Odour Profiling of Blood Training Aids for Blood-detection Dogs using Comprehensive Two-dimensional Gas Chromatography (GC×GC)

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Certificate of Authorship and Originality

I 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.

Signature of student:

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DEDICATION

In loving memory of my Dad

Dennis Rust

"Time can bring you down, time can bend your knees

Time can break your heart, have you begging please, begging please

Beyond the door there's peace I'm sure

And I know there'll be no more tears in heaven"

-Eric Clapton

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They say that success is not a destination but a journey, and as I come to the end of this adventure I hope that all the people I have met along the way, as mentioned above, know that they have had an integral and lasting effect on my life and future career in forensic science.

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Abbreviations

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^{1}D:	First dimension
1D:	One-dimensional
^{2}D:	Second dimension
2D:	Two-dimensional
3D:	Three-dimensional
BTEX:	Benzene, toluene, ethylbenzene, xylene
CAR:	Carboxen
DNA:	Deoxyribonucleic acid
DVB:	Divinylbenzene
EPA:	Environmental Protection Agency
F:	Fish ratio or Fisher ratio value
F_{crit}:	Critical value of F
GC:	Gas chromatography
GC×GC:	Comprehensive two-dimensional gas chromatography
HCA:	Hierarchical cluster analysis
HRD:	Human remains detection
HS-SPME:	Headspace solid phase microextraction
ID:	Internal diameter
IS:	Internal standard
K_2EDTA:	Dipotassium ethylenediaminetetraacetic acid
LCV:	Leuco-crystal violet
m/z:	Mass-to-charge ratio
MRI:	Magnetic resonance imaging
MS:	Mass spectrometry
MW:	Molecular weight
NADH:	Nicotinamide adenine dinucleotide
NIST:	National Institute of Standards & Technology
NSW:	New South Wales
PA:	Polyacrylate
PC-1:	First principal component

PC-2:	Second principal component
PC-3:	Third principal component
PCA:	Principal component analysis
PDMS:	Polydimethylsiloxane
PMI:	Post-mortem interval
ppm:	Parts-per-million
ppt:	Parts-per-trillion
S/N:	Signal-to-noise ratio
SPME:	Solid phase microextraction
STU-100:	Scent Transfer Unit – 100
TD:	Thermal desorption
TIC:	Total ion current
TOFMS:	Time-of-flight mass spectrometry
US:	United States
UTS:	University of Technology Sydney
VOC:	Volatile organic compound

Publications

Rust L, Nizio KD, Forbes SL. The influence of ageing and surface type on the odour profile of blood-detection dog training aids. Anal Bioanal Chem. 2016;408(23):6349-60.

Rust L, Buis RC. The scent of a crime. Australasian Science Magazine. 2015.

Conferences

Rust, L, Nizio, KD, Forbes, SL, Odour profiling of aged blood training aids for cadaver-detection and blood-detection dogs using comprehensive two-dimensional gas chromatography (GC×GC), 7th European Academy of Forensic Science (EAFS) Conference, Prague, Czech Republic, 10 September 2015

Rust, L, Nizio, KD, Forbes, SL, Investigating the inter-individual variation of blood odour detected by blood-detection dogs utilising GC×GC-TOFMS, 13th GC×GC Symposium, Riva del Garda, Italy, 30 May 2016

Rust, L, Nizio, KD, Forbes, SL, The odour of foul play – validating the detection limits of scent-detection dogs to blood evidence after crime scene clean-up, Australian and New Zealand Forensic Science Society 23rd International Symposium on the Forensic Sciences, Auckland, New Zealand, 20 September 2016

Rust, L, Nizio, KD, Forbes, SL, Investigating the inter-individual variation of blood odour detected by blood-detection dogs utilising GC×GC-TOFMS, Australian and New Zealand Forensic Science Society 23rd International Symposium on the Forensic Sciences, Auckland, New Zealand, 22 September 2016

Abstract

The use of blood-detection and cadaver-detection dogs as investigative screening tools to search for blood evidence is a contemporary addition to many law enforcement agencies around the world. The training protocols for these canines remain unstandardised and the specifics of the blood training aids implemented can vary between agencies. While there is a large field of research investigating the odour profiles of human remains, there has been very little research in a forensic context on specific tissue types such as blood, or that has linked variables in the blood odour with the response rate of these scent-detection dogs in the field.

The aim of this thesis was to investigate the chemical odour profile of blood training aids utilised for the training of blood-detection and cadaver-detection dogs and compare this directly to responses in training, in order to assist in the development of more effective and standardised training protocols. As part of this thesis, headspace solid phase microextraction (HS-SPME) was coupled with comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS), a novel technique that is also only recently being introduced into this field, to analyse fresh and degraded blood samples, from single and multiple donors, as well as latent blood samples.

Three studies were performed to investigate the chemical odour of blood under various conditions, which were compared with the responses of blood-detection and cadaver-detection dogs in scent line-ups conducted with local law enforcement. The first study examined the effect of ageing and analysed the volatile organic compound (VOC) profile of blood as it transitioned from fresh to degraded, over a 24-month period. Blood on two surfaces (non-porous aluminium tin and porous cotton) were compared and it was observed that fresh blood (collected within 24 hours) produced the most distinct profile irrespective of surface, and that as the blood degraded the odour became generalised across the two surfaces. However, comparing functional classes elucidated a common pattern between blood ages and surfaces. Similarly, results from the dog trials demonstrated that the blood-detection and cadaver-detection were more adept at locating fresh blood, with their efficacy becoming reduced as the blood aged. Notably, the samples on the non-porous surface posed a greater challenge for the canines to locate which is an important consideration for training protocols.

The second study expanded on the effect of ageing by replicating the first study over a 6-month period using four different blood donors, to determine if inter-individual differences exist in the

odour of blood. While the overall VOC profile of blood remained consistent across donors, some unique VOCs were identified between individuals as a result of variations in lifestyle, diet, health and environmental exposure. It was confirmed that the effect of ageing has a greater influence on the odour profile of blood and it was concluded that training aids should incorporate both fresh and degraded blood, with a single donor being sufficient. The blood-detection and cadaver-detection dog trials demonstrated the capability of the dogs to locate blood aged up to 6 months old regardless of the donor, but as the blood degraded, this efficacy reduced and displayed minor skewing for different donors.

The third study evaluated the baseline detection limits of the blood-detection and cadaver-detection dogs to latent blood when compared with current analytical instrumentation and a common chemical presumptive test – i.e. luminol. The experiment replicated a scenario in which a suspect may attempt to wash away a victim's blood on clothing to test anecdotal evidence of the sensitivity of scent-detection dogs. It was confirmed that the blood-detection and cadaver-detection dogs are much more sensitive than current analytical instrumentation, and are complementary screening tools to luminol for the detection of latent blood.

The overall results of this thesis provide a greater understanding of the odour of blood as a training aid for blood-detection and cadaver-detection dogs, and will assist in providing scientific support for their deployment as investigative tools if their use becomes challenged in court.