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A study of transfer and prevalence of organic gunshot residues

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Highlights

- Study of the distribution of OGSR on the shooter using a pistol
- High variability in OGSR production and transfer
- OGSR are not only transferred to the hand of the shooter, but also to other parts of the upper body
- The amount of OGSR is not proportional to the number of discharges
- Probability of occurrence of OGSR in the general population is low

Abstract

The main goal of the present study was to determine the amounts and distribution of organic gunshot residues (OGSR) on the shooter's upper body and clothing after discharging a pistol. A preliminary study was also performed to evaluate the prevalence of OGSR in the general population as well as in a

police laboratory environment. In the transfer study, results indicated that OGSR are not only transferred to the hand of the shooter, but also to other parts of the upper body. Thus, wrists and forearms also represent interesting targets as they are washed less frequently than hands. Samples from the face and hair of the shooters resulted in no OGSR detection just after firing. It was also observed that the concentrations recovered from clothing are generally higher compared to the same skin area. Prevalence in both general (n = 27) and police populations (n = 25) was low. No OGSR was detected in the samples from the general population and only two samples from the police population were found positive.

Keywords

Firearms; LC-MS; firearm discharge residues; distribution; stubs

1. Introduction

Chemical analysis of gunshot residues (GSR) can provide useful information for the reconstruction of events involving the use of firearms. This could include linking a person to such an event, an estimation of shooting distance and trajectory, bullet entry and exit point identification or simply determining the circumstances of a case and verifying the veracity of a testimony [1-3]. Additional information concerning the type of firearm and ammunition used as well as the time since discharge can also be useful [4-7]. GSR consists of a complex mixture of organic and inorganic material originating from the firearm, the ammunition and the combustion products formed during the discharge [8, 9]. GSR particles are not only propelled towards the target, but also in the direction of the shooter through the muzzle and, in significantly lower quantities, through other apertures of the firearm, such as the ejection port or the trigger notch. Therefore, GSR may be transferred to different parts of the shooter's body and also to other people or surfaces close to a firearm discharge [3, 10-12].

Current analysis methods for GSR focus mainly on the analysis of inorganic GSR (IGSR), which consist of metallic particles from the primer, the projectile, the cartridge and the weapon. Scanning electron microscopy (SEM) coupled to energy-dispersive X-ray spectroscopy (EDX) is the most commonly used analytical method for the detection of IGSR. However, the absence of inorganic particles on the surface in question, the prevalence of metal particles in the environment and the introduction of heavy metal-free or "non-toxic" ammunition has the capacity to produce false positives and negatives. Therefore, the analysis of complementary target molecules might prove necessary to further increase the probative value of the evidence [13]. The analysis of organic gunshot residue (OGSR) represents an interesting added value to operational practices. OGSR consist of completely burned, partially burned and unburned particles, mainly originating from the propellant and lubricants [4, 9, 14, 15]. More detailed information on the different formulations of the propellants and the various additives has been reviewed previously [9, 15, 16].

In recent years, numerous studies, which aimed to develop various analytical methods, as well as collection and extraction procedures for OGSR molecules, were published [15]. Among these, a wide range of protocols using liquid chromatography coupled to mass spectrometry (LC-MS) was proposed, allowing the identification and quantification of a number of propellant stabilizers and organic explosives [17-23]. Despite significant progress in the development of analytical methods regarding OGSR analysis, specific forensic questions still remain unanswered. Considering an OGSR research cycle, the determination of target compounds and development of analytical techniques are well documented, whereas only a few publications aimed at studying the transfer, persistence and prevalence of OGSR. However, these parameters are critical when interpreting analytical results [24]. Furthermore, knowledge about the persistence and prevalence of GSR in a specific environment are essential to assess the risk of possible contamination [25]. To date, most of the articles relative to these issues are based on

the study of IGSR and data still have to be collected for OGSR in order to implement reliably such approaches in practice.

Several studies have focused on transfer and persistence of explosive molecules [26-31]. While some of these compounds are present in OGSR, many others are structurally different so it is not possible to infer the transfer mechanism (and subsequent persistence) from studies involving explosives. Some studies specifically involving the transfer and persistence of OGSR on the shooter's body have also been reported and are summarized in Table 1.

A number of key observations and trends have emerged from the studies presented in Table 1. OGSR can be transferred to hands and clothing with the highest amounts detected for NG as it is a major component of smokeless powders. Stabilizer amounts (a minor component of smokeless powders) were present at lower levels and require an analytical method offering superior sensitivity. Nevertheless, no systematic study of the distribution of OGSR on the shooter's body has been conducted to date. Regarding persistence, a trend showing a longer persistence on clothing than on hands appeared. Some studies reported the absence of OGSR detection on hands 30 min to one hour after discharge [33, 36], whereas others obtained positive results after two or more hours [40, 44]. The difference in these results have been explained by several factors such as activities undertaken by the shooter following discharge, the sample collection methods applied or the instrumental technique used (sensitivity). On clothing, NG was detected up to five days showing far better persistence probably thanks to better retention from the fabric [32]. Again, this result is likely attributable to the larger amounts of this compound being transferred to the shooter. Due to the lack of a systematic study, no upper time limit for OGSR detection has been proposed yet. Studies showed that some OGSR compounds, mainly stabilizers, can be absorbed by the skin due to their lipophilic properties. Evaporation is also another mechanism for OGSR losses. According to these results, the best targets might be EC, 2-nitroDPA and 4-nitroDPA [43]. Finally, OGSR might be less susceptible to secondary transfer than IGSR due to these properties [41].

Besides the transfer and persistence of OGSR, some authors also studied the background prevalence of these compounds. Different population studies were conducted using various analytical protocols, as well as different target molecules [16, 45-49]. At several locations in England, a total of 337 specimens were taken in various public places, as well as from hotels, houses, vehicles and clothing. Additionally, 255 specimens were also obtained from police stations [45]. Using GC-TEA and GC-MS, the authors reported that four of the 337 specimens collected from public places were positive for ng levels of RDX, two for ng levels of NG and two for ng levels of 2,4-DNT and RDX. 24 of the 255 specimens collected from police sites were positive for ng levels of NG, three for RDX and one for PETN. A follow-up study was performed with 501 specimens collected in the four major UK cities, namely Birmingham, Cardiff,

Glasgow, and Manchester [46]. The authors reported that one of the 501 specimens collected from public places was positive for ng levels of RDX, two for ng levels of NG and one for ng levels of 2,4-DNT. Furthermore, over 300 specimens were collected from 28 cities across the U.S. and then analyzed by GC-TEA for explosives residues [47]. No organic explosives were detected. In 2016, 70 specimens were also obtained from police officers and from the furniture of four Pittsburgh police stations [48]. Using LC-MS/MS, EC was only quantified in four specimens, one from an officer and three from a police station. Regarding prevalence of OGSR on people, two studies are targeting OGSR on hands. A first prevalence study was performed in the U.S. on 100 volunteers from the general population with no positive results using micellar electrokinetic capillary electrophoresis (MEKC) [16]. Another prevalence study conducted on 73 people, belonging either to the general or to the police population of Morgantown, West Virginia, showed that the proportion of positive results for OGSR found in the specimens analysed by IMS was less than 5% [49]. All the authors of the aforementioned studies concluded that the detection of high explosive and OGSR traces is rare in public places and in the general population. The few positive results in police sites are not surprising, given that law enforcement personal are in regular contact with firearms.

While there has been significant focus on persistence and prevalence, minimal recent research has been conducted on the transfer and distribution of OGSR on the shooter's body and clothing. As the analytical techniques have rapidly evolved, typically leading to an increase in sensitivity and also selectivity, it might be unreasonable to base the interpretation of results on data acquired using less sensitive instrumentation. Moreover, the amount of data regarding the background prevalence of OGSR molecules useful in the interpretation at the source level is limited, because the studies were conducted in two countries only, whereas data from the country/region in question should be available for interpretation in actual casework. Finally, some studies targeted organic explosives and not organic stabilizers that are the main targets in OGSR analysis. Consequently, due to the differences in chemical structure, data cannot be extrapolated. Therefore, more data is required in order to evaluate the value of OGSR traces in a particular context with regard to competing hypotheses. As already mentioned, target compounds are well known and analytical techniques are sufficiently well developed to look further in the research cycle. Transfer, persistence and prevalence data are essential to build interpretation models and implement OGSR analysis in forensic laboratory routine.

The present study aims to examine the transfer and transferred amount of OGSR simultaneously collected on different parts of the shooter in order to assess optimal collection areas for detecting residues. Furthermore, a preliminary prevalence study was performed to determine if the target compounds are frequently found within selected populations, the first one generally not exposed to firearms and the second comprising staff from an operational forensic science laboratory.

2. Material and Methods

2.1 Experimental protocols

2.1.1 Transfer study

Shooting sessions were conducted in an indoor shooting range located in a specific building section with the ventilation turned off. Extraction and analysis of the specimens were performed in a separate laboratory in another section to minimise any potential contamination. A semi-automatic 9 mm Parabellum Sig Sauer P226 was used for all experiments. The firearm was completely dismantled, cleaned and lubricated before the study. After every OGSR collection, the outer parts of the pistol were cleaned using a piece of paper wetted with ethanol to avoid any contamination when touching the firearm. The ammunition used was 9 mm Parabellum from Geco (batch 61 SE). The shooters were asked to wash their hands with soap before entering the shooting range. A blank sample of both hands, as well as the pistol hand grip was taken to verify if they were clean. The pistol was loaded by a third person as the shooters were not allowed to touch any surface except the firearm at the time of firing. Then, the shooters were asked to hold the gun with both hands and fire one or three cartridges depending on the experiment. OGSR collection took then place outside the shooting range. After collection, they carefully washed their hands before starting the procedure once more. Five different shooters were involved in this study, one woman and four men, all aged between 25 and 40. Two men had facial hair, the other two were cleanly shaven.

Various items of clothing were used to investigate the effect that the fabric and its structure might have on the amount of OGSR transferred. Three pieces of each item were bought as replicates. The first item was a 100% cotton blue t-shirt from C&A, the second was a 70% cotton-30% polyamide grey pullover from H&M and the last was 100% acrylic black gloves from Maddison.

Five different experiments were performed (Table 2).

In Experiment 1, the aim was to determine what part of the hand should be targeted. The right hand was divided into four zones (Figure 1a), two for the back (thumb-index zone and rest) and two for the palm (same as back).

Experiment 2 investigated the transfer of one discharge onto various regions of the body of a woman shooter as well as clothing. Experiment 3 studied the same parameters, but with three discharges instead of one to observe the variation in the amount of OGSR with the number of discharges. Experiment 4 repeated the parameters from Experiment 3, but with men shooters (bearded or not). Finally, Experiment 5 considered shooters wearing gloves.

In Experiments 2 to 5, as the aim was to evaluate the distribution of residues, the collection zones were defined as follows. Specimens from the left and right side of the body were always taken separately as well as the back and palm of each hand, leading to four samples total for both hands (left palm, left back, right palm and right back) contrary to Experiment 1. The wrist zone extended to about one third of the forearm and the forearm zone was from one third of the forearm to the elbow. The upper part of the arm was never sampled as the shooter was always wearing a t-shirt. With regards to collection of the head region, three zones were targeted: cheeks and chin (beard if present), eyebrows and forehead, and hair for which a five centimetres wide band was collected from the top of the head to the ears. Thus, the whole length of the woman hair was not collected. For the experiments involving t-shirts, four specimens were taken: one for each sleeve and the front was divided into left and right as shown in Figure 1b. For the pullovers, eight specimens were taken (Figure 1c): front left and right, the sleeves were divided into three zones: wrist (about one third of the distance between the wrist and the elbow), lower part (two thirds left to the elbow) and upper part (from the elbow to the shoulder). In the case of gloves, each glove was separated into three zones: back, palm and wrist.

2.1.2 Prevalence study

A preliminary prevalence study was conducted to evaluate the amount of OGSR that can be detected on citizens who do not have regular contact with firearms, as well as people working in a forensic laboratory. For the general population, 27 individuals were collected. In the forensic laboratory, 25 individuals were collected. Each person had to answer a few questions (see SI) regarding its contact and use of firearms.

Two carbon stubs were used to collect OGSR from each subject: the first one for the hands (left and right, back and palm) and the second either for the wrists (left and right) or the wrist part of the sleeves if the person was wearing long sleeves.

2.2 Chemicals

ULC–MS grade water containing 0.1 % formic acid, methanol, formic acid (FA), and acetonitrile were purchased from Sigma-Aldrich (Buchs, Switzerland). Eight OGSR compounds were studied in this study (Table 3): diphenylamine from Fluka (Buchs, Switzerland); ethylcentralite, *N*-nitrosodiphenylamine, 4-nitrodiphenylamine, akardite II and *N*,*N*-diphenylformamide from Sigma–Aldrich (Buchs, Switzerland); 2-nitrodiphenylamine from Alfa Aesar (Karlsruhe, Germany); methylcentralite from MP Biomedicals (Illkirch, France). Standard solutions at 1 mg/mL were prepared in MeOH and stored at 4°C.

2.3 Instrumentation

Two different LC-MS systems were used in this study. A semi-quantitative approach was used. A calibration curve was measured for each sequence of experiments to account for instrument response variation from sequence to sequence. Analytical measurement uncertainty was considered as negligible (< 5%) compared to variability between specimens.

2.3.1 IMS-QTOF

The transfer study was carried out using an Acquity ultrahigh performance liquid chromatography (UHPLC) I-class system from Waters. The instrument was equipped with a binary pump, an autosampler, and a thermostatically controlled column compartment. Separation was performed using a C18 Kinetex core-shell column from Phenomenex. A SecurityGuard ULTRA cartridge with C18 selectivity was used to protect the analytical column. LC parameters are described in Table 4. The UPLC system was coupled to an ion mobility quadrupole time of flight (IMS-QTOF) mass spectrometer from Waters (Vion). Electrospray ionization was operated in positive mode. The $[M+H]^+$ of the target compounds were defined as the precursor ions except for *N*-nitrosoDPA that was fragmented in-source and was targeted as $[M+H-NO]^+$. Data acquisition using MS^E technology enabled identification of the target molecules. All data were acquired in *Sensitivity* mode. The source parameters were as follows: the source and desolvation temperatures were set to 120°C and 600°C respectively, the cone gas to 50 L/h and the desolvation gas to 1000 L/h. The capillary voltage was adjusted to 1 kV. The mass range was from 50 to 600 m/z with a scan rate of 0.2 s. A leucine enkephalin solution was used as a lock mass to correct for changes in environment or experimental conditions over the course of the analysis. Data acquisition, treatment and instrument control were monitored using UNIFI, Scientific Information System (Waters). Limits of detection for the target compounds were determined based on a signal to noise ratio equal to three (Table 3).

2.3.2 QTrap

The prevalence specimens were analyzed using an Agilent Infinity 1290 ultra-high performance liquid chromatography (UHPLC) from Agilent Technologies. The instrument was equipped with a binary

pump enabling a maximum delivery flow rate of 5 mL/min, an autosampler, and a thermostatically controlled column compartment. Separation was performed using a C18 Kinetex core-shell column from Phenomenex. A SecurityGuard ULTRA cartridge with C18 selectivity was used to protect the analytical column. The UHPLC system was coupled to a triple quadrupole mass spectrometer (5500 QTrap) from ABSciex. Electrospray ionization was operated in positive mode. The $[M+H]^+$ of the target compounds were defined as the precursor ions, and quantification was obtained from the SRM measurements. MS/MS parameters are given in Table 3. The source parameters were as follows: the desolvation temperature was set to 500°C, the nebulizer gas to 60 psig, the turbo gas to 50 psig and the curtain gas to 25 psig. The IonSpray voltage was adjusted to 5500 V. Data acquisition, treatment and instrument control were monitored using Analyst software. Limits of detection are indicated in Table 3.

2.4 OGSR collection and specimen preparation

Sampling was performed with carbon stubs from Plano (Germany). This collection device consisted of an aluminium stub 12.5 mm in diameter inserted in a plastic vial with a screwed cap. An adhesive carbon tab 12 mm in diameter was placed on the metallic part of the stub. Following recommendations from Zeichner et al [50], the stubs were dabbed 50 to 100 times on the skin and 200 to 300 times on hair, clothing and gloves.

After collection, the carbon adhesive was removed from the stub and transferred to a 20 mL scintillation vial containing 1 mL MeOH. The vials were then ultrasonicated during 15 minutes at room temperature to extract OGSR. Finally, the resulting solution was filtered through a 0.45 µm PTFE syringe filter (VWR). In order to monitor potential laboratory contaminations, blanks were also prepared for each extraction session.

3. Results and Discussion

3.1 Transfer study

The transfer study intended to study the distribution of OGSR on the shooter as well as the effect of some parameters, namely the number of discharges and fabric type. First, the propellant contained in Geco ammunition was analyzed and the following compounds were detected: DPA, *N*-nitrosoDPA, 2-nDPA, 4-nDPA, AK II, EC and *N*,*N*-DPF. The main compounds were DPA and *N*-nitrosoDPA. In this study, results were presented only if the molecule was detected in more than one specimen to enable comparison of the results based on a sufficient number of measurements.

Experiment 1 evaluated the distribution of OGSR on the right hand of the shooter. The aim was to determine if collecting OGSR only on the webbed region between the thumb and index was sufficient, or if the whole hand should be considered. Figure 2 shows that the largest amounts were detected from

the thumb-index zone, with the amounts recovered from both the back and palm side typically greater than the other part of the hand. Similarly to the propellant composition, DPA and *N*-nitrosoDPA were the most highly concentrated analytes in OGSR

These results indicate that most OGSR were deposited on the thumb-index zone of the hand. Similar results were obtained in a transfer study targeting IGSR by Vanini et al. [51]. Regarding the concentrations, it is logical to detect the highest concentrations on the back (thumb-index zone) as this part of the hand is completely exposed to residues during discharge. It is also the closest to the firearm. It was thus more surprising to find a significant amount of residues on the palm of the hand. However, fast redistribution of OGSR might occur and residues could be transferred to other parts of the hand already just after discharge. Consequently, it seems reasonable to collect residues from the whole surface of the hand starting with the thumb-index zone.

The following experiments intended to evaluate the transfer of OGSR to the upper-body. The influence of the shooter, the number of discharges and type of clothing was investigated. Results are presented separately for skin surfaces and clothing to enable a comparison of the amounts recovered from both types of surfaces.

Figure 3a illustrates the amount of OGSR recovered in Experiment 2, after a single discharge. An obvious observation is that OGSR were mainly transferred to the right side of the shooter. This can be easily explained by the construction of the firearm with the ejection port on the right side of the pistol. Moreover, OGSR were not only deposited on the hand, but also on the wrists and forearms. As a general rule, the amounts of OGSR detected decreased with the distance to the firearm with [back hand] > [wrist] > [forearm]. Much lower concentrations on the face and hair would then be expected. This was confirmed as OGSR were not detected on these surfaces.

The scenario for Experiment 3 (Figure 3b) involved increasing the number of shots of three to evaluate the influence of the number of discharges. By comparing the y-axis scales of both figures, it can be seen that the concentrations recovered were not proportional to the number of discharges. Indeed, even if more extreme outliers (*f.e*. DPA at 927 ppb) were present when discharging the pistol three times, the medians were only slightly higher for three discharges. As a consequence, the amount of OGSR could not be used to distinguish between numbers of discharges due to the low repeatability of OGSR production and transfer. However, it seems that the strong differences between left and right side of the shooter observed in Figure 3a were attenuated when three cartridges were discharged. For example, significant amounts were detected on the left wrist with three discharges, whereas nearly no residue was detected on the same zone with one discharge. Some hypotheses to explain that fact might be that the shooter spends more time in the plume leading to an extended exposure. Moreover, the size of the plume might also grow with the number of discharges leading to deposition on surfaces further than with one

discharge. Finally, the pressure and air perturbation induced by the subsequent discharges might also modify the shape of the plume, leading to more homogeneous deposition.

Experiment 4 repeated Experiment 3 but with four male shooters. A trend towards higher concentrations can be observed (Figure 3c). Overall, higher concentrations were recovered from the male shooters (except for the DPA outlier in Figure 3b). Interestingly, the concentration detected on the right wrist was particularly high as opposed to that of Figure 3b. A main difference between men and women regarding wrists and forearms was hairiness. If hair retained residues better, then more residues might be found on men than women. Another interesting point was the large amounts detected on the left palm. There is a straightforward explanation for this "anomaly": The shooters were asked to avoid manipulating the firearm after firing, but three shooters removed the magazine with their left hand before putting the pistol on the table (for security reasons). The amount transferred by this simple manipulation was substantial if compared to the same value in Figure 3b. Thus, secondary transfer occurring through handling a recently discharged firearm should be considered.

It is difficult to standardize experiments as individual characteristics such as skin type, hairiness density and presence of cosmetics might influence the OGSR retention characteristics even when collected just after discharge. A simple activity such as walking out of the shooting range for residue collection in another room can contribute to GSR loss. In spite of that, some trends were observed. Logically, as the distance from the firearm increased, smaller quantities of OGSR were detected. The hands received the highest amounts, followed by the wrist. Forearm concentrations were always much lower. Specimens from shooter's face and hair were also taken. Nevertheless, no OGSR could be detected, either with one or three discharges. Due to the larger distance from the pistol, the concentrations were probably too low to be detected by the instrument used in this study. Another important parameter that was previously discussed was the side of the ejector window influencing the spatial distribution (left/right) of the residues. Finally, due to different shooter characteristics, variable amounts of OGSR might be retained and detected. The main factor is probably hairiness that might better retain OGSR due to its surface structure. A second factor is skin moistness. If OGSR are absorbed by skin flakes and skin is dry, the process of dabbing the skin with a stub will remove a lot of dead skin flakes leading to detection of the absorbed OGSR. Women tend to keep their skin better hydrated using moisturizers, so less skin flakes might be collected from their skin leading to lower OGSR amounts detected. This remark does not hold for particulate residues that should not be influenced by this factor. Another element is the collected area. Men's hands for example are usually larger than women's. Consequently, a larger surface is available for OGSR deposition and subsequent collection. Such hypotheses should be further tested with specific experiments.

Figure 4 illustrates the results obtained from the various pieces of clothing sampled in Experiments 2 to 4. Three items were tested: a cotton t-shirt, a sweater and gloves. All items were bought in triplicate to

capture the expected variability. The sheddability of the textiles was not determined quantitatively. However, the shedding potential was estimated qualitatively by looking at the amount of fibers that were transferred to the adhesive after collection. It was the highest for the gloves, followed by the sweater and the t-shirt had the lowest shedding potential.

It can be observed than the amounts recovered from the woman shooter clothing (Fig4a-b) were generally higher than from men (Fig4c). However, different collection protocols were used. For the woman, the sweater and t-shirts were removed and dabbed on a table while for men, stubs were applied to clothing while the shooter was wearing it. Losses were expected by removal of clothing. Nevertheless, dabbing directly on the shooter might influence the results due to the uneasiness of the procedure (the person in charge of sampling might be reluctant to use higher pressure when sampling directly from a person). Thus, the higher number of positive samples in the case of the woman shooter relative to men (Figure 4a-c) indicates that clothing should be removed as delicately as possible before collection on a table. The results observed in Figure 4a and b show that deposition was also highly irreproducible when the same collection protocol was used. With three discharges, greater levels of OGSR were detected on the t-shirts than with only one discharge while the opposite was observed with the sweaters. For the t-shirts, more OGSR was detected on the front of the t-shirt compared to the sleeves. For the sweater, more residues were detected on the sleeves than on the front. It is thus difficult to draw a conclusion from these results. It seems that the distribution and deposition of residues are even more irreproducible than what was observed on the skin. Nevertheless, a logical trend was observed. With the sweaters, a higher amount of residues was detected on the sleeve zones, closest to the firearm, similarly to what was observed on skin. Comparison of these results with those from Figure 3 on skin, it is possible to see that the range of concentration was similar to what was detected on skin. However, when comparing the results for the forearms to the values for the sleeves (zone Z2) (Figure 3a vs Figure 4a), it can be seen that more residues were detected on clothing than on its skin counterpart. Thus, clothing seems to retain higher amounts of OGSR compared to skin. This was confirmed by the experiment involving gloves. The concentrations collected on the back of the right glove were less dispersed than for hands and the median was much higher than for skin. Nevertheless, the distribution of OGSR on the gloves was similar to what was observed for hands with more residues on the right side than on the left, and the highest concentration detected on the back of the hand, closely followed by the wrist. Interestingly, very low amounts of OGSR were detected on the gloves' palms. OGSR collection on clothing using stubs might not be the best option to target that type of surfaces. Indeed, many studies successfully used vacuuming to recover GSR [34, 35, 38, 52, 53]. In this study, stubs were chosen for sake of straightforward comparison with results on skin, but in practice better efficiencies might be obtained using other collection methods.

In summary, results showed that OGSR were not only transferred to the hands of the shooter, but also to wrists, arms, and clothing. It is thus recommended to also consider other surfaces and not only hands. In case the suspect is not apprehended immediately after the incident, this is even more important, as redistribution of the residues might happen through secondary transfer from hands to other skin surfaces/clothing or OGSR might be lost due to hand washing or other activities, leading to more chances to detect OGSR on body surfaces or items of clothing that were not washed.

3.2 Prevalence study

A sample of individuals from a population who is not regularly in contact with firearms was chosen to conduct a preliminary prevalence study. The goal of this study was to assess if the OGSR compounds listed in Table 3 might be frequently encountered in the general population. 27 people were considered. At the time of collection, some questions regarding potential contact with firearms were asked. Among these 27 individuals, only six had infrequent contact with firearms, either for hunting or within the army framework. None of them had discharged a firearm recently (the last discharge was more than one month ago). One of them manipulated a firearm five days before. No target compounds were detected in any of the specimens. As a consequence, even if some of these people had contact with firearms some time ago, the persistence of OGSR was limited and nothing could be detected. A study involving 73 individuals by Bell and Seitzinger in West Virginia (USA) concluded that the proportion of positive samples was less than 5% and consequently the background level was not a concern for assay development [49]. The present results agree with their conclusions. According to the data of the present study, the probability of occurrence of such compounds in the general population is low, sustaining, at the source level, that the detection of OGSR compounds supports the hypothesis of a recent contact with firearms. To approach the activity level, more data regarding secondary transfer and persistence are necessary to interpret OGSR traces. However, this study confirms that it is worth looking for OGSR, as these can be considered a rare occurrence in the population.

The second part of the prevalence study involved a population of 25 people from a forensic laboratory who were collected for OGSR using the same protocol. Among them, eight individuals had never discharged or handled a firearm. Among the remaining 17 individuals, only two reported having discharged a pistol recently (two hours before sampling and one the day before sampling). Four others mentioned handling a firearm between one and three hours before without discharging it. Most of the people washed their hands between the last discharge/manipulation and the time of collection except for two people who did not wash their hands after a manipulation. The results from the stubs were positive for only two individuals. A small amount of DPA (0.58 ppb) was detected on the wrists of the first one. However, this person indicated never discharging or handling firearms. In this case, secondary transfer

through contact with a colleague or a contaminated surface is the likely source of the positive result. As only DPA was detected at low concentration, an environmental source such as those proposed in the literature might also be considered [54]. The second individual indicated having discharged a pistol two hours before residue collection. Both hands and wrists stub were positive for a range of target analytes. DPA, *N*-nitrosoDPA, 2-nDPA, 4-nDPA, EC and *N*,*N*-DPF were detected at relatively high concentrations ([DPA] was 24.2 ppb on the hands and 71.9 ppb on the wrists). The number of cartridges discharged was between 25 and 50, and despite hand washing after firing, the amount of OGSR remained significant. Thus, working in an environment where people handle and discharge firearms does not imply the presence of OGSR on all workers. Only one person was tested positive without handling a firearm and another was positive after having recently handled a pistol. A study by Ali *et al*. in Pittsburgh police stations showed the presence of EC in many of the specimens and they concluded that the potential for secondary transfer does exist and should be studied [48]. In the future, it is planned to extend the prevalence study to a larger population as well as including specimens from facilities that might be contaminated.

4. Conclusions

The present study had two main goals. The first one was to study OGSR transfer and more precisely to determine where OGSR are transferred on a shooter when a pistol is discharged. Results indicated that OGSR are not only transferred to the hand of the shooter, but also to other parts of the upper body. Thus, wrists and forearms are also interesting targets as they are washed less frequently than hands. Specimens from the face and hair of the shooters were also taken. However, no OGSR were detected on these surfaces just after firing. Transfer was also investigated onto clothing. It was observed that the concentrations recovered from clothing are generally higher compared to the same skin area. OGSR might persist longer on clothing due to trapping of OGSR into the fabric. Consequently, in a real case, specimens collected from a suspect should not be limited to hands, but should also include other skin surfaces such as forearms, face as well as clothing. It should be noted that the OGSR distribution is likely to depend on the firearm type and similar studies using rifles and shotguns might lead to different observations.

The second aim of the present work was to determine the prevalence of OGSR on the hands and wrists of individuals from the general population (n = 27) and from a forensic science laboratory population (n = 25). In our study, no OGSR were detected among the 27 individuals from the general population (rarely in contact with firearms). In the forensic science laboratory population (more likely to be in contact with firearms), only two individuals were found positive for OGSR, one of them having discharged a firearm two hours before sampling. The other positive case (amount close to LOD) is likely due to secondary transfer or external contamination as the person never had a direct contact with a

firearm. Although one must be cautious not to over-interpret these results due to the limited number of specimens, our study indicates that the prevalence of OGSR is overall low (3.8%). A larger prevalence study is planned in the future to extend the amount of data and include them in an interpretation model. Prevalence data are important to assess the background occurrence of OGSR compounds and thus the probability of testing positive in the absence of contact with firearms. As highlighted by Maitre et al. in a recent review [24], the non-application of an interpretation framework is mainly due to the lack of available data relevant to questions of transfer, persistence and background. As a consequence, prevalence studies should be encouraged to gather as much data as possible. Such data should also be completed by a persistence study as suspects are rarely arrested "in flagrante delicto".

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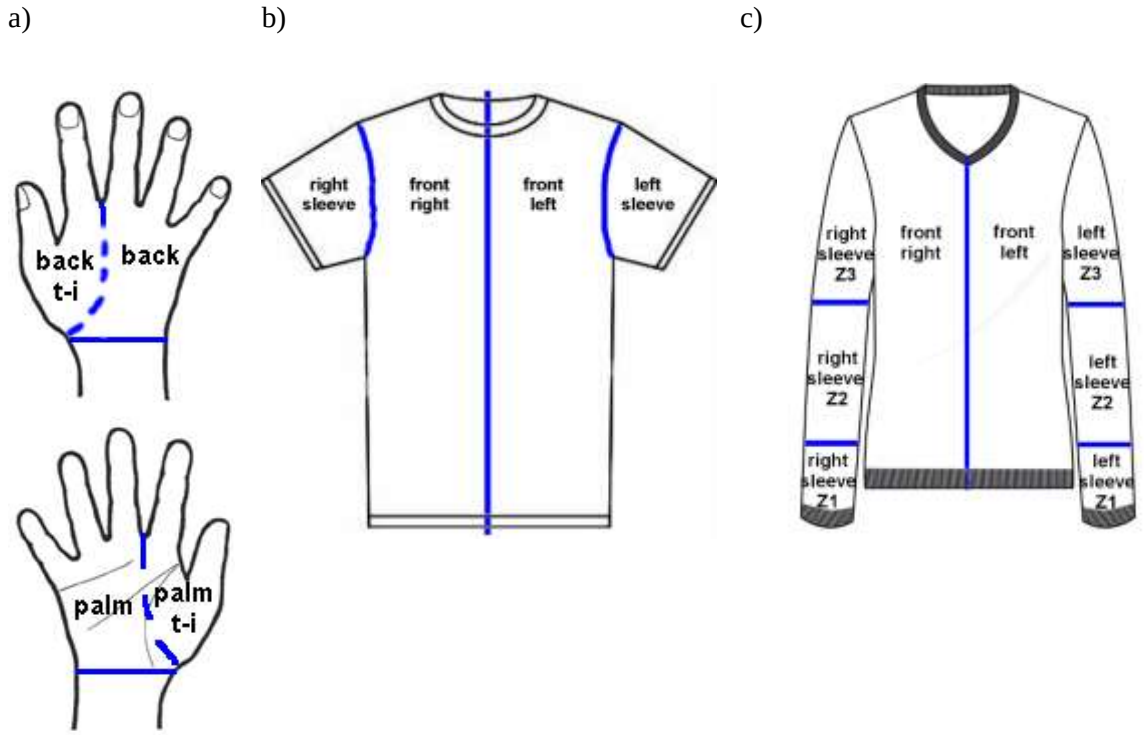

Figure 1: Schemes of the zones selected for OGSR collection. a) Right hand in Experiment 1 (four zones), b) T-shirt (four zones), c) Pullover (eight zones). T-I is used for thumb-index. Only the front of clothing was targeted.

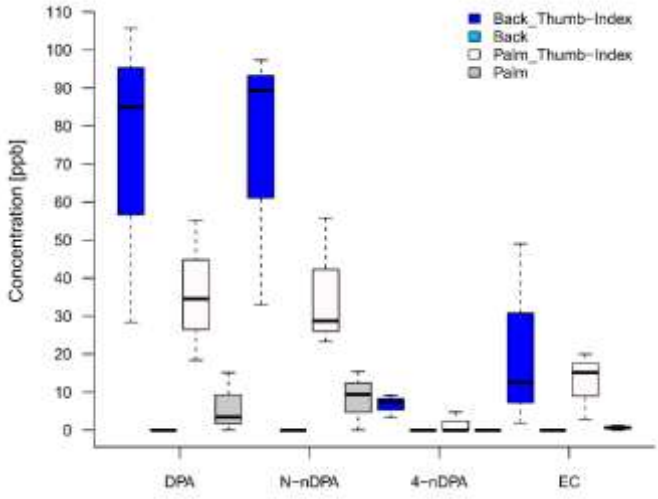

Figure 2: Experiment 1. Boxplots representing the amount of OGSR recovered from four zones of the right hand of the shooter after one pistol discharge (n = 3). Geco ammunition was used.

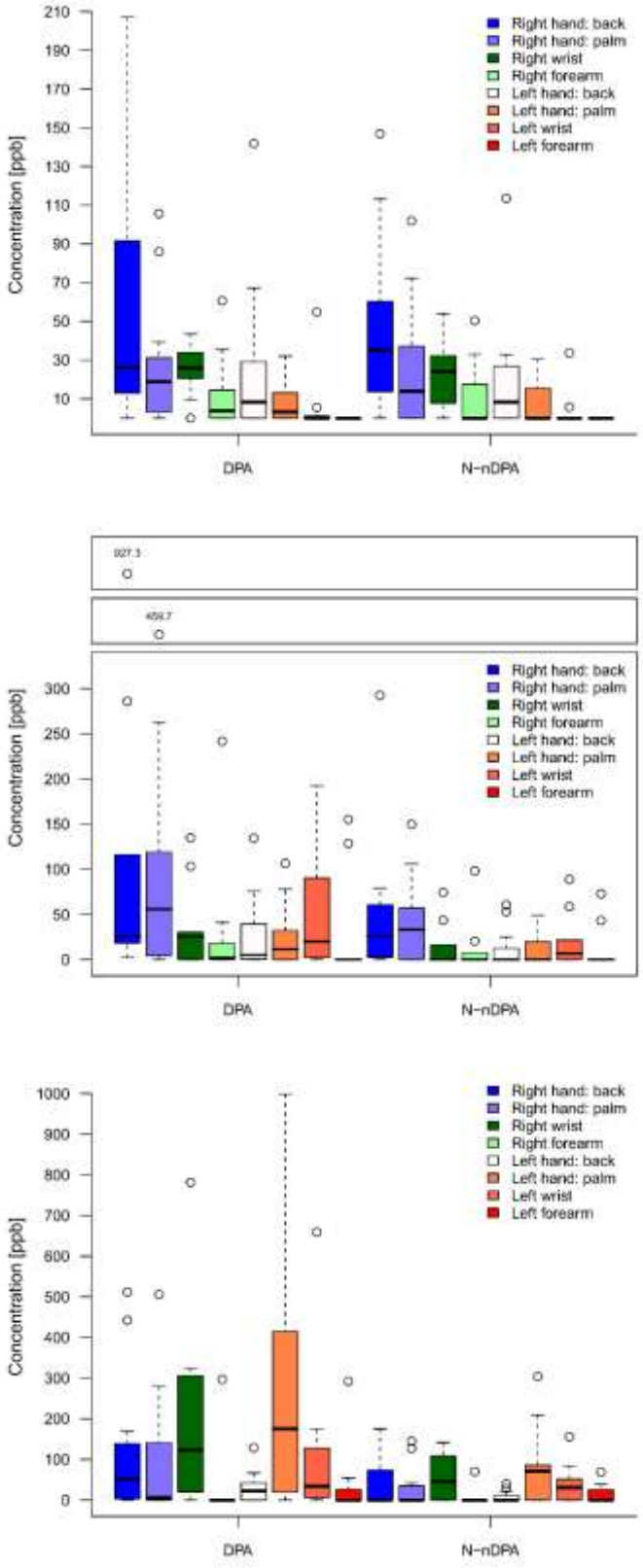

Figure 3: Experiments 2-4: OGSR recovered from skin surfaces. Boxplots representing the amount of OGSR recovered from the hands, wrists and forearms of a shooter. a) Woman shooter, one pistol discharge (Experiment 2). b) Woman shooter, three pistol discharges (Experiment 3). c) Men shooters, three pistol discharges (Experiment 4). Geco ammunition was used. For number of replicates, refer to Table 2. The results for 4-nDPA and EC can be found in SI.

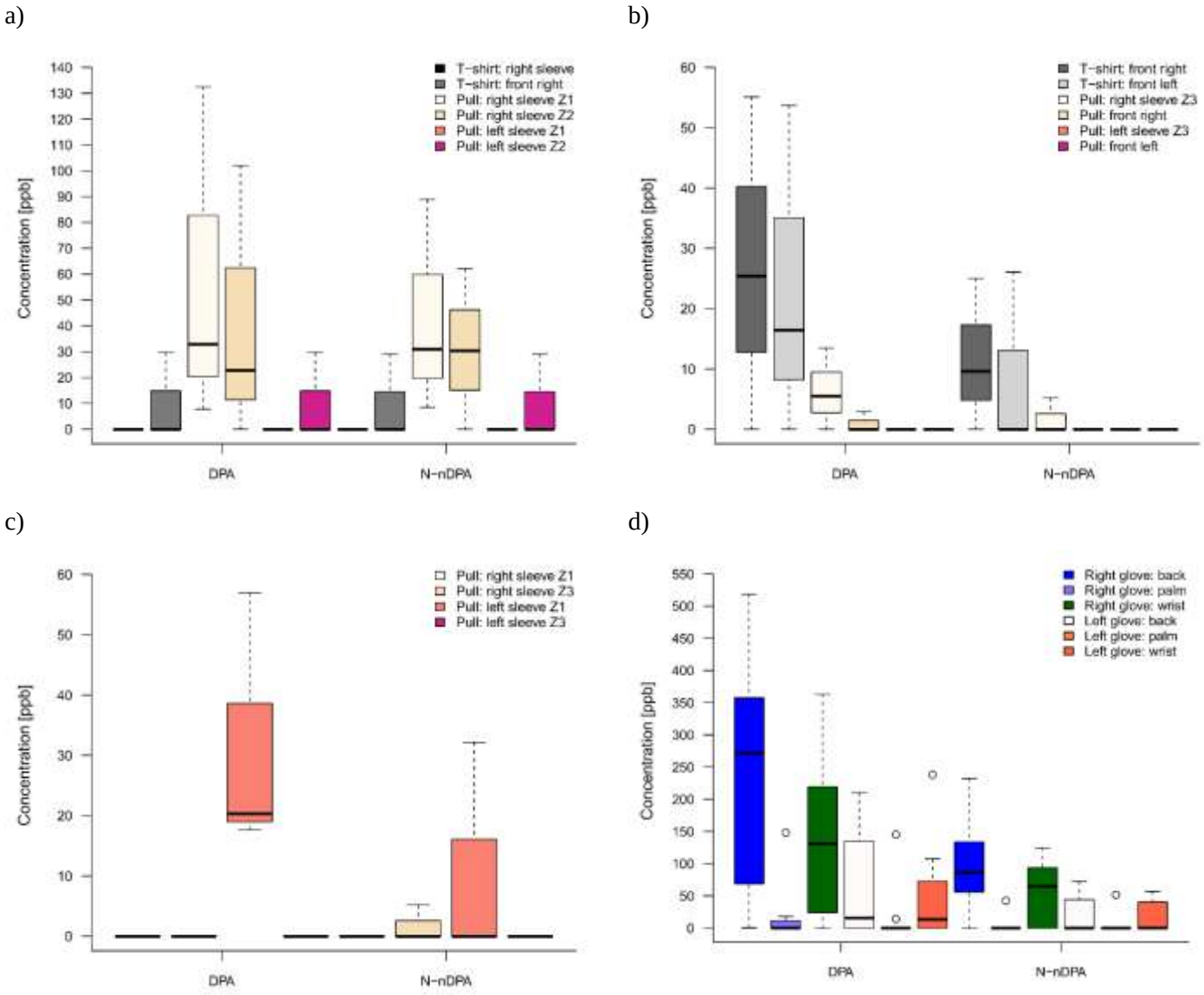

Figure 4: Experiments 2-4: OGSR recovered from clothing surfaces. Boxplots representing the amount of OGSR recovered from a) clothing from a woman shooter (Experiment 2: one discharge, n = 3), b) clothing from a woman shooter (Experiment 3: three discharges, n = 3), c) clothing from four men shooters (Experiment 4: three discharges, n = 3) and d) gloves (three discharges, n = 9). Geco ammunition 9 mm Parabellum was used. The results for 4-nDPA and EC can be found in SI if available.

Table 1: Summary of OGSR transfer and persistence studies

Reference ***Type of study***	**Target compounds**	**Surfaces sampled**	**Collection technique**	**Analytical technique**	**LO**
Lloyd, 1986 [32] *Transfer and persistence*	NC, NG, DPA	Hands, Neck, Face, Clothing	Swabbing (hands) Vacuuming (clothing)	SEC-PMDE (NC) HPLC-PMDE (NG) HPLC-Coulometric detector (DPA)	ng 0.1 ng
Douse and Smith, 1986 [33] *Transfer and persistence*	NG	Hands, Clothing	Swabbing Vacuum Sampling	Capillary GC-ECD	pg
King, 1993 [34] *Casework*	NG, 2, 4-DNT	Hands, Clothing	Swabbing Vacuum Sampling	HPLC-PMDE trapped to GC-TEA	1 n
Speers et al., 1994 [35] *Transfer*	NG, 2,4-DNT, DPA, EC, MC	Clothing	Vacuum Sampling	HPLC-PMDE GC-MS	50 10
Northrop, 2001 [36] *Transfer and persistence*	Not detailed	Hands	Tape lifting	MEKC	0.9
MacCrehan et al., 2003 [37] *Method development and transfer*	NG, EC	Hair	Combing	MEKC	N.
Zeichner et al., 2003 [38] *Persistence*	NG, 2,4-DNT, 2,6-DNT, DPA, dinitroDPA, N-nitrosoDPA, EC	Clothing	Vacuum sampling	IMS GC-TEA GC-MS	0.3 0.0 Lo
Zeichner and Eldar, 2004 [39] *Transfer and persistence*	NG, 2,4-DNT, 2,6-DNT	Hands, Hair	Tape lifting	IMS GC-TEA	0.1
Zhao et al., 2008 [40] *Method development and persistence*	EC, MC	Hands, Hair	-	DESI-MS/MS	8 t
Arndt et al., 2012 [41] *Persistence*	DPA	Hands	Swabbing	IMS	-
Moran and Bell, 2013 [42] *Skin permeation study*	DPA, N-nitrosoDPA, 2-nDPA, EC	PDMS membrane	-	IMS	N.
Moran and Bell, 2014 [43] *Skin permeation study*	DPA, 2- and 4-nDPA, DMP, EC	PDMS membrane	-	GC-MS	N.
Gassner et al., 2016 [44] *Transfer and persistence*	DPA, N-nitrosoDPA, 2- and 4-nDPA, EC, MC, AK II, N,N-DPF, 1,3-DPU	Hands, Face, Hair, Clothing	Swabbing Tape lifting	LC-MS	0.0

*AK II, akardite II ; DESI, desorption electrospray ionization ; DMP, dimethyl phthalate ; 2,4-DNT, 2,4-dinitrotoluene ; 2,6-DNT, 2,6-dinitrotoluene ; DPA, diphenylurea ; EC, ethylcentralite ; ECD, electron capture detector ; GC, gas chromatography ; HPLC, high performance liquid chromatography ; IMS, ion mobilit capillary electrophoresis; MS, mass spectrometry ; NC, nitrocellulose ; NG, nitroglycerine ; N-nitrosoDPA, N-nitrosodiphenylamine ; 2-nDPA, 2-nitrodiphenyla PMDE, pendant mercury drop electrode detector ; SEC, size exclusion chromatography ; TEA, thermal energy analyzer ; UHPLC, ultrahigh performance liquid ch

Table 2: Description of the various experiments carried out in the transfer study. All the discharges were performed holding the pistol with two hands

Experiment number	Shooter number	number of discharges	n (replicates)	Surface sampled
1	1 (woman)	1	3	Right hand (4 zones)
2	1 (woman)	1	12	Hands
			9	Wrists and forearms
			3	Face and hair
			3	T-shirt
			3	Pullover
3	1 (woman)	3	12	Hands
			9	Wrists and forearms
			3	Face and hair
			3	T-shirt
			3	Pullover
4	2,3,4,5 (men)	3	12	Hands
			9	Wrists and forearms
			5	Face and hair
			3	T-shirt
			3	Pullover
5	1 (woman),2 (man)	3	9	Gloves

Table 3: Target compounds and MS parameters

Target Compounds	Vion (Waters)		QTrap 55oo (ABSciex)			
	Expected neutral mass	**LOD [ng/mL]**	**SRM transitions**	**LOD [ng/mL]**	**Declustering potential [V]**	**Collision energy [V]**
Akardite II (AK II)	226.1106	0.2	227.1 → 170.1 227.1 → 91.9	0.01	120	27 36
Methylcentralite (MC)	240.1263	0.1	241.2 → 134.1 241.2 → 105.9	0.01	125	24 36
***N*,*N*-diphenylformamide (*N*,*N*-DPF)**	197.0841	0.1	198.1 → 92 198.1 → 65	0.02	130	30 54
Ethylcentralite (EC)	268.1576	0.1	269.2 → 147.9 269.2 → 120	0.005	120	20 33
2-nitrodiphenylamine (2-nDPA)	214.0742	2	215.1 → 197 215.1 → 180.1	0.02	80	14 23
4-nitrodiphenylamine (4-nDPA)	214.0742	1	215.1 → 197.8 215.1 → 167.1	0.02	60	18 47
Diphenylamine (DPA)	169.0891	0.1	170.1 → 93 170.1 → 66	0.5	200	32 58
***N*-nitrosodiphenylamine (*N*-nitrosoDPA)**	198.0793	1	199.1 → 169 199.1 → 66	0.02	60	15 30

Table 4: LC parameters

LC parameters	**IMS-QTOF Vion (Waters)**			**QTrap 55oo (ABSciex)**		
Column type	C18 (2.6 µm, 2.1 mm × 150 mm)			C18 (2.6 µm, 2.1 mm × 100 mm),		
Column temperature	40 °C			40 °C		
Flow rate	0.35 mL/min			0.25 mL/min		
Injection volume	5 µL			5 µL		
Gradient table	**t / min**	**% A H_2O+0.1%FA**	**% B ACN+0.1%FA**	**t / min**	**% A H_2O+0.1%FA**	**% B ACN+0.1%FA**
	0	80	20	0	65	35
	0.5	80	20	0.5	65	35
	2	60	40	6	20	80
	8.5	20	80	7	0	100
	9	0	100			