

■ REVIEW ARTICLE

## Persistence & Detection of Organic Gunshot Residue in a Forensic Investigation: A Review

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### ABSTRACT

A method of identifying organic gunshot residue (GSR) is proposed as an alternative tool for unique identification in the forensic context. In recent trends of uses of non toxic/heavy metal free (HMF) ammunition, there must have been challenges in identification and detection of inorganic gunshot residue (GSR). The organic GSR preliminary emanate from the propellant of smokeless powder. Smokeless powder consists of either single base which is composed of nitrocellulose, double base which is composed of nitrocellulose and nitroglycerine or triple base which consists of nitrocellulose, nitroglycerine and nitro-guanidine. In order to preserve and lengthen the service life of smokeless powder different additives like stabilizer, plasticizer, flash inhibitors, coolant, moderate and surface lubricants etc were used.

Through this article review, we have summarized the study of the detection of diphenylamine (DPA), ethylcentralite (EC), methylcentralite (MC), dimethyl phthalate (DMP) and N-nitrosodiphenylamine (N-nDPA), which are generally found in the stabilizer, flash inhibitors, plasticizers and degradation products of the smokeless powder which could be separated and detected from the UPLC-MS (Ultra performance liquid chromatography – tandem mass spectrometer). This persistence and detection of organic GSR will be helpful for the forensic experts for testimony in a court of law.

**KEYWORDS** | organic gunshot residue particles, non-toxic ammunition

### INTRODUCTION

**T**O OVERCOME THE CHALLENGES of frequent uses of non-toxic/heavy metal free (HMF) ammunition in the market space, the detection of organic GSR particles are very much useful for the forensic experts. Organic component of GSR generally comes from the propellant contained in ammunition and along with propellant to conserve and sustain the life time of the propellant some additive mentioned in Table 1, where added like stabilizer, plasticizer, flash inhibitors, coolant, moderator and surface lubricants etc,<sup>2</sup> which guides to enlighten organic GSR particle. However, the detection and

analysis of GSR is foremost confirmation of evidence in the forensic ballistics fields and there are exigencies to develop more sensitive and selective technique that result in the more in number of cases. The gunshot residue particles are mainly referred to as inorganic particles deposited on the shooter's hands, surrounding areas of the firearm after discharge and travels through the discharged bullet with generally also lean at the target along with bullet residues.

The inorganic GSR particle originated from the primer contain in the percussion cap of a cartridge case. Customarily, the presence of GSR is confirmed by the

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distinctive constituent of Barium (Ba), lead (Pb), antimony (Sb) and bullet residues like copper (Cu), lead, zinc (Zn) nickel (Ni) etc., for supporting purposes.

The organic component in the GSR can be called as organic GSR originated from the propellant powder contain in the cartridge case which are composed of smokeless powder. Smokeless powders are categorized by their compositions single, double and triple base powder. Single base powder contained of nitrocellulose (NC), double Base consist of nitrocellulose (NC) nitroglycerine (NG) and triple base consist of nitrocellulose (NC) nitroglycerine (NG) and nitro-guanidine.<sup>3</sup>

Findings of inorganic elements in the GSR particle are facing challenges by the number of probability of run across casework including lead and heavy metal free (HMF) contained ammunition. So that organic component in GSR plays very important role to pinpoint the findings of GSR particles on the shooter's hands and at the target. In the present review we focused mainly on five compounds which are component of additives contained in gun propellant powder were inspected that are ethylcentralite (EC), methylcentralite (MC), diphenylamine (DPA) originated from stabilizer dimethyl phthalate (DMP) originated from plasticizer and N-nitrosodiphenylamine (N-nDPA) (as shown in Fig. 1) originated from the metabolites or degeneration product of the powder.<sup>1,2</sup>

Many other analytical techniques were employed for separation and detection of organic component of GSR mainly from them, gas chromatography (GC), Raman spectrometry, Micellar electro-kinetic capillary electrophoresis (MEKC), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and desorption electro-spray ionization-mass spectrometry (DESI-MS). Moreover, there are also other environmental presence and findings of OGSR additives such as EC, MC, DPA, DMP and N-nDPA which requires better understanding of forensic scientist about OGSR evidences in a context beyond the elementary questions of detection by analytical methods. Indeed progression of awareness about the persistence of such evidences is crucial when analyzing gun powder additive as a routine procedure for case work.<sup>6</sup> The technique of tandem mass spectrometry (MS) attached with chromatography has

always been favorable due to the highly sensitivity and selectivity. The UPLC-MS was equipped with electro-spray ionization (ESI), adopting multiple reactive monitoring (MRM) modes to determine diphenylamine, ethylcentralite, methylcentralite, dimethyl phthalate, and N-nitro-sodiphenylamine, in the present article review. Ultra-performance liquid chromatography (UPLC) is one of the efficient liquid chromatography (LC) methods to deploy for the separation of different constituents in compound. It is additionally utilized for the recognition and quantitative analysis of compounds and has become trends over the world since many years. Moreover accomplish the considerable improve in speed, resolution and affectability in liquid chromatography, a critical progression in the column and instrumentation technique (column particle size and column measurement) were organized. To overcome the above goal, one of the analytical methods on the basis of small porous particles is Ultra-Performance Liquid Chromatography (UPLC).<sup>26</sup>

#### Techniques and their Analysis

As per Maitre<sup>6</sup> et al., the chromatographic separation of mixture was executed on a UPLC instrument. The mobile phases were used as a methanol having 1% formic acid and Milli-Q water, formic acid of 0.1% with the help of gradient method given in Table-II below, which comprise a 4.6% rise of methanol per minute. Thermostatically saturated column at 43°C and an injection volume of 2µL throughout were deployed.

Additionally evolution of LC-MS is concluded at UPLC with the optimized tandem mass spectroscopy method approach the identification of positive and negative ions which allows the detection of compound desired in even single run. There is an engagement of two ionization source called electrospray ionization (ESI) which is capable in both positive and negative mode and another one is atmospheric pressure chemical ionization (APCI) which is capable in negative mode and switching between them at high speed. This desires the detection of eighteen compounds in only 8 minutes running time taken.<sup>7</sup>

Other several forensic analysts have been separately examined organic constituent in gunshot residue particles by the technique of liquid chromatography assembled with mass spectrometry

(LC-MS) for segregation and identification. The exhibit evidence absorbed during examination is only disadvantage in the method of liquid – chromatography.

Maitre et al.,<sup>6</sup> ethylcentralite (EC), methylcentralite (MC), diphenylamine (DPA), dimethyl phthalate (DMP) and N-nitrosodiphenylamine (N-nDPA) (as shown in Fig. 1) were picked out as the compounds of interest deployed in the manuscript [15]. They may be the most important and typical compounds present in modern gun propellant and consequently the higher side probability of detection in OGSR samples.

Benito et al.<sup>2</sup> used liquid chromatography quadruple time of flight mass spectrometry (LC-QTOF-MS) to separate and detect 18 supplementary agents present in gunpowder (given in table – I) by using MS/MS mode.

Taudte et al.<sup>15</sup> derive and evaluate the development of an ultra high performance liquid chromatographic method (UHPLC) for less time dissociation of 32 compound inherently found in organic part of GSR (including EC, MC, DPA, DMP and N-nDPA). The total time taken for the analysis was 27 minute along with the limit of detection ranging from 0.03 to 0.21ng at 214nm.

As per studies undertaken Mucha et al.<sup>9</sup> the protocol of sampling of OGSR and technique of evaluation was carried for detection of following compounds: tri-nitroglycerine, nitroguanidine,

2,4-dinitrotoluene, stabilizers: diphenylamine and n-nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenyl stabilizer/plasticizer: ethylcentralite, methylcentralite, akardite-I, akardite-II, plasticizer: dibutylphthalate, dimethylphthalate, triethylcitrate and tributylcitrate.

As per Taudte et al.<sup>10</sup> a gradient UHPLC method was evolve for the quantitative analysis of approx. 32 organic components of gunshot Residue using artificial neural networks (ANNs) for rapid optimization. Identities of the compound detected by UHPLC analysis with UV detection where confirm by mass spectrometric detection and which is further associated to an atmospheric pressure chemical ionization (APCI) ion source. Multiple reaction monitoring (MRM) mode was engaged which yields high degree of authenticity in the identification of compound of interest i.e. DPA, N-nDPA, 2 naphthol 4-NDPA, DNG, 2-NDPA, EC and MC in APCI+ mode and NG, DNG in APCI- mode.<sup>10</sup>

Gassner et al.<sup>11</sup> experimented ultra high performance liquid chromatography (UHPLC) equipped with binary pump for maximum delivery separation was performed using kinetex core-shell column from phenomenex (2.6um, 2.1mm x 100mm) with a column C18 stationary phase. UHPLC system was bridged to a triple quadruple mass spectrometer. Electro-spray ionization was operated in positive mode. The [M+H]<sup>+</sup> of the

Explosives	Sensitizers	Stabilizers	Fresh Inhibitors	Plasticizers	Degradation Products
Nitrocellulose	Trinitrotoluene (TNT)	Diphenylamine	2, 4-dinitrotoluene	Diethyl Phthalate	2-Nitrodiphenylamine (2-NDPA)
Nitroglycerine	Pentaerythritoltetranitrate	Methyl centralite	Nitroguanidine	Diethyl Phthalate	4-nitrodiphenylamine (4-NDPA)
Nitroguanidine	--	Ethylcentralite	Nitroguanidine	--	2,4-Nitrodiphenylamine (2,4-NDPA)
Cyclonite (RDX)	--	--	--	--	N-nitrosodiphenylamine (N-NDPA)
Octogen (HMX)	--	--	--	--	2-amine-4,6-dinitrotoluene (2-ADNT)
2,4-dinitroanisole (DNAN)	--	--	--	--	4-amine-2,6-dinitrotoluene (4-ADNT)

**Table 1** Organic additives found in modern smokeless gun-propellant

Sl. No.	Curve	Rate of Flow (mL/min)	Mobile Phases (Water) (+0.1% v/v formic acid)	Mobile Phases (Methanol) (+0.1% v/v formic acid)	Run Time (in Mins.) (In Mins)
1	6	0.8	70%	30%	0.00
2	6	0.8	14.8%	85.2%	12.00
3	6	0.8	70%	30%	15.00
4	6	0.8	70%	30%	17.00

**Table 2** UPLC Gradient Conditions<sup>6</sup>

target compound were explained as the precursor ions and quantification was obtained from SRM measurements MS/MS parameter and along with target compounds AKII, 1,3-DPU, MC, n-NDPA, EC, 2-NDPA, 4-NDPA, DPA were detected.

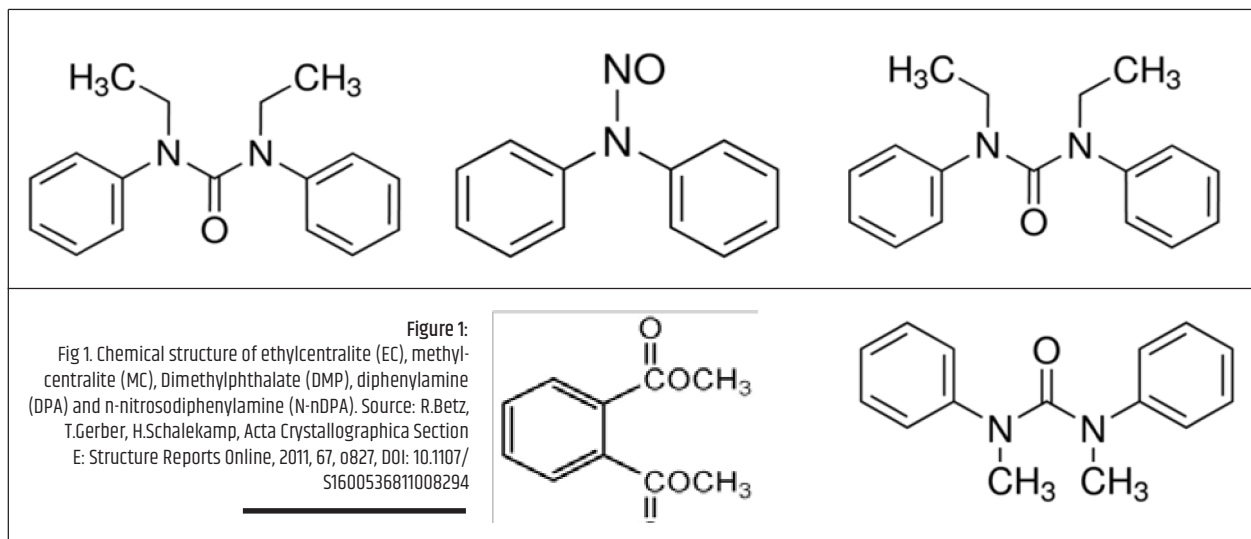
As per the studies by Leah ali<sup>12</sup> liquid chromatography coupled to tandem mass spectrometry (LC-MS /MS) has achieved a worldwide extensive method of separation and evaluation of gun-propellant GSR and explosive because of its higher level of sensibility and accuracy of Liquid Chromatography along with MS/MS mode from its potential to go through product and precursor ion analyze.

Laza *et al.*, engaged LC-MS /MS to analyze commonplace gun-propellant powder stabilizers (table -I) it was concluded that this technique is appropriate for the investigation of organic constituents of GSR. Although the samples had been lifted with the help of cotton swab and were de-

DPA, DMP, N-nDPA, 2-nDPA and 4-nDPA.<sup>28</sup>

Optimization of tandem Mass Spectroscopy, conditioned with a multiple reaction monitoring (MRM) system was begun and optimized to abolish probable interference and enhance the detection sensitivity for the compound of interest included in Table-I below along with certified reference material. The method MRM scanning was chosen to enlighten the lower limit of detection by monitoring of particular ions continuously in shorter time.<sup>12</sup>

According to Leah Ali *et al.*,<sup>12</sup> with increasing uses of heavy metal or lead free ammunition, it is very much necessary to build up methods for the detection of propellant GSR besides the routine inorganic primer contained in GSR particle. Optimized LC parameter and optimized MRM scrutinizing method were evolved to determine the higher side sensitivity for compound present as an additive in organic component of GSR



tected for gunpowder.

Morelato *et al.*, observed that the primary and most effective group to investigate both primary contained particles and propellant GSR on aluminum adhesive stubs. The authors analyze the findings of organic GSR especially EC, MC and DPA first via the instrument desorption electro spray ionization mass spectrometry (DESI-MS). The existence of elements composition in percussion cap of cartridge case were detected through SEM/EDS. Gradient timetable was built to get complete segregation of desired compounds i.e. EC, AKII,

that is AKII, EC, DPA, DMP, N-nDPA, 4-nDPA, 2-nDPA.

Thomas *et al.*,<sup>12</sup> a reverse phase gradient UH-PLC-MS/MS practice has been established for the identification and detection of additive in gun propellant. Approximately, 20 compound present as an additive in organic component of GSR which was segregated by UPLC as well as recognized by the mode of MS/MS in multiple reaction monitoring system. Particularly compounds of interest including diphenylamine, centralite, nitrotoluene, nitroglycerine and various compound of phthalate

group. Indeed electrospray ionization (ESI) with positive and negative modes were engaged along with negative atmospheric pressure chemical ionization (APCI) to evaluate all compounds in a single run time and analyzed within eight minute.

Wide spread smokeless powder propellant additives including both single and double base powder (Table-1) had been separated and detected to determine the relative compositions. Standard GSR mixture was spotted on C18 column and as well as detected in Ultra Violet light within approximately eight minute time taken.<sup>13</sup>

In accordance with capacity factor found by LC analyzer and their existence in two MRM indicators could be reliably recognized the various organic constituents present as additive in the smokeless gun-powder. Studies by Thomas et al. the technique of UPLC-MS/MS approx 18 compound could be separated via C18 column, the compounds of interest detected including EC, MC, DPA, DMP, n-NDPA and which might be individually distinguished by their capacity factor in LC with MS data and when detectable at low nano-gram level, this can be seen as an betterment over LC-MS/MS method published worldwide on account of the broad variety of analyst that can be separated and detected in a fast and effective mode. Moreover the working procedure is monitored with MRM transition for peak confirmation.<sup>13</sup>

Low limit of detection acquired with the UHPLC-MS/MS method should allow its applicability to follow up assays along with GSR samples lifted from the shooter's hands, which would be the one of the best subjects of future research.

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#### RESULT & DISCUSSIONS

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Many of the author's research work has focused on the detection of organic component of GSR via UHPLC-MS/MS along with MRM scanning method and compounds of interest mainly used in stabilizer, plasticizer, flash inhibitors and degradation products etc of gun powder.

The component DPA, EC, MC, DMP and N-nDPA (Fig.1) are basically present in the smokeless powder in modern ammunition which is mainly used as burning rate moderator and stabilizer for smokeless powder. Moreover, EC and MC can be considered as a signature compound for GSR. Separation and detection of these com-

pounds by the method of UHPLC have made headway in the GSR detection.

Confirmation for the GSR would be after analysis of primer GSR as well as the majority of uses heavy metal free (HMF) ammunition, the detection of organic GSR increasingly should be taken on trends for analysis and also useful in evidence for the court of law by providing a trustworthy proof for existence of gunshot residue found in the sample for examination.

By the use of UHPLC-MS/MS method there is also less time consuming for separation and detection of each sample due to its feature of highly sensitivity, linearity, repeatability and authenticity for the validation of UHPLC-MS/MS method is more authentic and reliable. The proficiency to characterize various types of gun propellant additives, which are recognized on the basis of capacity factor and MRM chromatogram.

Maitre *et al.*, analyze all the target compound of interest whenever abundant was got the limit of detection (LOD). Whereas for N-nDPA -  $5.64 \times 10^{-3}$  ppm, for MC -  $1.75 \times 10^{-4}$  ppm, for DPA -  $2.09 \times 10^{-3}$  ppm and for EC -  $3.82 \times 10^{-4}$  ppm are found their LODs.

Benito *et al.*, various organic supplement material were analyzed in GSR involve the plasticizer DEP (Diethyl phthalate) and DMP (Dimethyl phthalate), the stabilizer ethylcentralite (EC), methylcentralite (MC) and degrading product of diphenylamine (DPA). These findings are consonant with the usual organic component of GSR.

It is also known as DEP degradation product of DPA, 4-NDPA and centralite are some popular compounds in GSR. The types of phthalate ester (PAE) actually are diethyl phthalate (DEP) and their also uses in some industrial material in plastics, painting, pesticide, cosmetics and many more. Therefore the detection of DEP as additive in an organic gunshot residue is not sufficiently authentic for GSR as it could be originated from any product of materials made-up of plastic. The separation and detection of degradation product of DPA and centralite are evidential clue of having firearm discharged or having in the contiguity of a firearm discharged event. DPA is treated as stabilizer for propellant and explosive that extends the storage time. Diphenylamine reacts with nitric and nitrous acid leading to the post-combustion

oxidation of nitrocellulose and nitroglycerin.

Diphenylamine (DPA) is further transferred in mono-, di- and trinitro DPA imitative, which are individual character of gun-propellant. Centralites product are absolute to smokeless gunpowder reaction behind their uses are restricted to the ammunition.<sup>2,4,16,17</sup> It is therefore investigation provides strong evidence in gunshot residue analysis.

With the aid of detection of these compounds as well as less time consuming we could overcome the challenges of non-toxic (NT)/heavy metal free (HMF) ammunition use in the current scenario. It is therefore suggestion that with the detection of EC, MC, DPA, DMP and N-nDPA etc. Forensic expert would secondary conformed about the Gunshot Residue (GSR) and firearm discharged event, which would be more beneficial for the forensic investigations.

However, UPLC is one of the most analytical method and primitive tools in the field of chemistry which increases the speed, resolution, as well as their sensitivity of the chromatographic technique and reduces the time of analysis, less consumption of solvent and economical. There is less noise and better signal to noise ratio for the peaks prevailed through UPLC. It yields very sharp and narrow peaks of interest in almost any desired compound.<sup>26</sup> [IJFMP](#)

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