter 1 - This chapter includes some parts of the literature review:
R.V. Taudte, A. Beavis, L. Blanes, N. Cole, P. Doble, C. Roux, Detection of Gunshot Residues Using Mass Spectrometry, ***BioMed Research International***, 2014, Article ID 965403, http://dx.doi.org/10.1155/2014/965403

Chapter 2 – R.V. Taudte, A. Beavis, L. Wilson-Wilde, C. Roux, P. Doble, L. Blanes**,** A portable explosive detector based on fluorescence quenching of pyrene deposited on coloured wax-printed μPADs**, *Lab Chip***, 2013**, 13,** 4164-4172, DOI: 10.1039/C3LC50609F

Chapter 3 – This chapter has been submitted to the ***Journal of Mass Spectrometry***:
R. V. Taudte, C. Roux, D. Bishop, C. Fouracre, A. Beavis, High-throughput screening for smokeless powders and gunshot residues using RapidFire® with tandem mass spectrometry

Chapter 4 – R.V. Taudte, C. Roux, D. Bishop, L. Blanes, P. Doble, A. Beavis, Development of a UHPLC method for the detection of organic gunshot residues using artificial neural networks, ***Analytical Methods***, 2015, **7**, 7447-7454, DOI: 10.1039/C5AY00306G

Chapter 5 – R.V. Taudte, C. Roux, L. Blanes, M. Horder, K.P. Kirkbride, A. Beavis, The Development and Comparison of Collection Techniques for Inorganic and Organic Gunshot Residues, ***Analytical and Bioanalytical Chemistry,*** 2016, **408**, 2567-2576, DOI: 10.1007/s00216-016-9357-7

Chapter 6 - This chapter has been prepared for submission to ***Forensic Science International:***

R.V. Taudte, C. Roux, A. Beavis, Stability of smokeless powder compounds on collection devices

LIST OF CONFERENCES

The research conducted during this project was presented at several international conferences listed below.

Year	Conference	Presentation
2015	7th European Academy of Forensic Science Conference, Prague (Czech Republic)	The Development and Comparison of Procedures for the Combined Collection of Organic and Inorganic Gunshot Residues
2014	22st International Symposium on the Forensic Sciences, Adelaide (Australia)	The Development and Comparison of Procedures for the Combined Collection of Organic and Inorganic Gunshot Residues
2012	21st International Symposium on the Forensic Sciences, Hobart (Australia)	Development of Procedures for Casework Sample Collection and Processing for Organic Gunshot Residue Analysis

ACKNOWLEDGEMENT

Once upon a time, a girl moved to Australia and started a PhD at the University of Technology Sydney. A few years later…she was finally about to finish. The end of an exciting, mind broadening, fulfilling and sometimes exhausting journey is coming close and I would have never made it to this stage without the help and support of many wonderful people.

First and foremost, I would like to express my deepest appreciation to my supervisors, Associate Professor Alison Beavis and Professor Claude Roux. Thank you for giving me the opportunity to undertake this project, supporting me throughout this incredible journey, encouraging me when encouragement was needed, giving me the freedom to try and follow my ideas and the opportunity to grow as a researcher. Thank you so so much Alison, for spending weekends with me on the shooting range, being incredibly supportive and positive throughout this project and making me always feel accomplished after all our meetings. Thank you Claude! I am indebted to you for your support and trust that made it possible for me to come to UTS and the many opportunities you gave me along the way. Both of you have given invaluable contributions to the preparation of the research output from my doctoral work and I cannot put in words how grateful I am for everything you have done for me.

I would like to thank Dr Lucas Blanes, Dr David Bishop and Professor Philip Doble for their constant help and support especially in regards to micropads and analytical chemistry. Thank you Lucas for the many Brazilian barbecues I enjoyed very much and inspiring me to finally learn the guitar.

I am truly grateful to Sergeant Mark Horder. Without you, this research would not have been possible!

In addition, I would like to thank Elizabeth Chan from NSW Health and Joanna Pryke who initialised this project and provided me with valuable feedback specifically in regards to case work applicability.

I would also like to thank Katie McBean from the Microstructural Analysis Unit (UTS), Dr Richard Wuhrer at the University of Western Sydney and Ken Mason for the support with the SEM and the automated gunshot residue software. My apologies for the many desperate emails I sent you when my computer skills repeatedly abandoned me and I experienced issues with the software.

I would like to show my gratitude to everyone involved in the preparation and review process of manuscripts resulting from this research and in the preparation of this thesis. Your constructive feedback greatly improved the written work related to this PhD and I am extremely grateful for your time and efforts.

I would like to thank Microsoft for developing such an amazing program as office. I am in awe of everyone who had to write a PhD thesis without the possibility to automatically update List of Figure and Tables, as well as references using endnote.

I would like to thank my fellow PhD candidates and colleagues at the university: Ali, Fiona, Nadine, Matt, Dan, Anna, Scott, Marie, Joyce, and many more. Our communal lunches, coffee breaks, Friday bar evenings and other social activities made the last four years more than enjoyable and I am so grateful to have found wonderful friends in many of you.

A big thank you to Ronald and the whole Shimoninski family! Every morning I go to work with a big smile on my face – and this is because of you!

Thank you Claire and everyone involved in the UTS Volleyball Club! You managed to distract my mind from PhD work for at least a few hours per week and reminded me that there is a life outside university.

I would like to thank Maiken, who showed me that every obstacle in life is conquerable and who found surprisingly fun in counting particles.

I want to thank my partner Gabriele for his support throughout the whole time. I know the last few years have not been easy for you and I greatly appreciate all the sacrifices you made to make my life easier even though they made your life harder. You have been there for me, always provided constructive feedback and advices. You have been my motivation and inspiration for undertaking a PhD and I greatly appreciate everything you have done for me! I love you so so much, ti amo, LoYuMuMo! You mean the world to me! Always remember how beautiful you are.

Words cannot express how grateful I am and how much I love my family and friends back home in Germany. Despite the 14,000 kilometres including an ocean separating us, you have been there for me every step of the way. You have given me the strength and endurance to not give up when it became difficult and always focus on the positives. You have always believed in me, even when I doubted myself. You have been my rock, my hope and my strength! I could not think of any better support system and cannot thank you enough for your love and friendship.

To all PhD candidates out there:

As my mum would say:”Bleib übrig!”.

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ABBREVIATIONS

1,2-DNG	1,2-Dintiroglycerine
1,3-DNG	1,3-Dinitroglycerine
2,3-DNT	2,3-Dinitrotoluene
2,4-DNDPA	2,4-Dinitrodiphenylamine
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
2-NDPA	2-Nitrodiphenylamine
3,4-DNT	3,4-Dinitrotoluene
3-NT	3-Nitrotoluene
4-A-2,6-DNT	4-Amino-2,6-dinitrotoluene
4-nDPA	4-Nitrosodiphenylamine
4-NT	4-Nitrotoluene
AAS	Atomic absorption spectroscopy
ACN	Acetonitrile
ANN	Artificial Neural Network
APCI	Atmospheric chemical ionisation
AUS	Australia
Ba	Barium
CE	Collision energy
DBP	Dibutyl phthalate
DCM	Dichloromethane
DDNP	Diazodinitrophenol

DEP	Diethyl phthalate
DMP	Dimethyl phthalate
DNB	2,3-Dinitrobenzene
DNT	Dinitrotoluene
DPA	Diphenylamine
EC	Ethyl centralite
EGDN	Ethylene glycol dinitrate
EtOH	Ethanol
FID	Flame ionisation detector
GC	Gas chromatography
GC-TEA	Gas chromatography coupled to thermal energy analyser
GSR	Gunshot residue/s
h	Hour/s
HMF	Heavy-metal free
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tatrazocine
HPLC	High performance liquid chromatography
IED	Improvised explosive device
IGSR	Inorganic gunshot residues
IMS	Ion mobility spectroscopy
IPA	Isopropanol
ISTD	Internal standard
ISDN	Isosorbide dinitrate
kΩ	Kiloohm

LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LC	Liquid chromatography
LC-QTOF-MS	Liquid chromatography coupled to quadrupole time of flight mass spectrometry
LED	Light-emitting diode
LF	Lead free
LOD	Limit of detection
LOQ	Limit of quantification
MC	Methyl centralite
MECE	Micellar electrokinetic capillary electrophoresis
MeOH	Methanol
mg	Milligram
min	Minute/s
mL	Millilitre
MLP	Multilayer perceptron
mm	Millimetre
mm^2	Square millimetre
MPP	Minimum peak pair
MRM	Multiple reaction monitoring
ms	Milliseconds
MS	Mass spectrometry/ mass spectrometer
MS/MS	Tandem mass spectrometry
MTBE	Methyl tert-butyl ether
NAA	Neutron activation analysis

NB	Nitrobenzene
NC	Nitrocellulose
ng	Nanogram
NG	Nitroglycerine
NGU	Nitroguanidine
nm	Nanometre
N-nDPA	N-Nitrosodiphenylamine
N,N’-DPU	N,N’-Diphenylurea
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide
NPD	Nitrogen phosphorus detector
NSW	New South Wales
NSWPF	New South Wales Police Force
OGSR	Organic gunshot residues
Pb	Lead
PDMS/DVB	Polydimethylsiloxane/divinylbenzene
PETN	Pentaerythitol tetranitrate
pg	Picogram
PMDE	Pendant mercury drop electrode
ppm	Parts per million
PTFE	Polytetrafluoroethylene
QQQ-MS	Triple quadrupole mass spectrometer
R^2	Coefficient of determination
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
resorcinol	1,3-Benzenediol

rpm	Revolutions per minute
RSD	Relative standard deviation
RT	Room temperature
Sb	Antimony
SDS	Sodium dodecyl sulfate
sec	Seconds
SEM-EDX	Scanning electron microscopy with energy dispersive x-ray spectroscopy
SOP	Standard operating procedure
SPE	Solid phase extraction
SPME	Solid phase microextraction
Sr	Strontium
SD	Standard deviation
TEA	Thermal energy analyser
tetryl	2,4,6-Trinitrophenylmethylnitramine
TNB	1,3,5-Trinitrobenzene
TNT	2,4,6-Trinitrotoluene
UHPLC	Ultra-high performance liquid chromatography
UP	Ultrapure
USA	United States of America
UV	Ultraviolet
TOF-SIMS	Time of flight secondary ion mass spectrometry
W_H	Width after heating
W_N	Nominal width
W_P	Width after printing

µg	Microgram
µL	Microlitre

ABSTRACT

The detection and interpretation of gunshot residues (GSR) plays a crucial role in the investigation of firearm related events. Specimens are commonly collected using GSR stubs with double sided adhesive carbon tape. After collection, the stubs can directly be analysed using scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDX), which is widely used for the detection of inorganic gunshot residues (IGSR) as it provides simultaneous elemental and morphological information of discrete particles. Since SEM-EDX analysis focuses on the detection of characteristic GSR particles incorporating the elements lead, antimony and barium, the relatively recent introduction of lead free (LF) and heavy-metal free (HMF) ammunition challenges the current standard operating procedure (SOP) for GSR analysis. Other problems arise from the recent findings of GSR-like particles from environmental and occupational sources. The incorporation of organic gunshot residues (OGSR) into the current SOP can provide additional and complementary information that is alleged to overcome these limitations. This project focused on the detection and incorporation of OGSR to current GSR SOPs on different levels.

A screening technique was developed for the in-field detection of compounds potentially present in smokeless powders and GSR. The technique was based on microfluidic paper-based analytical devices (µPAD) and fluorescence quenching of pyrene and showed promising results for detecting energetic compounds in OGSR. A portable µPAD reader was built and showed potential for in-field detection of GSR (and explosives).

A second screening technique was developed based on solid phase extraction (SPE). This technique can allow pre-concentration and clean-up of samples before

OGSR analysis, which might be necessary considering the low amounts of OGSR that are commonly detected on specimens directly collected after discharge. A proof-of-concept study using a completely automated on-line SPE robot, the RapidFire®, connected to a triple quadrupole mass spectrometer (QQQ-MS) was conducted showing promising results for the pre-concentration and/or screening of OGSR.

To allow the detection of a broad range of OGSR, an ultra-high performance liquid chromatography (UHPLC) method with ultraviolet (UV) detection and mass spectrometric confirmation using a QQQ-MS was developed using a statistical approach (Artificial Neural Networks (ANN)). This approach was applied for the first time to GSR analysis. The network was trained and used for the prediction of retention times of the target compounds in relation to different gradients. The final UHPLC-UV method was fully validated and tested using simulated case specimens collected at an indoor firing range. It proved sufficiently sensitive and selective for the detection of OGSR from hands and the establishment of smokeless powder profiles.

Three different collection protocols for the recovery of OGSR and IGSR from hands were conceptualised to enable both subsequent OGSR analysis by UHPLC-UV and IGSR analysis by SEM-EDX. Comparing the two superior protocols, the extraction efficiencies of OGSR from alcohol swabs and GSR stubs were found to be comparable, while GSR stubs proved to be more efficient in collecting OGSR. Testing the protocols using simulated case specimens taken at the shooting range confirmed that GSR stubs followed by liquid extraction are more suitable than wipes for a combined collection of OGSR and IGSR.

Finally, the stability of OGSR on collection devices, i.e. alcohol swabs and GSR stubs, was investigated for a time period of 63 days. Interestingly, energetic compounds were found to be relatively stable, while stabilisers, often the target compounds for OGSR, degraded mostly following a negative logarithmic curve. This could be problematic for the developed SOP for the collection and analysis of both OGSR and IGSR, since SEM-EDX analysis is preceding OGSR analysis causing the degradation of compounds of interest.

In summary, an SOP for GSR collection and analysis was developed that could potentially overcome problems arising from LF and HMF ammunitions. Further research studies into persistence and background are necessary to test the value of the developed SOP in a forensic framework.