Toxicological analysis of bloodstains and traces

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Aims: The use of liquid blood in forensic toxicology is an approved and accepted practice. In this study, long term stability of selected licit and illicit drugs and the ability to reproduce analytical results obtained from blood, dried after spotting onto different surfaces, were investigated. **Materials and Methods:** Whole blood was spiked with 17 different licit and illicit drugs. Aliquots of 100 µL of this blood were spotted onto five different surfaces: tiles, bed sheet, parquet flooring, wallpaper and carpet. The blood spots were left to dry at ambient temperature and under refrigeration at an average temperature of 4 °C respectively for six months. The samples were analyzed on days 1, 3, 8, 15, 22, 29, 43, 57, 71, 85, 113, 141, 169 and 197 after incubation. Different extraction methods were used for the different materials. Extracts were measured by means of an LC-MS/MS device. **Results and Discussion:** All drugs analyzed for were stable over the evaluated period. The most favorable results were obtained from those surfaces where the blood could easily be collected, e.g. from the tiles and parquet floor and due to the method used for collection, least amount matrix effects could be obtained. For the carpet and bed sheet where the entire stain was cut out, obvious matrix effects could be observed. The quantification for half the analytes was done with deuterated internal standards, based on the known blood volume (100 µL). The other analytes were quantified with the use of a general internal standard and therefore only semi-quantitative results could be obtained. During our study, other dried fluids than blood containing pharmaceuticals or illicit drugs have also been examined including urine, infusion liquid and rubbings. **Conclusion:** The results obtained from the study and other examined fluids show the diversity and possibilities of the analysis of dried bloodstains and other traces from crime scenes.

1. Introduction

Since the beginning of the 20th century, when Ivar Christian Bang discovered a method to measure glucose concentrations in blood taken from an ear vessel of a rabbit [1] the analysis of dried blood spots has continued to develop and is an accepted practice in clinical toxicology today. However, the use of this alternative matrix is not widely spread in the field of forensic toxicology. A number of studies have been reported that demonstrate the improved stability of various drugs in dried blood on a filter card, compared to storage in liquid blood [2]. Yet little is known about toxicological analysis of blood, dried on other surfaces [3]. In this study, the long-term stability of selected licit and illicit drugs and the ability to reproduce analytical results obtained from blood, dried after spotting onto different surfaces, were investigated. Furthermore some cases from routine toxicology are presented here to demonstrate the potential of the analysis of dried blood spots.

2. Materials and Methods

Whole blood, obtained from a voluntary person without any drug treatment was used to spike a blood pool with 17 different licit and illicit drugs in concentrations shown in table 1.

Benzoylecgonine (as metabolite of cocaine) and methamphetamine were chosen as commonly used stimulants. From the broad range of opioids morphine, codeine, methadone and fentanyl were selected.

Tab. 1. Concentrations of the 17 analytes in the blood pool for the long-term study.

Analyte	concentration [ng/mL]
Benzoylecgonine	150
Methamphetamine	50
Morphine	50
Codeine	50
Methadone	150
Fentanyl	5
Nordazepam	400
Lorazepam	30
Doxepin	100
Citalopram	100
Quetiapine	250
Mirtazapine	100
Diphenhydramine	100
Zolpidem	100
Bisoprolol	50
Metformin	1500
Ramipril	5

The two benzodiazepines, nordazepam and lorazepam were chosen because of their wide legal and illegal application in Germany. Some antipsychotics and antidepressant drugs were selected and also bisoprolol, metformin and Ramipril as common medication in Germany.

Whole blood was then spiked with the above mentioned drugs in the given concentrations and stirred for 2.5 hours. Afterwards the homogeneity of the pool was tested by taking six specimens from different locations of the sample and analyzed for the 17 drugs as follows: 100 aliquots of 100 µL each were spotted on five different surfaces. Tiles, parquet floor, wall paper, a bed sheet and a carpet were used to simulate different materials that might represent real crime scenes. Half of the samples were stored at ambient temperature and the other half in a refrigerator at approximately 4°C for 6 months. The samples were then analyzed for the spiked analytes at irregular intervals. At the beginning of the study, samples were analyzed more frequently because instability and degradation is more likely to occur within the first days and weeks of storage.

The extraction of the samples was different for each surface and was performed as follows: Blood spots from tiles and parquet were scraped with a scalpel, from wall paper blood was wiped off with a wet swab. Blood stains on the carpet and bed sheet were cut out entirely. All of the samples were reconstituted with different amounts of purified water (100 µL for the blood from tiles and parquet, 500 µL for wall paper, 1 mL for bed sheet and 2 mL for carpet, respectively), due to the absorbency of the respective material. A mixture of internal standards was added to all samples, after which they were shaken for 20 minutes. A protein precipitation with acetonitrile was carried out, the samples were centrifuged and the supernatant was evaporated to dryness under nitrogen flow. The residue was reconstituted with buffer solution and measured via LC-MS/MS.

3. Results

All 17 drugs could be detected after six months of storage on five different materials at two different temperatures. The analytes for which a deuterated internal standard was used could be quantified, based on the known applied volume of 100 µL blood. These results are shown exemplarily for morphine in figure 1.

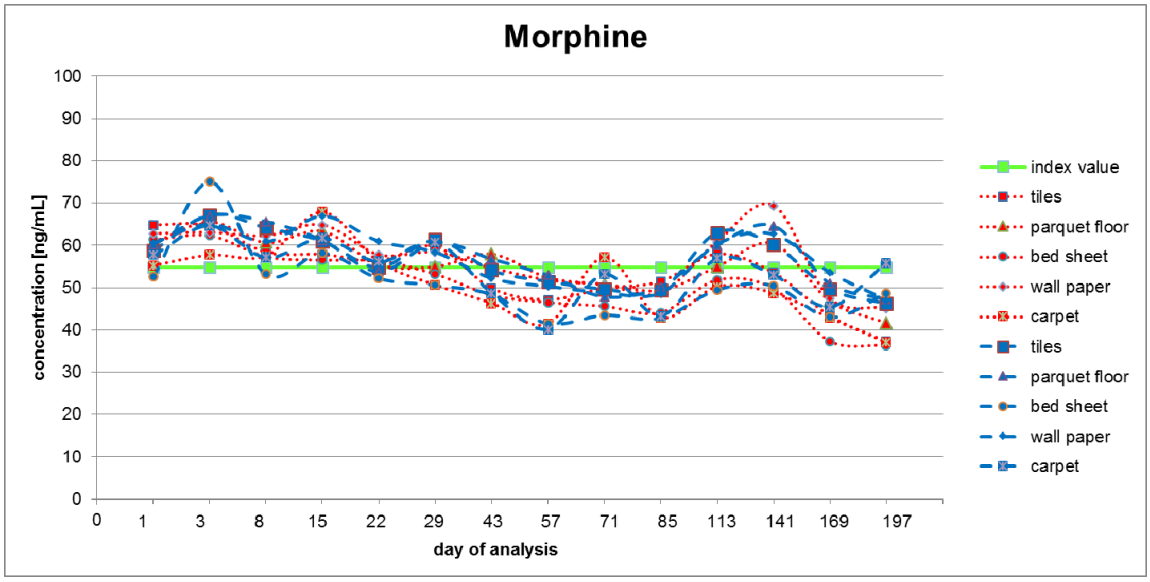

Fig. 1. Long-term-stability of morphine over a period of six months.

Semi-quantitative results could be obtained for the other substances from tiles and parquet. Here quantification could be achieved by using MPPH as internal standard. Those surface materials which were cut out entirely, i.e. bed sheet and carpet and also the swabs taken from the wall paper produced no clean spectra. High noise levels were visible and suppression effects could be seen. From these materials the analytes without deuterated internal standard could not be quantified. These results are shown exemplarily for quetiapine in figure 2.

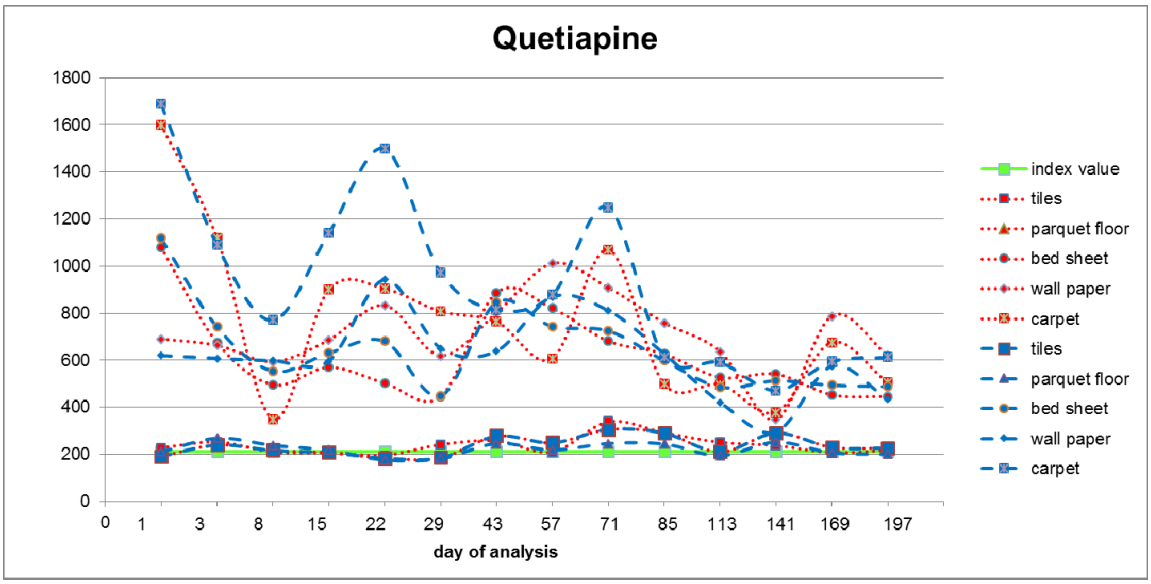

Fig. 2. Long-term-stability of quetiapine over a period of six months

4. Routine Forensic Cases

During the time of the study, several cases occurred where analysis of dried blood but also other dried traces was in focus. The four presented cases show the diversity and potential of the analysis of dried stains and the possible applications for this diverse alternative matrix.

Case 1: The first case involves the analysis of dried blood for ethylglucuronide from different garments. Two men were arguing in an apartment, both had drunk alcohol. One of them then dragged a knife and stabbed the other one. During this, he injured his own hand and blood of both men was spilled over the shoes of the offender. Both survived this incident. Toxicological analysis of the blood of both men yielded only alcohol in high amounts. No further drugs could be detected. We were asked to analyze the bloodstains on the t-shirt of the victim and one shoe of the offender. Parts of the stains on the t-shirt were cut out, as well as unstained parts, which served as comparison. Water and a mixture of internal standards were added and the samples were shaken for 20 minutes. A protein precipitation with acetonitrile was carried out, the supernatant evaporated to dryness and the sample was reconstituted in buffer solution. The blood stains on top of the shoe were identified to be from the offender, as was determined by DNA-analysis. Wet swabs were used to rub the blood from the shoe and also from an unstained part of the shoe. To these swabs water and a mixture of internal standards were added and they were treated as described above. Toxicological analysis via LC-MS/MS yielded ethylglucuronide for both garments. The comparative samples taken from unstained sites from the garments were negative for ethylglucuronide. Additionally the samples taken from the bloodstained part of the shoe of the offender yielded traces of cocaine, but neither benzoylecgonine nor ecgonine methylester were found. Surprisingly samples taken from the non-stained part of the shoe (with no visible marks) yielded traces of cocaine, too. Therefore we assume that the shoes must have been in contact with cocaine powder, because no metabolites could be detected and liquid blood of both men was negative for any drug other than alcohol. This shows that even swabs with a low amount of blood or no visible traces can be useful if sensitive techniques are used.

Case 2: This case involves the use of pepper spray by the police. A man was to be arrested by the police. However, he became aggressive and dragged a knife. The policemen made use of their pepper sprays to incapacitate the man. Nevertheless he went on towards one officer and was about to attack him with the knife. The officer felt forced to shoot the offender, who later died in hospital. Our task was to confirm whether the use of pepper spray could be proven. Prior to autopsy, samples were taken from the face of the man by using wet swabs. These swabs were then treated with ethanol and shaken for five minutes. The samples were analyzed by means of an LC-QTOF-device and yielded capsaicin. Thus the use of pepper spray could be demonstrated even though the face of the man had been cleaned in hospital due to vomit and blood.

Case 3: The last case presented here involved a stain on a bed sheet. An old woman died in a retirement home. Post-mortem toxicological analysis yielded a lethal amount of lidocaine, as well as toxic concentrations of mirtazapine, zolpidem and zopiclone in samples from the femoral blood of the subject. Residues of a liquid containing these substances were found in a glass at the bedside table. On the bed sheet a stain in a location and color that pointed to urine as liquid was detected. We were asked to confirm whether the stain was either caused by urine or by spilled liquid from the glass.

To verify if the liquid dried on the linen was urine, parts of the stained as well as the unstained areas of the bed sheet were cut out. As comparison, samples with apple juice, black tea and urine of voluntary persons were spiked on a non-stained part of the sheet. These parts were treated in an identical manner as the samples obtained from the stain: water was added and samples were shaken for 20 minutes, after which they were analyzed for creatinine and urea at a medical laboratory. Creatinine and urea could only be detected in urine samples and in the stained part of the sheet.

As second confirmation that the spot was urine from the old woman, a toxicological analysis of the stain was performed. Two different methods were used to prepare the stain. Parts of the

stained part as well as parts of the unstained part as comparison were cut out. For simple preparation, 1 mL of reconstitution buffer was added, the samples shaken for 20 minutes and 100 µL of the liquid, which became slightly yellow, were pipetted into an HPLC-vial, to which an internal standard mix was added. One mL of water was added to the rest of the samples; these were also shaken for 20 minutes, however internal standards and glucuronidase were added afterwards to 100 µL of the liquid, in order to cleave glucuronides. Both samples were analyzed via an LC-MS/MS device. Results are shown in table 2.

Tab. 2. results of toxicological analysis of the bed sheet.

Sample	Mirtazapine	Zolpidem	Zopiclone	Lidocaine
Sheet with cleavage	6.22 ng/4 cm²	1.57 ng/4 cm²	82.2 ng/4 cm²	2780 ng/4 cm²
Sheet without cleavage	2.59 ng/4 cm²	2.70 ng/4 cm²	29.1 ng/4 cm²	596 ng/4 cm²

All substances found in post mortem blood and urine sample of the old woman could be detected in the stains from the bed sheet. Additionally, an LC-TOF-MS-analysis was performed and the carboxy acid metabolite of Zolpidem and Hydroxymirtazapine, and thus also the in-vivo metabolism of the two drugs could be verified. We could thus prove that the stain on the sheet was urine from the old woman. Furthermore we could disprove the statement of the nurse who suggested the stain was originally from the liquid in the glass, which was found at the bedside table.

5. Conclusion

All analytes investigated during the long-term study were stable for six months without noteworthy degradation. The only exception that occurred in an experiment prior to the long-term study was 7-Aminoflunitrazepam, which was not detectable even in dried spots after a few days. Even though the study shows the behavior of the substances under controlled conditions, the cases presented here show the relevance and potential of these alternative matrices in forensic toxicology. Different surface materials, different matrices, different storage conditions and different amounts of traces were successfully investigated and are worth taken into consideration in forensic casework.

6. References

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