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ORGANIC GUNSHOT RESIDUE FROM LEAD-FREE AMMUNITION

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"Organic Gunshot Residue from Lead-Free Amunition"

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I.

Introduction

The analysis of gunshot residue (GSR) often provides crucial information in criminal investigation. Detection and identification of GSR are commonly performed with the aim of determining whether or not a person fired a gun. GSR are composed of burned and unburned particles from the propulsive charge, as well as components from the primer, the bullet, all the other components of the cartridge (see annexes 1 and 2) and the firearm itself.

The scanning electron microscope equipped with an energy dispersive X-ray analyser (SEM-EDX) can isolate and help identify individual gunshot residue particles through both morphological and elemental characteristics. The morphology and the chemical composition of particles produced by the explosion of cartridges containing lead, antimony and barium in the primer are distinctive. Wolten *et al.* [1979] and later Wallace and McQuillan [1984] proposed to classify GSR particles in categories, based on their possible sources. The GSR particle whose only known source is the explosion of a primer mixture were called “unique” particles by Wolten *et al.* [1979].

In 1982, Hagel and Redecker patented a primer used for the manufacturing of a new ammunition, developed to minimize airborne lead levels and possibly other metallic residue such as barium and antimony [Hagel and Redecker, 1982]. The new ammunition was called SINTOX® and was commercialized by Dynamit Nobel. The primers for this kind of ammunition are produced without the elements presents in the “unique” particles (lead, barium, antimony) and their explosion does not produce “unique” or specific particles. Therefore the contribution of SEM-EDX analysis can be poor in forensic cases when such cartridges are used [Romolo and Margot, 2001], while organic GSR detection could be fundamental in criminal cases when lead-free ammunition is shot. Some

SEM-EDX analyses of particles produced by lead-free primers are collected in annexe 3. In annexe 4 there are the X-ray diffraction analyses of a traditional primer (containing antimony sulphide, barium nitrate and lead styphnate) and of a lead-free primer (containing diazodinitrophenol and strontium nitrate).

The organic gunshot residue (O-GSR) is mainly the residue of the propellant contained in the cartridge. Propellants used in cartridges are known as smokeless powders. They are essentially low explosives, in that they have a reaction rate slow enough to allow its use as a propellant for projectiles. Smokeless powders are either single-base (nitrocellulose), double-base (nitrocellulose plus nitroglycerine) or triple-base (nitrocellulose, nitroglycerine, nitroguanidine) [Meyer *et al.*, 2002]. In propellant manufacturing one or more stabilizers are always employed, due to their chemical structure which prevents the spontaneous, exothermic and acid-catalyzed decomposition of nitrocellulose, nitroglycerine and similar nitric acid esters. Diphenylamine (DPA) reacts with the oxides of nitrogen formed by the slow decomposition of the nitrocellulose (NC), thereby being converted into the corresponding N-nitroso and nitro-derivates. DPA is a pure stabilizer, while other substances, such as methylcentralite (MC) and ethylcentralite (EC), can exert both a stabilizing effect and a gelatinizing effect, simplifying the manufacturing of smokeless powders. Other compounds are introduced into propellant formulations for specific purposes, among them dinitrotoluenes are used as burn modifiers and phthalates as plasticizers (see annexe 2).

The analysis of diphenylamine, methylcentralite, ethylcentralite or others stabilizers and their degradation products in bulk propellants permit the study of the behaviour of propellants during ageing and the estimation of the probable shelf life of the powder. Another aspect of the separation and identification of stabilizers and their reaction products in smokeless powders can be the characterization of a sample for forensic purposes, because these derivatives reflect not only the production of the gunpowder, but also its storage conditions and thermal history following manufacture [Espinoza and Thornton, 1994]. Smokeless powders are commonly used to prepare

pipe bombs and analysis of bulk materials, post-blast samples, suspects' clothes and swabs from individuals, cars or other surfaces can give a substantial contribution to the investigation [Smith *et al.*, 1999; Wallace and Midkiff, 1993; Kee *et al.*, 1990; Dahl and Lott, 1987].

A large number of organic GSR identification and characterization methods were extensively reviewed by Meng and Caddy [1997] but are nowadays used only in a limited number of laboratories [MacCrehan, 2003]. Analysis of organic GSR in casework (skin surfaces and clothing) was started at the beginning of nineties in the Forensic Science Service Laboratory in Birmingham and in the Northern Ireland Forensic Science Laboratory [King, 1995, Speers, *et al.*, 1994; Wallace and McKeown, 1993]. The Division of Identification and Forensic Science of the Israeli Police begun analyzing organic GSR on clothes from real cases at the end of 2002 [Zeichner, 2003].

Promising results were obtained using micellar electrokinetic capillary electrophoresis (MEKC) [MacCrehan, *et al.*, 2002; MacCrehan, *et al.*, 2001; Northrop, 2001a; Northrop, 2001b; Reardon *et al.*, 2000; MacCrehan, *et al.*, 1998] but procedures are quite complex and time consuming. Several procedures for explosive traces and organic gunshot residue detection are described in annexe 5. There are analytical techniques giving very good results in trace detection of explosives, needing further research to be used in organic GSR detection. Such a need was considered to develop the research presented in this work.

II.

Aim of the research

Lead-free primers do not contain lead, antimony and barium and their explosion produces particles having poor evidential value. Their market is increasing and manufacturers are continuously introducing on the market new lead-free primed cartridges. Winchester Ammunition (East Alton, IL, USA) started producing 9 mm Luger Super Unleaded during the nineties [Haag, 1995]. In April 2003, the Winchester catalogue on internet includes two lines of reduced hazard and non-toxic training ammunition: Super Clean NT® and WinClean®. These cartridges are listed in the following tables.

Super Clean NT®	
.357 Magnum	105 gr. (Tin) Bullet
.357 Sig	105 gr. (Tin) Bullet
.38 Special	110 gr. (Tin) Bullet
.40 Smith & Wesson	140 gr. (Tin) Bullet
.45 Automatic	170 gr. (Tin) Bullet
9 mm Luger	105 gr. (Tin) Bullet

Table 1. List of Winchester Super Clean NT® ammunition [Winchester].

WinClean®	
.357 Magnum	125 gr. Jacketed Soft Point Bullet
.357 SIG	125 gr. Brass Enclosed Base Bullet
.380 Automatic	95 gr. Brass Enclosed Base Bullet
.38 Special	125 gr. Jacketed Soft Point Bullet
.40 Smith & Wesson	165 gr. Brass Enclosed Base Bullet
.40 Smith & Wesson	180 gr. Brass Enclosed Base Bullet
.45 Automatic	185 gr. Brass Enclosed Base Bullet
.45 Automatic	230 gr. Brass Enclosed Base Bullet
9 mm Luger	115 gr. Brass Enclosed Base Bullet
9 mm Luger	124 gr. Brass Enclosed Base Bullet
9 mm Luger	147 gr. Brass Enclosed Base Bullet

Table 2. List of Winchester WinClean® ammunition [Winchester].

The contribution of organic gunshot residue detection and identification is therefore fundamental and bound to become increasingly needed with an extended use of lead-free ammunition. There are other reasons suggesting the need for developing new procedures for detection and identification of organic gunshot residue.

1. Some cartridges contain primers producing “not-unique” GSR particles [Haag, 1996; Heard, 1990; Tassa *et al.*, 1982; Meyers and Kopec, 1976]. In a database recording the chemical and physical features of seventy different types of .22 calibre rimfire ammunition, only 16%

of the cartridges contain primer with the three elements Pb, Sb and Ba [Wrobel *et al.*, 1998]. In these cases, where "unique particles" are not produced, evidential value of particle analysis performed using SEM/EDX is poor.

2. Ammunition having mercury fulminate-based primers are commonly manufactured by Eastern European countries and used extensively in the Middle East. Russian and Egyptian 7.62x39 cartridges or 9 mm Luger ammunition for Israeli Sub Machine Gun have primer mixtures which consist mainly of mercury fulminate, potassium chlorate and antimony sulphide, and their explosion does not produce "unique" particles [Zeichner *et al.*, 1992; Tassa *et al.*, 1982]. Moreover, Wallace [1998] investigated the presence of mercury in discharge residue particles from mercury-containing ammunition. He found that mercury did not make a significant contribution to the elemental composition of the GSR because a high percentage was released into the atmosphere and was not detectable by SEM.

3. Using organic techniques, it is possible to develop more sensitive methods than SEM/EDX, which was reported to be unable to identify inorganic residue in several test firings whereas the results for organic residue were positive [Lloyd, 1986a].

4. It is possible to further characterize the ammunition fired. The limited number of elements detected by SEM/EDX produces data which are rarely sufficient for determining the type of ammunition involved in a firing case [Moauro and Falso, 1993; Heard, 1990]. The results of analysis in an actual case are considerably influenced by the effect of different ammunitions used in the same firearm in the past [Khanmy and Gallusser, 1995; Zeichner *et al.*, 1991]. Increasing the number of substances detected in a real case can help determine the ammunition shot.

5. Organic analysis can be faster than SEM/EDX analysis, the screen-up method for explosives used in the Forensic Science Service Laboratory in Birmingham takes around

three hours for skin surface samples (taken by the swabbing kit) and around one hour to examine an item of clothing [King, 1995].

The aim of the present research work was to develop a new procedure for detecting and identifying organic GSR produced by lead-free ammunition, where organic analysis is of great importance. The results were expected to be relevant in all cases involving gunshot residue detection and in the field of explosive traces analysis.

III.

Structure of the research

1. The first step of the project was to identify target molecules to be detected. To date, no systematic work on propellants used in lead-free ammunition has been published. The smokeless powders obtained from commercial lead-free cartridges were analyzed using gas chromatography with mass spectrometric detection (GC-MS). The MS detector was chosen because of its capability of identifying unknown substances, comparing electron impact (EI) mass spectra with database results and giving information on molecular weight and structures. This step is described in chapter IV.

2. After determining the composition of the smokeless powders it was necessary to develop a procedure to sample organic GSR from skin surfaces and another to sample from clothes. "Swabbing is the commonest technique used for collecting organic residue from hands, whereas vacuum lifting is widely used in sampling organic residue from clothing" [Meng and Caddy, 1997]. The published procedures had to be optimized for the present research and used in a series of shooting tests. Samples from people and objects having no relationship with firearms and explosives were taken to obtain data necessary to the forensic interpretation of results. Shooting tests and sampling procedures are described in chapter V.

3. Ion Mobility Spectrometry (IMS) is a highly sensitive analytical technique able to detect a wide range of chemical compounds (both organic and inorganic) at trace levels in gas phase. In chapter VI are described the studies relative to the application of an IMS apparatus (IONSCAN® Model 400) for detection of nitroglycerine in organic GSR. Preliminary results of this research were presented at the 16th meeting of the International Association of Forensic Science, held in

Montpellier [Romolo and Margot, 2002] and at the 3rd meeting of the European Academy of Forensic Science, held in Istanbul [Torre *et al.*, 2003].

4. Thermedics Inc. (Woburn, MA, USA) developed a system for the detection of explosive traces mainly for airport security activity commercialized with the name of EGIS. It is based on high-speed gas chromatography combined with a highly selective and sensitive chemiluminescence detector. In chapter VII the studies relative to the application of the EGIS system for detection of nitroglycerine in organic GSR are illustrated. Preliminary results of this research were presented at the 16th meeting of the International Association of Forensic Science, held in Montpellier [Romolo and Margot, 2002] and at the 3rd meeting of the European Academy of Forensic Science, held in Istanbul [Torre *et al.*, 2003].

5. Atmospheric pressure ionization interface (API) coupled with a tandem MS spectrometer can perform both mild ionization of thermolabile explosive compounds [Yinon, 2001] and was expected to allow sensitive detection of stabilizers used in smokeless powder manufacture. The works of Casetta and Garofolo [1994] and Garofolo *et al.* [1996a] showed the value of LC-MS-MS in the field of analysis of explosives. They analyzed different explosives such as cyclonite (RDX), nitroglycerine (NG), dinitrotoluene (DNT), pentaerythritol tetranitrate (PETN) and nitroguanidine (NQ), among which are molecules of interest in organic GSR analysis. In this step of the research the molecules indicated by the analysis described in the first stage of the project were separately analyzed using a high performance liquid chromatograph equipped with an API ion source and a triple-quadrupole mass spectrometer detector (HPLC-MS-MS). The analytical conditions for the best limit of detection (LOD) of each molecule were determined. Subsequently the parameters governing the chromatographic separation of the compounds of interest were studied to develop a method able to perform their identification and quantitation in a single run. A practical method should be based on a minimum number of steps and analyses. The aim of this part of the research included the development of the analytical conditions to determine

the maximum number of molecules in a single run with the best limit of detection. In chapter VIII are described the studies about HPLC-MS-MS analysis and the validation of the method. Preliminary results of this research were presented at the 15th meeting of the International Association of Forensic Science, held in Los Angeles [Romolo and Margot, 1999] and at the 3rd meeting of the European Academy of Forensic Science, held in Istanbul [Torre *et al.*, 2003].

6. The discussion about the interpretation of results is an essential part of the research. The chemical information obtained by the method developed cannot be used in real cases without extensive studies on firing tests. Only the study of analytical results of samples realized in well-defined situations can help build up the experience and the framework necessary to apply a new method to real casework. In forensic science, this experimentation is as valuable as development work brought by a new analytical method, because it is necessary to understand the forensic meaning of chemical information and to evaluate the related evidence. The interpretation of results was studied both relative to qualitative and to quantitative results. The interpretation of qualitative results was performed determining the diagnostic sensitivity and specificity [Hino *et al.*, 2003; Loong, 2003; Ferrara *et al.*, 1994; Spiehler *et al.*, 1988] of the procedures developed (Ionscan, EGIS, HPLC-MS-MS) in well determined shooting conditions. The interpretation of quantitative results was limited to the HPLC-MS-MS analysis, studying the effect of different parameters (weapons, cartridges, number of shots, etc.). This last stage of the research permitted to determine the applicability of the developed procedures to real cases and to formulate proposal for further developments in the field of forensic analysis of organic gunshot residue. The discussion about the interpretation of results is in chapter IX. Parts of the discussion were presented at the 15th meeting of the International Academy of Forensic Science, held in Los Angeles [Romolo and Margot, 1999] and parts at the 16th meeting of the International Academy of Forensic Science, held in Montpellier [Romolo and Margot, 2002].

IV.

The analysis of smokeless powder in lead-free ammunition

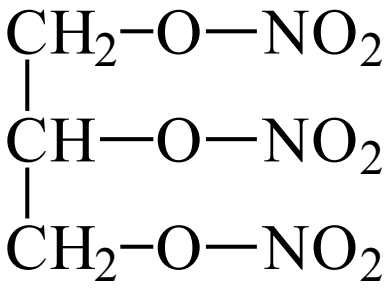
4.1. Introduction

It was not possible to find any publication about the smokeless powders used in lead-free ammunition. The Winchester catalogue reported that Super Clean NT® featured a clean burning propellant [Winchester, 2002] without giving further information. A primer composition wherein manganese dioxide and zinc peroxide or strontium peroxide are used as oxidizers in place of barium nitrate [Krampen *et al.*, 1986; Hagel *et al.*, 1982] is known to possess a low flame temperature which, on occasion, creates performance problems [Bjerke *et al.*, 1990]. To date, no research work on propellants used in lead-free ammunition was found in published scientific literature and target molecules to be detected for the present research needed to be identified.

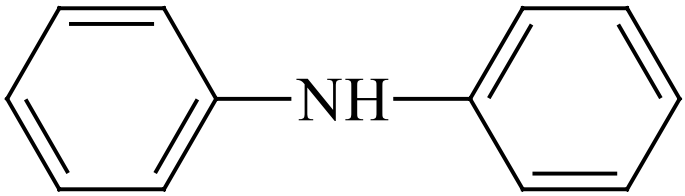
4.2. Experimental

4.2.1. Chemicals and reagents

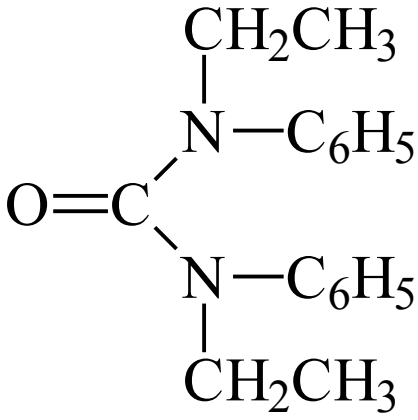
The chemical structures and properties of nitroglycerine (NG), diphenylamine (DPA) and ethylcentralite (EC) are shown in Fig. 1 and in Table 3, 4 and 5 [Meyer *et al.*, 2002]. DPA 99% and EC 99% were purchased from Sigma Aldrich S.r.l. (Milano, Italy). Methanol "plus" of gradient grade was obtained from Carlo Erba (Milan, Italy). NG in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). The stock solutions of all the other compounds were prepared by dissolving 10 mg of each substance in a 100 ml volumetric flask with methanol. All the stock solutions were stored at –20°C. The smokeless powders found in the cartridges listed in Table 6 were analyzed.



NG



DPA



EC

Fig. 1. Chemical structures of nitroglycerine (NG), diphenylamine (DPA) and ethylcentralite (EC).

NITROGLYCERINE	[55-63-0]	
1,2,3-propanetriol trinitrate		
formula	$C_3H_5N_3O_9$	
physical aspect	liquid	
molecular weight	227,1	
energy of formation	-369.7 kcal/kg	
enthalpy of formation	-392.0 kcal/kg	
oxygen balance	3,50%	
nitrogen percentage	18,50%	
volume of detonation gases	762 l/kg	
heat of explosion	1617 kcal/kg	(H_2O liq.)
	1507 kcal/kg	(H_2O gas)
density	1.591 g/cm^3	
solidification point	+13.2 °C stable modification	
	+ 2.2 °C stable modification	
lead block test	520 cm^3/10 g	
detonation velocity, confined	7600 m/s	
impact sensitivity	0.02 kp m	
friction sensitivity	up to 36 kp	
critical diameter of steel sleeve test	24 mm	
vapour pressure	millibar	temperature °C
	0,00033	20
	0,0097	50
	0,13	80
	0,31	90

Table 3. Properties of nitroglycerine [Meyer *et al.*, 2002].

DIPHENYLAMINE	[122-39-4]
formula	$C_{12}H_{11}N$
physical aspect	solid
molecular weight	169,2
energy of formation	+186.1 kcal/kg
enthalpy of formation	+164.9 kcal/kg
oxygen balance	-278,90%
nitrogen percentage	8,28%
density	1.16 g/cm^3
melting point	54 °C
boiling point	302 °C

Table 4. Properties of diphenylamine [Meyer *et al.*, 2002].

ETHYLCENTRALITE	[85-98-3]
1,3-diethyl-1,3-diphenylurea	
formula	$C_{17}H_{20}N_2O$
physical aspect	solid
molecular weight	268,4
energy of formation	-68.2 kcal/kg
enthalpy of formation	-93.5 kcal/kg
oxygen balance	-256,40%
nitrogen percentage	10,44%
density	1.112 g/cm3
melting point	71.5-72 °C
boiling point	326-330 °C

Table 5. Properties of ethylcentralite [Meyer *et al.*, 2002].

4.2.2. Equipment

The cartridges were opened by a kinetic impact bullet puller to recover the smokeless powder. The balance used for weighing the smokeless powder was a Sartorius Basic (Göttingen, Germany) capable of measuring to 0.1 mg. The ultrasonic bath was an Elma T 420 (Übach-Palenberg, Germany). The vortex mixer was a Heidolph Reax Top (Kelheim, Germany). The centrifuge was an ALC 4225 (Milano, Italia). A Hewlett-Packard 6890 Series GC System gas chromatograph equipped with a 5973 Mass Selective Detector (Hewlett-Packard, Palo Alto CA, USA) was used. The GC conditions were as follows: injection port temperature, 50°C for 2 minutes; then programmed 180°C/min to 260°C, final time 20 min; inlet pressure = 40 psi for 1 minute, then 10,99 psi (pulsed split mode); carrier gas, helium; flow 1,3 ml/min, split flow, 50 ml/min; column HP-5 MS (30 m x 0.25 mm i.d.), 95% dimethyl - 5% diphenyl polysiloxane, film thickness 0.25 μm; column temperature, 50°C for 2 minutes; then programmed 15°C/min to 260°C, final time 5 min; transfer line temperature, 260°C.

4.2.3. Sample preparation

One cartridge from every box was opened and a 10 mg portion of smokeless powder was weighted and transferred into a 15 ml centrifuge glass tube. 1.0 ml of methanol was pipetted into each tube. The samples were vortex-mixed for 10 seconds and ultrasonicated for 15 minutes. The tubes were then vortexed for another 10 seconds and finally centrifuged for 5 minutes at 2000 rounds per minute. An aliquot of 1 μl of methanolic solution was analyzed by GC-MS. Complete recovery by this extraction approach was tested by a second extraction of the smokeless powder sample with fresh solvent which yielded no additional recovery of the components [Reardon *et al.*, 2000].

4.3. Results and discussion

The smokeless powders obtained from commercial lead-free cartridges were analyzed using gas chromatography with mass spectrometric detection (GC-MS). The MS detector was chosen because of its capability of identifying substances in the mixture, comparing electron impact (EI) mass spectra with database results and giving information on molecular weight and structures. All identifications obtained from the database were confirmed after analysis of a standard solution of the compounds of interest. Ethylcentralite was not present in the GC-MS database and its identification was based on the retention time and on the mass spectrum of the standard solution. The gas chromatogram of the powder found in the FIOCCHI 9 mm Luger Leadless cartridge and the mass spectrum of nitroglycerine are shown in Fig. 2. NG has a base peak at low mass (*m/z* 46). The gas chromatogram of the powder found in the FEDERAL 9 mm Luger cartridge and the mass spectrum of diphenylamine are shown in Fig. 3. The gas chromatogram of the powder found in the CCI 9 mm Luger cartridge and the mass spectrum of ethylcentralite are shown in Fig. 4. DPA and EC show significant molecular peaks. The gas chromatogram of the powder found in the HIRTENBERGER 9 mm Luger cartridge and the mass spectrum of dibutylphthalate (DBP) are shown in Fig. 5. The identification of DBP, based on the spectra database of the GC-MS system, was never confirmed with a separate analysis of a pure standard. The powder from the GECO 9 mm Luger SX contained a different phthalate with different retention time and mass spectrum, possibly di-isopentylphthalate, following the same spectra database. Analytical results are summarized in Table 7, where the presence of a compound is indicated by a "+". Where "+" is absent the compound was not detected. Most of the smokeless powders contained nitroglycerine and only three were single base (Fiocchi 9 x 21 IMI Lead less, Fiocchi 9 mm Luger Zero Pollution and RWS/GECO .38 Special). The only explosive compound in single base smokeless powders is nitrocellulose (NC). It is possible to analyze NC by size-exclusion chromatography [Lloyd, 1986c; Lloyd, 1984] but not by using HPLC with reversed-phase columns or with GC-MS. For this reason the NC was not considered in the research. In all the powders at least one stabilizer is present:

diphenylamine or ethylcentralite. In six cases dibutylphthalate was found. The analytical results permitted to take into account four target molecules for the following steps of the research: nitroglycerine, diphenylamine, ethylcentralite and dibutylphthalate. The last compound was ruled out due to its widespread diffusion in plastic products. Phthalic acid diesters (PAEs) are widely used as plasticizers to impart softness and flexibility to normally rigid plastics. They can be found not only in polyvinyl chloride (PVC) plastics, in other resins such as poly vinyl acetates, cellulosics and polyurethanes but also in paints, adhesives, cardboard, lubricants and fragrances [Cadogan, 2002; Staples *et al.*, 1997]. PAEs are observed not only in landfill leachate water [Bauer *et al.*, 1998; Bauer *et al.*, 1997; Öman and Hynning, 1993], but also in sewage sludge [Furtmann, 1996; Painter and Jones, 1990], surface and fresh water [Vitali *et al.*, 1997; 7, Furtmann, 1996; Ritsema *et al.*, 1989] and sediment [Furtmann, 1996; Parkman and Remberger, 1995; Rice *et al.*, 1993].

1	CCI	Blount, Inc.	Lewiston, ID, USA	Blazer® Lead-free	9 mm Luger	124 gr. TMJ*
2	CCI	Blount, Inc.	Lewiston, ID, USA	Blazer® Lead-free	.38 Special + P	158 gr. TMJ*
3	FEDERAL	Federal Cartridge Co.	Anoka, MN, USA	BallistiClean®	9 mm Luger	100 gr. JSP*
4	FIOCCHI	Fiocchi Munizioni	Lecco, ITALY	Leadless	9 mm Luger	115 gr. FMC*
5	FIOCCHI	Fiocchi Munizioni	Lecco, ITALY	Leadless	9 x 21 IMI	123 gr. FMC*
6	FIOCCHI	Fiocchi Munizioni	Lecco, ITALY	Zero Pollution	9 mm Luger	123 gr. FMC*
7	GECO	Dynamit Nobel	Troisdorf, GERMANY	SINTOX®	9 mm Luger	124 gr. FMJ*
8	HIRTENBERGER	Hirtenberger AG	Hirtenberg, AUSTRIA	Schadstoff freie Anzundung	9 mm Luger	123 gr. FMJ*
9	Fabrique Fédérale de munitions	RUAG Munition	Thoune, SWITZERLDAND	Lead-free priming	9 mm Luger	123 gr. FMJ*
10	RWS/GECO	Dynamit Nobel	Troisdorf, GERMANY	No Lead - No Barium - Non erosive	9 mm Luger	124 gr. SFJ*
11	RWS/GECO	Dynamit Nobel	Troisdorf, GERMANY	No Lead - No Barium - Non erosive	.38 Special	158 gr. SPFN*
12	SINTOX	Dynamit Nobel	Troisdorf, GERMANY	SINTOX®	9 mm Luger	123 gr. SFJ*
13	SPEER LAWMAN	Blount, Inc.	Lewiston, ID, USA	CLEAN FIRE®	9 mm Luger	124 gr. TMJ*
14	WINCHESTER	Winchester Olin	East Alton, IL, USA	SUPER-X® SUPER UNLEADED	9 mm Luger	115 gr. FMJE*
15	WINCHESTER	Winchester Olin	East Alton, IL, USA	SUPER-X® SUPER UNLEADED	.38 Special	130 gr. FMJE*
16	WINCHESTER	Winchester Olin	East Alton, IL, USA	SUPER-X® SUPER UNLEADED	.40 Smith & Wesson	180 gr. FMJE*

* See following page

Table 6. List of cartridges examined.

FMC Full Metal Case
FMJ Full Metal Jacket
FMJE Full Metal Jacket Encapsulated
JSP Jacketed Soft Point
SFJ Special Full Jacket
SPFN Soft Point Flat Nose
TMJ Totally Metal Jacketed

			NG	**DPA**	**EC**	**P***
1	CCI	9 mm Luger	+		+	
2	CCI	.38 Special + P	+		+	
3	FEDERAL	9 mm Luger	+	+	+	
4	FIOCCHI	9 mm Luger	+		+	
5	FIOCCHI	9 x 21 IMI		+		
6	FIOCCHI	Zero Pollution		+		
7	GECO	9 mm Luger	+	+		+*
8	HIRTENBERGER	9 mm Luger	+	+	(+)**	+
9	Fabrique Fédérale de munitions de Thoune	9 mm Luger	+	+	(+)	+
10	RWS/GECO	9 mm Luger	+	+	(+)	+
11	RWS/GECO	.38 Special		+		
12	SINTOX	9 mm Luger	+	+	(+)	+
13	SPEER LAWMAN	9 mm Luger	+		+	
14	WINCHESTER	9 mm Luger	+	+	(+)	+
15	WINCHESTER	.38 Special	+		+	
16	WINCHESTER	.40 Smith & Wesson	+	+	+	

Table 7. Results of the analyses of smokeless powders.

* Phthalate (P) is dibutylphthalate except for GECO.

** (+) indicates a component whose peak is less than 1/10 of the main peak of the chromatogram.

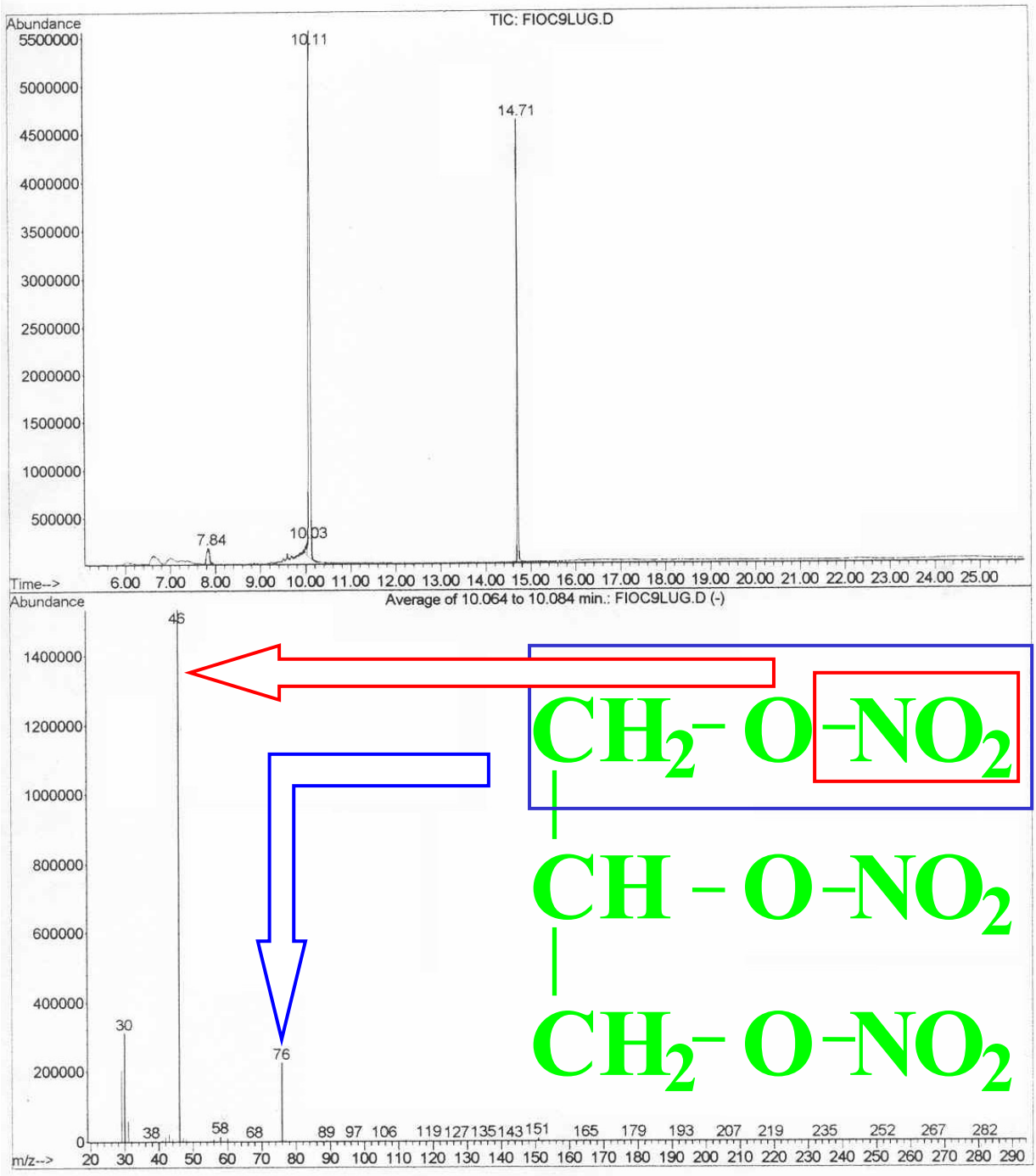


Fig. 2. GC-MS analysis of the powder found in the FIOCCHI 9 mm Luger Leadless, GC peak of nitroglycerine (10.11) and ethylcentralite (14.71). The arrows indicate the origin of two fragments.

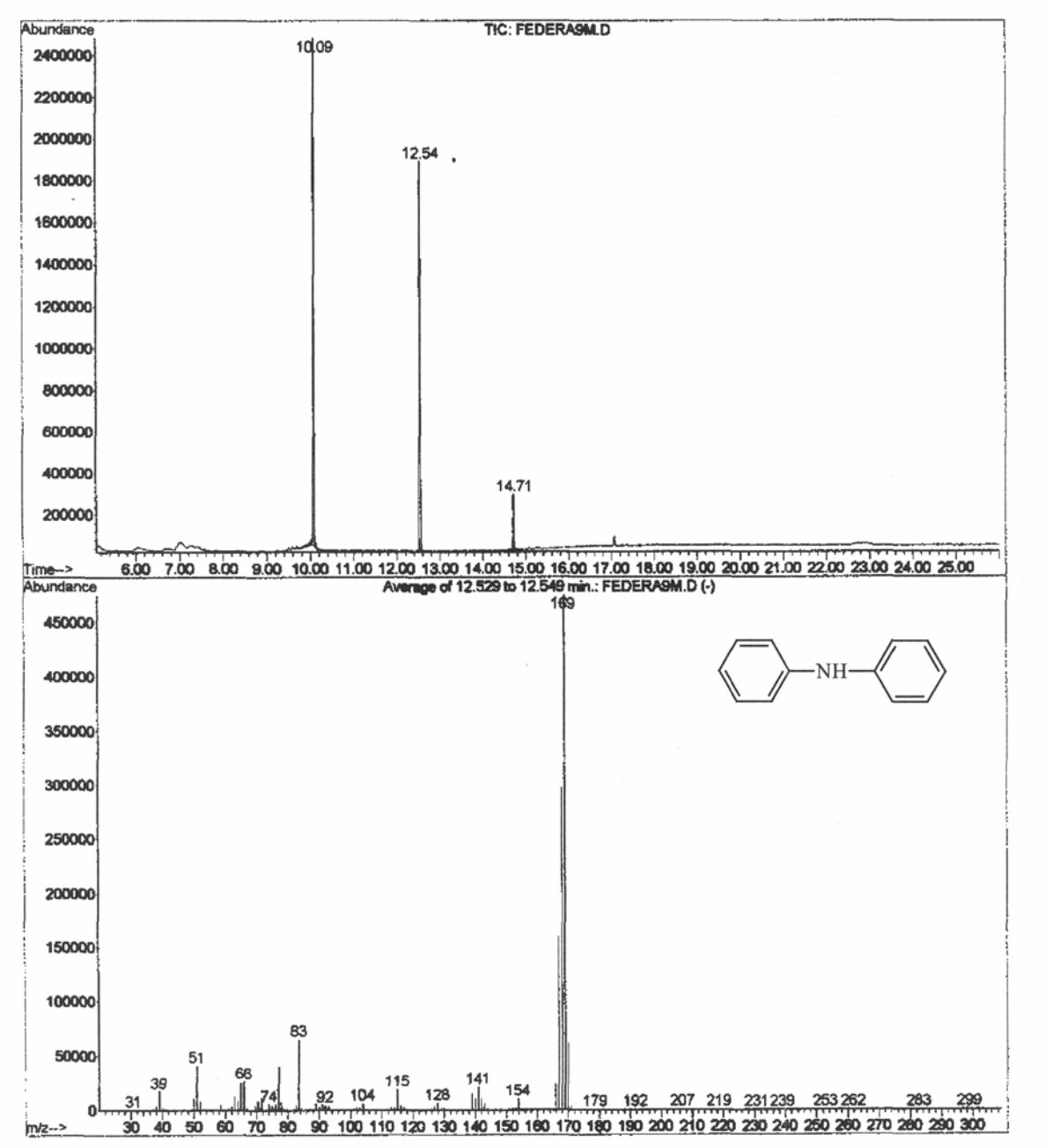


Fig. 3. GC-MS analysis of the powder found in the FEDERAL 9 mm Luger cartridge, GC peak of nitroglycerine (10.09), diphenylamine (12.54) and ethylcentralite (14.71).

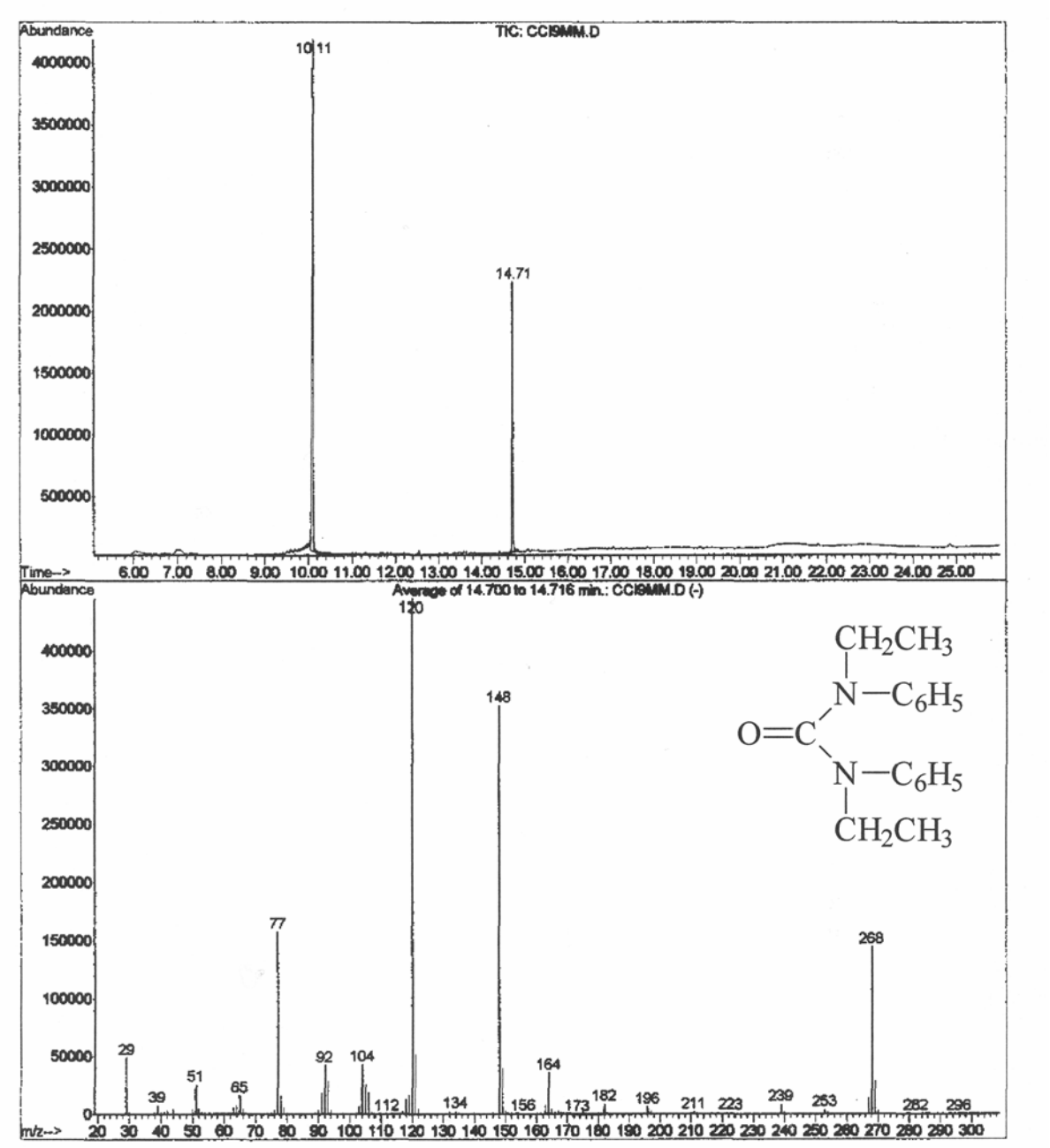


Fig. 4. GC-MS analysis of the powder found in the CCI 9 mm Luger cartridge, GC peak of nitroglycerine (10.11) and ethylcentralite (14.71).

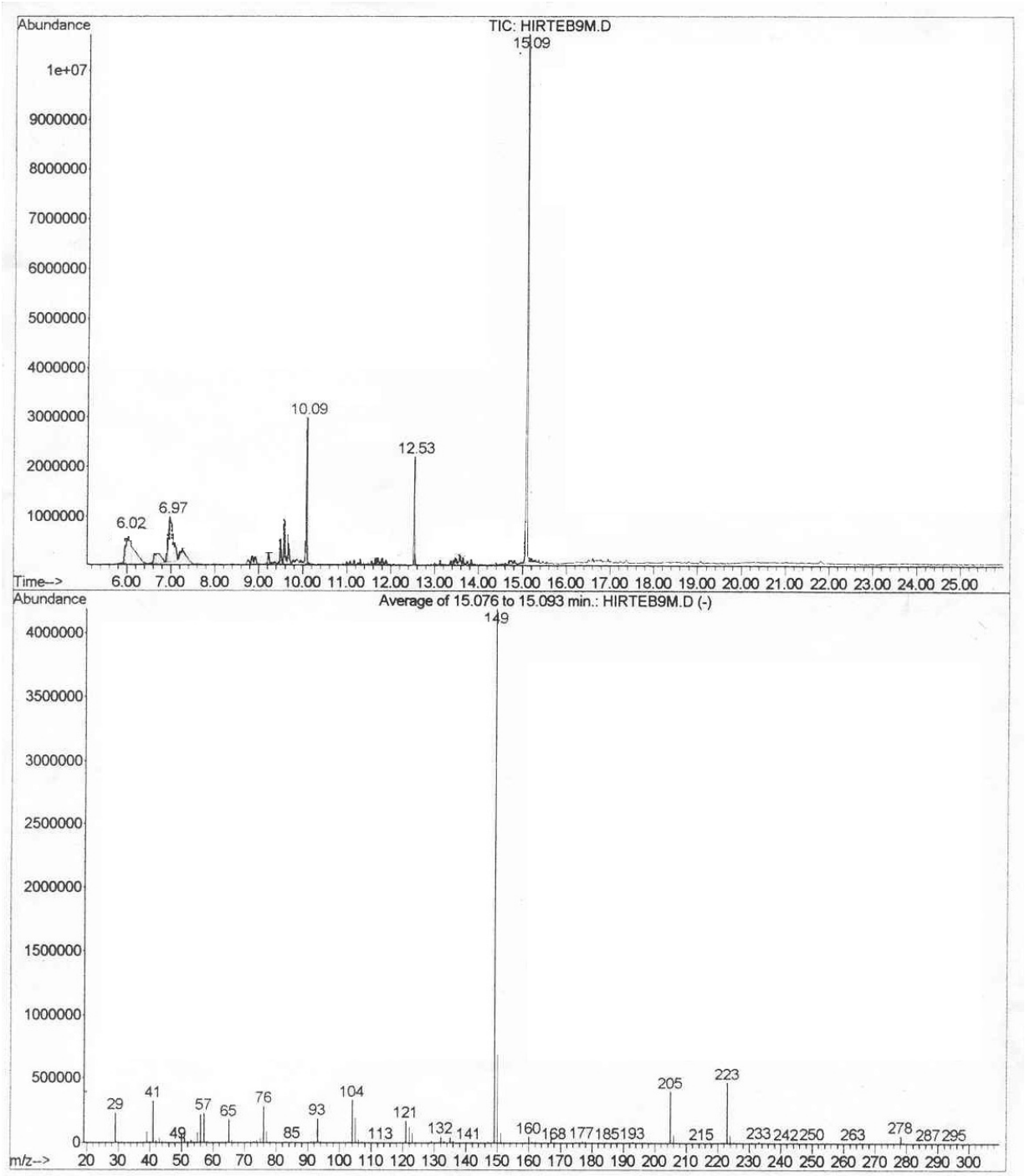


Fig. 5. GC-MS analysis of the powder found in the HIRTENBERGER 9 mm Luger cartridge, GC peak of nitroglycerine (10.09), diphenylamine (12.53) and dibutylphthalate (15.09).

V.

Shooting tests and sampling procedures

5.1. Introduction

The choice of a correct sampling method is critical for detection and identification of gunshot residue (GSR), traces of explosives and related organic compounds. The sampling method chosen depends on the nature of the material to be examined (skin surfaces, clothes, etc.). For smooth surfaces a piece of material can be soaked in a solvent and rubbed over the surface being examined. This procedure, called **swabbing**, is the method of choice for skin, work surface, floors and smooth fabrics such as leather or plastic. On fabrics with an open weave, such as tweed or loosely knitted fabrics, ***vacuum lifting*** is generally preferred. Traces are trapped on a filter using a flexible tube and a vacuum pump. Collection of particles by adhesive ***tape lifting*** on surfaces is the favourite sampling approach for scanning electron microscope (SEM) analysis [Romolo and Margot, 2001].

Different materials for swabbing suspects' hands were tested: cotton-wool, synthetic wool, filter paper, non-woven cotton cloth, acrilan. An ideal swabbing system should efficiently remove the residual explosive from the hands with as little co-extracted interferents as possible. Also, the explosive should remain stable in the swabbing solvent. In water solution, for example, explosives can be degraded by hydrolysis and bacterial activity. The boiling point of the solvent is another important factor: Douse [1982] used ethyl ether to avoid loss of EGDN during the concentration step and later methyl tert-butyl ether [Douse, 1985].

Different swabbing systems developed are shown in Table 8.

N°	material	solvent	reference
1	cotton wool (40 mg)	diethyl ether	Douse, 1982
2	cotton wool (100 mg)	0.5 ml ethanol	Lloyd, 1983b
3	cotton wool (300 - 500 mg) viscose wool (300 - 500 mg) acrilan wool (300 - 500 mg)	acetone diethyl ether ethanol	Twibell *et al.*, 1984
4	non woven cotton (Litex)	ethanol	Russell, 1984
5	cotton wool (10 mg)	methyl tert-butyl ether	Douse, 1985
6	non woven cotton cloth (4 x 6 cm) Litex-10 from LIC Medical (Sweden)	1 ml isopropanol/water (8/2 v/v)	Lloyd and King, 1990
7	acrilan	isopropanol	Wallace and McKeown, 1993
8	non-absorbent cotton wool	ethyl acetate	Meng and Caddy, 1994
9	cotton wool from Gardan Disposables (UK)	ethanol/water water	Warren *et al.*, 1998
10	cotton wool Q-tip from Cheesebrough-Ponds (USA)	acetone water isopropanol/water	Thompson *et al.*, 1999

Table 8. Swabbing systems.

It is difficult to evaluate which is the best swabbing system. The main reason is that swabbing coextracts several interfering compounds from hands and organic gunshot residue are made up of chemically different substances. The acetone swab technique, tested for diphenylamine [Mach *et al.*, 1978], showed a low recovery efficiency (6,5% recovered for 80 ng diphenylamine on the hand). Another reason is that a good solvent can be unsuitable for particular analysis (e.g. water containing solvent systems are not suitable for GC analysis). Twibell *et al.* [1982] tested 8 different solvent systems for swabbing nitroglycerine from hands and found ethanol as the best compromise organic solvent. Optimization studies of the swab material showed that non-woven cotton "Litex" swabs, used pre-wetted with ethanol, were the most efficient [Russell, 1984]. In a later work Twibell *et al.* [1984] tested different swabbing system (three materials and three solvents) for swabbing nitroglycerine, trinitrotoluene and RDX. The results of tests indicated that the material and the solvent had little effect on extraction efficiency and they used for final tests ready-made swabs (Vernail Small Cotton Wool Balls, Vernon Carus Ltd, England) with ethanol. Lloyd and King [1990] proposed the use of non woven cotton cloth (4 x 6 cm) Litex-10 from LIC Medical (Sweden) pre-wetted with 1 ml of propan-2-ol and water (8:2). Meng and Caddy [1994] found ethyl acetate better than ethyl ether for the detection of ethylcentralite. Warren *et al.* [1998] used cotton wool from Gardan Disposables Ltd. (UK) wetted with ethanol or ethanol/water mixture to sample both organic traces of explosives and inorganic ions and sugars. Thompson *et al.* [1999] developed a water based procedure for processing cotton swabs (Q-tip brand from Cheesebrough-Ponds USA Co.) wetted with water, acetone or isopropanol/water mixture.

The recovery of organic gunshot residue from swabs can be obtained with different techniques. The main problem is using a minimum amount of solvent, in order to avoid problems associated with concentration of the sample like impurities present in the solvent and loss of the more volatile compounds. The first technique was **direct extraction** [Douse, 1982], washing the swab with small portions of ether, resulting in a total volume of extracts of 12 ml. Later a **constricted tube**

technique, a **squeeze method** and a **syringe elution** were developed. In the first technique the swab was washed and compressed in a test tube with a narrow hole in the bottom. In the squeeze method the swab was pressed against the wall of a vial using forceps. In the syringe elution the swab was compressed in a 5 ml glass syringe. These three methods produce a total volume of about 5 ml, repeating the extraction with different aliquots of solvent to ensure the maximum recovery. The constricted tube extraction gives more variable recovery efficiency. Using a **centrifugal extraction** of swabs, over 70% of nitroglycerine, TNT and RDX could be extracted in about 1 ml of the solvent and about 50% with the first spin [Twibell *et al*., 1984]. It is also possible to use a Teflon membrane filter during centrifugation and separate the inorganic GSR particles to be examined by SEM-EDX [Speers *et al.*, 1994].

Northrop and MacCrehan [1992] tested some sampling procedure to be used for subsequent micellar electrokinetic capillary chromatography (MEKC). They tested alcohol-cleaned cotton, polyester and PTFE wool, moistened with ethanol or acetone. Each swab was then ultrasonicated for 15 minutes in 500 μl of ethanol containing 1% ethylene glycol. The extract was concentrated to about 2 μl under a stream of nitrogen. The remaining gelatinous concentrate, due to unwanted quantities of skin fats and oils, created a very high sample viscosity resulting in peak shape distortion, or prevented redissolution of the analytes in the running buffer. Collection of the GSR by ***tape lifting*** provided a much more appropriate sampling approach. A binocular micro-stereoscope was used to examine each lift, looking for particles to be extracted. A 2 cm^2 section from the tape was then extracted by ultrasonic agitation for 30 minutes with 50 μl ethanol and 1 μl ethylene glycol. MacCrehan *et al.* [1998] tested a "water soluble" and an "alcohol-soluble" tape, but they weren't found to be suitable for subsequent analysis by MEKC, producing noisy electropherograms and distorted peaks.

Adhesive tapes are not very suitable for the collection of GSR from clothing, because the loss of stickiness restricts the area from which such particles can be collected. ***Vacuum lifting*** is generally

used for sampling gunshot residue and explosive particles from clothing, inside of bags, pockets, etc.. Jane *et al.* [1983] used a glass-fibre disk in a syringe barrel attached to the laboratory vacuum line. Residues were then extracted with ether. Andrasko and Pettersson [1991] developed a double filtration system constructed from a 25 mm diameter Nucleopore aerosol holder connected to an ordinary vacuum cleaner with a porous nylon prefilter with a pore size of 20 μm and a membrane filter made of polycarbonate with a pore size of 0.8 μm. The collected particles were examined by scanning electron microscopy. The Forensic Science Laboratory of Northern Ireland developed an efficient vacuuming system for the recovery of organic and inorganic cartridge discharge residue (CDR) [Speers *et al.*, 1994; Wallace and McKeown, 1993]. Suction sampling apparatus consists of a 25 mm diameter in-line "Deldrin" filter holder and a 25 mm diameter fluoropore membrane filter. After sampling and recovering of traces, it may be necessary to concentrate the solution obtained before analysis. Ether can be easily evaporated to near dryness (5-10 μl) using a stream of nitrogen, allowing the last traces of ether to evaporate at room temperature to avoid loss of EGDN [Douse, 1982]. Lloyd [1983b] used an air flow (75 ml/min) followed by 30 minutes at 20°C to evaporate ethanol. He found that 86% of EGDN was lost when concentrating the extract to 1/5. A water bath at 90°C was used by Twibell *et al.* [1984] to reduce volume extracts to 1 ml. Addition of a small percentage of a non-volatile substance like ethylene glycol (boiling point = 198 °C) prevents losses during the evaporative concentration [Northrop and MacCrehan, 1992]. Recently MacCrehan *et al.* [1998] used a heated centrifuge system to remove ethanol by controlled evaporation under reduced pressure.

The experimental activity described in the present chapter was the performing of shooting tests and the sampling of residue for analysis. The sampling procedures described by Wallace and McKeown and adopted in the Forensic Science Agency of Northern Ireland were followed with minor modifications [Speers *et al.*, 1994; Wallace and McKeown, 1993]. **The research helped optimize**

the recovery of organic GSR in solutions suitable for analysis, leaving the opportunity to analyze inorganic GSR particles by SEM.

5.2. Experimental

5.2.1. Materials and equipment

Two weapons were used for shooting tests, a Colt Detective Special .38 Special (Hartford, CT, USA) and a SIG Sauer P226 9 mm Luger (Eckernförde, Germany). Three different lead-free primed cartridges were used. They were Winchester .38 Special Super-X® Super Unleaded, Winchester 9 mm Luger Super-X® Super Unleaded and GECO SX 9 mm Luger. The swabs used for sampling from skin surfaces were Alco-Prep® kindly provided by H&W cv (Glabbeek, Belgium). The swabs contained a mixture called "isopropyl rubbing alcohol" made with Isopropyl Alcohol USP (United States Pharmacopea) and Purified Water USP, without any other compounds that could interfere with the following analysis [H & W cv, 2003]. Other swabs tested were prepared using cotton pads commonly used to remove make-up from three different brands: Johnson and Johnson from Consumers Companies, Inc., DEMAK-UP from Fort James Italia (Genova, Italy) and Naturaline from COOP (Basel, Switzerland). Empty glass columns (6 ml) were from Supelco INC. (Bellafonte, PA, USA). Isopropyl alcohol RS for HPLC, methanol RS for HPLC and acetone RPH were Carlo Erba (Milano, Italy). For vacuum lifting 25 mm diameter 0.5 μm fluoropore membrane filters FHLP 02500 were used, purchased from Millipore (Watford, UK) with Deldrin filter holders 1109 purchased from Gelman (Northampton, UK) and an Edwards vacuum pump (Crawley, UK). Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). Isosorbide dinitrate (ISDN) containing 25% of lactose was from Menarini (Firenze, Italia) and kindly provided by Istituto Luso Farmaco S.p.A. (Milano, Italy). Nylon 11 bags, 60.0 x 90.0 mm, film thickness 40 μm, were purchased from M&Q Plastick Products Ltd. (Limerick, Ireland). The centrifuge was an ALC PK131R (Cologno Monzese, Italy).

5.2.2. Shooting tests

Shooting tests were performed in the shooting range of the Institut de Police Scientifique et de Criminologie (IPSC), of the Université de Lausanne (CH). All the tests are summarized in Table 9. Before every shooting test the shooter washed both his hands, took the weapon prepared by someone else and left on the desk in the shooting range, shot and was sampled by a third person outside the shooting range. The shooter did not touch any surface after washing his hands and before sampling except for the weapon. The cloth worn by the shooter during the shooting were taken and stored in nylon bags before sampling to avoid NG loss.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE
V1	Colt	Win .38	1	laboratory coat
V2	Colt	Win .38	1	woollen jacket
V3	Colt	Win .38	1	sweatshirt
V4	Colt	Win .38	1	cotton shirt
S5	Colt	Win .38	6	hands
SV6	Colt	Win .38	4	cotton shirt + hands
V7	Colt	Win .38	4	cotton shirt
V8	Colt	Win .38	4	cotton shirt
V9	Colt	Win .38	4	cotton shirt
V10	Colt	Win .38	4	cotton shirt
V11	Colt	Win .38	4	cotton shirt
V12	Colt	Win .38	4	cotton shirt
V13	Colt	Win .38	4	cotton shirt
V14	Colt	Win .38	4	cotton shirt
V15	Colt	Win .38	4	cotton shirt

Table 9. Shooting tests.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE
V16	Colt	Win .38	4	cotton shirt
V17	Colt	Win .38	4	cotton shirt
V18	Colt	Win .38	4	cotton shirt
V19	Colt	Win .38	4	cotton shirt
V20	Colt	Win .38	4	cotton shirt
V21	Colt	Win .38	4	cotton shirt
V22	Colt	Win .38	4	cotton shirt
V23	Colt	Win .38	4	cotton shirt
V24	Colt	Win .38	4	cotton shirt
V25	Colt	Win .38	4	cotton shirt
V26	SIG Sauer	Win 9 mm	4	cotton shirt
V27	SIG Sauer	Win 9 mm	4	cotton shirt
V28	SIG Sauer	Win 9 mm	4	cotton shirt
V29	SIG Sauer	Win 9 mm	4	cotton shirt
V30	SIG Sauer	Win 9 mm	4	cotton shirt
V31	SIG Sauer	Win 9 mm	4	cotton shirt
V32	SIG Sauer	Win 9 mm	4	cotton shirt
V33	SIG Sauer	Win 9 mm	4	cotton shirt
V34	SIG Sauer	Win 9 mm	4	cotton shirt
V35	SIG Sauer	Win 9 mm	4	cotton shirt
SV36	SIG Sauer	GECO 9 mm	4	cotton shirt + hands
SV37	SIG Sauer	GECO 9 mm	4	cotton shirt + hands
SV38	SIG Sauer	GECO 9 mm	4	cotton shirt + hands
SV39	SIG Sauer	GECO 9 mm	4	cotton shirt + hands
SV40	SIG Sauer	GECO 9 mm	4	cotton shirt + hands

Table 9. Shooting tests.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE
SV41	SIG Sauer	GECO 9 mm	3	cotton shirt + hands
SV42	SIG Sauer	GECO 9 mm	3	cotton shirt + hands
SV43	SIG Sauer	GECO 9 mm	3	cotton shirt + hands
SV44	SIG Sauer	GECO 9 mm	3	cotton shirt + hands
SV45	SIG Sauer	GECO 9 mm	3	cotton shirt + hands
SV46	SIG Sauer	GECO 9 mm	2	cotton shirt + hands
SV47	SIG Sauer	GECO 9 mm	2	cotton shirt + hands
SV48	SIG Sauer	GECO 9 mm	2	cotton shirt + hands
SV49	SIG Sauer	GECO 9 mm	2	cotton shirt + hands
SV50	SIG Sauer	GECO 9 mm	2	cotton shirt + hands
SV51	SIG Sauer	GECO 9 mm	1	cotton shirt + hands
SV52	SIG Sauer	GECO 9 mm	1	cotton shirt + hands
SV53	SIG Sauer	GECO 9 mm	1	cotton shirt + hands
SV54	SIG Sauer	GECO 9 mm	1	cotton shirt + hands
SV55	SIG Sauer	GECO 9 mm	1	cotton shirt + hands
S56	SIG Sauer	GECO 9 mm	3	hands
S57	SIG Sauer	GECO 9 mm	3	hands
S58	SIG Sauer	GECO 9 mm	3	hands
S59	SIG Sauer	GECO 9 mm	3	hands
S60	SIG Sauer	GECO 9 mm	3	hands

Table 9. Shooting tests.

5.2.3. Sampling procedures

Two different methods were used for sampling. Swabbing was used for skin surfaces. Vacuum lifting was the method chosen for clothes. People and objects not related to shooting were sampled in order to get blank controls. The sampling forms shown in the following page were filled for every sampling. The details about each shooting test (weapon, cartridge, number of shots) were described in the line of the description of the occupation of the last two hours.

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Université de Lausanne

Institut de police scientifique et de criminologie (IPSC)

SAMPLING FORM

Test number _____ Sampling date _____

Sampling time _____

Sampling operator _____ Analysis date _____

Normal occupation _____

Sex	M	F

Hand generally used	R	L

Sampled cloth _____

Colour _____

Was the occupation in the last two hours the normal one?	YES	NOT

If NOT describe _____

Was used a firearm in the last 24 hours?	YES	NOT

or simply touched?

	YES	NOT

If in the last 24 hours a firearm was used or touched, were the hands washed after?	YES	NOT

The subject would like to be contacted with the analytical results?	YES	NOT

Address _____

Telephone _____

NOTE _____

SAMPLES	conditions	results
Cloth		
Right hand		
Left hand		
Control		

5.2.3.1 Sampling procedures for skin surfaces

Two different swabbing systems were used. Commercial cotton round pads were cut in four pieces to prepare four cotton swabs. Swabs from cotton pads were dampened with 1.0 ml isopropyl alcohol and then shaken to remove the excess solvent. One swab was used to sample each hand. The swab is firmly rubbed numerous times over the entire surface of the hand i.e. palm, fingers, thumb, back and wrist. This isopropanol based sampling procedure was used for ion mobility spectrometry (see chapter VI) and EGIS analysis (see chapter VII). H&W Alco-Prep® swabs were used for samples to be analyzed with HPLC-MS-MS (see chapter VIII). After sampling, each swab was immediately enclosed into a 7 ml amber glass vial and sealed using a crimped teflon coated septum and stored at -20°C before the extraction.

5.2.3.2 Sampling procedures for clothes

Vacuum lifting was described by Wallace and McKeown [1993]. Deldrin filter holders were closed with plastic caps on the ends and stored at -20°C before the extraction. The main advantage of the vacuum lifting procedure employed is the possibility to isolate GSR particles to be analyzed by scanning electron microscopy after organic GSR extraction.

5.2.4. Sample preparation

5.2.4.1 Preparation of swabs from skin surfaces

Samples obtained as described in paragraph *5.2.3.1* were treated in slightly different ways, depending on the subsequent analysis. The internal standard (IS) was not used for ion mobility spectrometry (see chapter VI) and EGIS analysis (see chapter VII) and was added only in sample analyzed by HPLC-MS-MS (see chapter VIII). The IS used was RDX in the analysis performed between 1997 and 2001 and ISDN since 2002. A stock solution of ISDN (1 mg/ml) was prepared by

dissolving the compound in methanol and stored at -20°C. The standard solution containing 500 μg/ml of RDX and the standard solution containing 500 μg/ml of ISDN were prepared by diluting the RDX solution or the ISDN stock solution. The procedure for the extraction of the swab is in the following stepwise description.

a. Wash hands and wear new disposable gloves.

b. Wash the bench working surface with acetone.

c. Take swabs of the bench surface and hands (controls).

d. Put vials with swabs on a different bench, away from the working surface.

e. Fill the analysis form (see page 40), with a blank between each sample (see further details in chapter VIII for HPLC-MS-MS analysis).

f. Label the small vials (1.5 ml for autosampler).

g. Crimp the small vials with teflon coated septa.

h. Pierce the septa of the small vials with a disposable scalpel blade.

i. Insert one empty glass column into each pierced septum on the small vials.

j. Take every swab with new disposable tweezers and put it in the column inserted into the corresponding labelled vial. Use always the same hand to touch the large vials (7 ml amber glass vial).

k. Wash hands.

l. Pipette 10 μl of the 500 μg/ml IS solution onto the swab (only for analysis with HPLC-MS-MS described in chapter VIII).

m. Wash the internal walls of the large vials with 1 ml methanol.

n. Transfer the methanol onto the corresponding swab with a disposable pipette. Use always the same hand to touch the large vials.

o. Close the glass columns with plugs.

p. Centrifuge for 5 minutes at 3000 rounds per minute at T = 5°C.

q. Wash hands and wear new disposable gloves.

r. Decap vials and seal with new crimped septa.

s. Place the vials in the autosampler tray of the HPLC-MS-MS in the order of the analysis form (see details in chapter VIII).

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ANALYSIS FORM

Test/Case number _____
Operator _____
Extraction area _____
Instrument type _____
Instrument number _____

Extraction date _____
Extraction time _____
Analysis date _____
Analysis time _____

Sample number	Sample description	Results	Notes

Date _____ Signature _____

5.2.4.2 Preparation of filters from clothes

Samples obtained as described in paragraph *5.2.3.2* were treated in slightly different ways, depending on the subsequent analysis. The internal standard was not used for ion mobility spectrometry (see chapter VI) and EGIS analysis (see chapter VII) and was added only in sample analyzed with HPLC-MS-MS (see chapter VIII). The IS used was RDX in the analysis performed between 1998 and 2001 and ISDN since 2002. A stock solution of ISDN (1 mg/ml) was prepared by dissolving the compound in methanol and stored at -20°C. The standard solution containing 500 μg/ml of RDX and the standard solution containing 500 μg/ml of ISDN were prepared by diluting the RDX solution or the ISDN stock solution. The procedure for the extraction of the filters is in the following stepwise description.

A. Wash hands and wear new disposable gloves.

B. Wash the bench working surface with acetone.

C. Take swabs of the bench surface and hands (controls).

D. Put Deldrin with filters on a different bench, away from the working surface.

E. Fill the analysis form (see page 40), with a blank between each sample (see further details in chapter VIII for HPLC-MS-MS analysis).

F. Label the 7 ml amber vials.

G. Close the vials with plastic caps.

H. Pierce the plastic caps of the vials with a disposable scalpel blade.

I. Take one Deldrin filter holder, take out the cap from the lower end and insert the lower end of the Deldrin into the pierced cap of the corresponding vial.

J. Take out the cap from the upper end.

K. Tighten the Deldrin unit.

L. Wash hands and wear new disposable gloves.

M. Return to point I. for the following Deldrin filter holder.

N. Pipette 10 µl of the 500 µg/ml IS solution into the upper end of the Deldrin filter holders inserted into the vials (only for analysis with HPLC-MS-MS described in chapter VIII). Take care not to touch the sides to avoid cross contamination (otherwise, the pipette tip must be replaced).

O. Pipette 1 ml methanol into the upper end of the Deldrin filter holders inserted into the vials. Take care not to touch the sides to avoid cross contamination (otherwise, the pipette tip must be replaced).

P. Cap the Deldrin filter holders and allow dissolving any organic residue for five minutes turning around the vial in a 45° position.

Q. Insert the vials into centrifuge holders on top of the lower end of a Falcon tube cut 3.0 cm long (see Fig. 6).

R. Centrifuge for 5 minutes at 2000 rounds per minute.

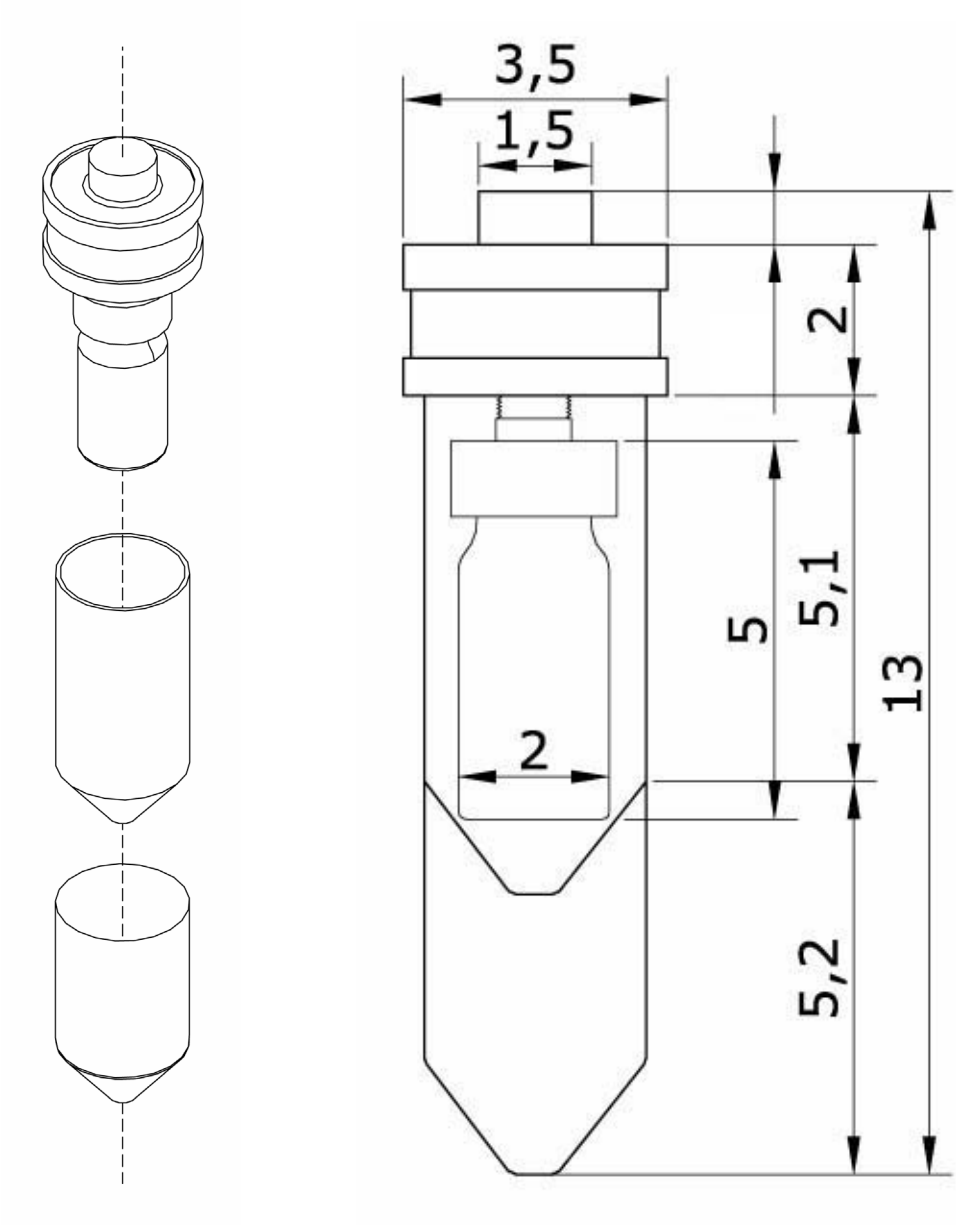


Fig. 6. The position of the Deldrin filter holder and the vial in the centrifuge [cm].

5.3. Results and discussion

5.3.1 Cotton pads for swabs

The cotton pads commonly used to remove make up were chosen because they are more resistant than common cotton wool and very easy to find on the market. H&W Alco-Prep® swabs were expected to give more reproducible sampling and to be better for using in a contamination-free procedure, due to the package. In the box with 100 items, every swab is in a separate sealed small envelope, to be opened by tearing before the use. H&W Alco-Prep® swabs were not used for ion mobility spectrometry and EGIS analysis due to the presence of water.

To determine the variability of the weight of cotton pads in a pack, 20 round pieces for brand where weighted. The arithmetic mean, the sample standard deviation (s) and the coefficient of variation (CV) were calculated [Skoog *et al.*, 1996]. In Table 10 are shown the results [g] for Johnson and Johnson, DEMAK-UP and COOP. The Naturaline from COOP pads were preferred because they gave the lower CV.

	Johnson & Johnson	DEMAK-UP	COOP
	0,7070	0,6376	0,5548
	0,6269	0,6124	0,5695
	0,7091	0,5535	0,5799
	0,6395	0,5962	0,5730
	0,7187	0,6396	0,5467
	0,7100	0,6413	0,5548
	0,7418	0,6008	0,5552
	0,5548	0,6203	0,5346
	0,4883	0,5822	0,5527
	0,6915	0,6201	0,5487
	0,7566	0,6329	0,5660
	0,8793	0,7223	0,5469
	0,6868	0,5642	0,5546
	0,5825	0,6029	0,5651
	0,7607	0,6137	0,5694
	0,6643	0,6264	0,5854
	0,5126	0,6173	0,5961
	0,5733	0,5965	0,5883
	0,7223	0,5303	0,5969
	0,6802	0,5659	0,5883
Mean	**0,6703**	**0,6088**	**0,5663**
s	0,0934	0,0404	0,0180
CV	14	7	3

Table 10. Weight, arithmetic mean, sample standard deviation (s) and coefficient of variation (CV) of 20 round pads from 3 packs of different brands [g].

5.3.2 Solvent recovery from filters

The solvent recovery procedure from the Deldrin filter holder was studied and optimized. Speers *et al.* [1994] purged the Deldrin unit with nitrogen for 20 s after adding the solvent. The purging procedure was compared with three centrifuge procedures adding 1 ml of methanol into a Deldrin holder containing a fluoropore filter. The Deldrin unit was capped and the vial was turned around in a 45° position for five minutes before solvent recovery. The amounts of solvent recovered from the four different recovery procedure in µl are reported in Table 11. The centrifuge for 5 minutes at 2000 round per minute at T = 5°C was preferred because of the volume results, the possibility to process more samples in the same time and the limited handling, resulting in a lower risk of contamination.

	Purging with nitrogen	Centrifuge for 3 minutes at 2000 r.p.m. (T room)	Centrifuge for 5 minutes at 2000 r.p.m. (T room)	Centrifuge for 5 minutes at 2000 r.p.m. (T = 5°C)
	580	600	680	700
	720	640	620	640
	620	680	700	720
	570	670	680	690
Mean	**620**	**650**	**670**	**690**
S	69	36	35	34
CV	11	6	5	5

Table 11. Amounts of solvent recovered, arithmetic mean, sample standard deviation (s) and coefficient of variation (CV) from four different recovery procedures [µl].

VI.

Analysis with ion mobility spectrometry

6.1. Introduction

Ion Mobility Spectrometry (IMS) is a high sensitive analytical technique able to detect a wide range of chemical compounds (both organic and inorganic) at trace levels in gas phase. It was first introduced by Cohen and Karasek in 1970 under the name plasma chromatography [Cohen and Karasek, 1970]. An IMS apparatus is capable of measuring the ionic mobilities of compounds in the gaseous phase and at atmospheric pressure, under the influence of a weak electric field. Most of the applications of the IMS are in the military, security and forensic fields (chemical warfare agents, explosives and illicit drugs) [Karpas, 1989]. However, there is an increasing trend towards the civilian applications, like industrial process monitoring and detection of pollutants.

The analytical capabilities of IMS include solid sample introduction on a swab or filter, fast analyses, high sample throughput, high sensitivity, ease of use. The IMS instruments can be rugged enough to be field-portable and easy to use to enable non-scientific personnel to operate it under strictly controlled conditions. For these reasons it is widely used not only in laboratories but on the scene of crime, in airports control and custom zones too. IMS has become one of the key analytical techniques used to detect concealed explosives, including detection both in the vapour phase and of low-vapour-pressure explosives by sampling sub-nanogram traces and analyzing with a suitable device for introduction of samples [Kolla, 1997].

An IMS system comprises an inlet section, an ionization region, an ion filtering zone, an output section for ion species detection and a control device.

Molecules in gas phase can be directly introduced into the ionization section, but a preconcentrator can be used to enhance the sensitivity. A preconcentrator is basically a filtering device that allows a small amount of the compound of interest in a large incoming air flow to be concentrated into a much smaller air volume via adsorption and desorption, prior to ionization.

Particles of explosive with low vapour pressure on surfaces can be collected using wipe samples, made with filter materials, and thermally desorbed into the IMS apparatus. Another way to volatilize explosive materials for analysis by IMS is laser desorption [Huang *et al.*, 1987].

In conventional apparatus a ^{63}Ni emitter permits the ionization of analytes mixed with a carrier gas (purified air) and a reactant gas, which enhance ion formation in the ionization region [Lawrence *et al.*, 2001; Fetterolf and Clark, 1993]. During the past decade, improvements have occurred in IMS on the understanding of reactant gas chemistry, the influence of temperature on ion stability and sampling methods [Ewing *et al.*, 2001]. More recently a non-radioactive ion source, called variable ionization potential (VIP) or electron lamp, was developed to replace current radioactive ionization sources [Doring *et al.*, 2001]. Laser multiphoton ionization (MPI) was used to produce ions from explosive vapours at atmospheric pressure in air for analysis by ion mobility spectrometry too [Clark *et al.*, 1995]. The possibility of using negative corona discharge as the ionization source for negative ion mobility spectrometry (IMS) was studied as well [Khayamian *et al.*, 2003; Tabrizchi and Abedi, 2002; Turner *et al.*, 1997].

Trace analytes are characterized after ionization by determining the mobilities of the ions in a weak electric field of an ion filtering zone, called drift tube. The different ions travel in the ion drift tube at ambient pressure and in the presence of a counter current drift gas. The ions with a higher mobility traverse the drift region in a shorter time before reaching the output section for ion species detection. The plot of the ion current intensity of the detector versus the time, recorded by the control section, gives rise to an ion mobility spectrum or plasmagram. The ion mobility spectrum of an analyte can be characteristic and identifiable. Unfortunately, IMS spectra can contain compounds

having similar behaviour with the target molecules, interfering with the analysis [Daum *et al.*, 2001]. Rapid temperature programming coupled with chemometrics was shown as a useful tool for the separation of analytes from interferents [Buxton and Harrington, 2001].

Ion mobility spectrometry (IMS) has to date been successfully utilized for several analytical problems, from the detection of prescription and illicit drugs as well as for rapidly screening human hair samples for the stimulant methamphetamine and for the entactogens MDMA ("ecstasy") and MDEA ("Eve") [Keller *et al.*, 1998]. Ion mobility spectrometry is a promising approach for monitoring vapours of highly electronegative species such as chlorinated compounds [Chen and Chen, 2002; Walls *et al.*, 1999], to perform analyses for industrial process control [Li *et al.*, 2002], for detection of the accumulation of impurities in gas switches for hydroelectric generating stations and for measuring the levels of volatile organic compounds in the life-supporting atmosphere of the International Space Station [Eiceman, 2002]. Recent studies indicate that this technique has potential for detection of biogenic amines present in vaginal fluid to diagnose bacterial vaginosis [Karpas *et al.*, 2002] and for detection of specific components of bacterial cells to identify and differentiate bacterial strains and species [Vinopal *et al.*, 2002].

The importance of IMS in counterterrorism investigations to detect explosive traces both before and after an explosion was early recognized [Fetterolf, 1993; Fetterolf and Clark, 1993; Yelverton, 1988; Spangler *et al.*, 1983]. Commercial apparatus permit the collection of trace physical evidence transferred to hands or surfaces through contact or post-blast residue. The low detection limits are provided by efficient ionization process, optimized drift tube conditions and sensitive ion detectors [Denson *et al.*, 2002]. Filter material properties such as pore size, surface roughness and porosity; flow rate and explosive vapour pressure are parameters that can affect the IMS response [Su and Babcock, 2002a; Mina *et al.*, 2001; Ritchie *et al.*, 1994]. Samples can be dissolved in a solvent and vaporized using a sample dispenser in the reaction compartment of the IMS. In this way, the sample dispenser can be operated at a lower temperature [Adler, 1999]. The gas phase ion chemistry of

most explosives is mediated by the fragile C-ONO_2 bonds or by the acidity of protons and can follow complicated pathways. However, once ions are formed, they appear to have stabilities on time scales equal to or longer than ion drift times, between 5 and 20 ms [Lawrence *et al.*, 2001; Kolla, 1997; Fetterolf and Clark, 1993; Karpas, 1989].

Calibrations in the picogram to nanogram range of explosive vapour generators for 2,4,6-trinitrotoluene (TNT), cyclo-1,3,5-trimethylene-2,4,6-trinitramine (RDX), and pentaerythritol tetranitrate (PETN) were obtained using an ion mobility spectrometer. IMS was then chosen as the calibrating instrument to build and to support an independent validation and verification facility for explosive detection systems for the US Federal Aviation Administration [Eiceman *et al.*, 1997; Davies *et al.*, 1993].

In 1996 an Ionscan Model 250/350 was used to perform rapid quantitative determination of 2,4,6-trinitrotoluene [Garofolo *et al.*, 1996b]. A calibrated Barringer Ionscan 400 ion mobility spectrometer was used to analyze repeatable and well-characterized simulation for detection of trace explosive residue [Phares *et al.*, 2000]. Calibration helps in maintaining data quality by recording peak amplitudes and mobility data from quality control (QC) check solutions before proceeding with analyses. Analyses of QC data are helpful to provide an early indication of potential problems [Poziomek *et al.*, 1998].

A special attention was paid to application of IMS in airport security activity. The coupling of an airline passenger personnel portal with a high-flow (HF), high-resolution (HR) ion mobility spectrometry (IMS) was described [Wu *et al.*, 2002]**.** The first study to identify possible interfering air contaminants common in airport settings by IMS was published in 2001 [Matz *et al.*].

IMS has been used in more complex analytical procedures. Garofolo *et al.* analyzed HPLC fractions by IMS after removal of solvent. The sensitivity (200 pg to 1 ng) and reliability were increased compared with results obtained using HPLC-UV alone [Garofolo *et al.*, 1994]. Solid phase micro-extraction (SPME) methods were combined with ion mobility spectroscopy to provide rapid and

sensitive qualitative and quantitative detection of trinitrotoluene (TNT) and dinitrotoluene (DNT) in water and soil. These sampling systems, when combined with field-portable IMS, were developed as a means of classifying buried or submerged objects as explosive ordnance [Chambers *et al.*, 1999]. The integration of a solid phase micro extraction inlet system into an ion mobility spectrometer resulted in a portable, sensitive explosives detection system. A standard operating procedure for the analysis of more than 10 explosives in less than 60 seconds at the low ppb level of detection was developed, avoiding the need for long sample preparation procedures [Wu *et al.*, 2000]. Another application of IMS was in the development of a land mine detector prototype based on ion mobility spectrometry [Desilets *et al.*, 1998]. In many areas of the world, the marine environment is being contaminated by organic explosives. The sources of this contamination, which is primarily TNT and RDX, are abandoned munitions and polluted aquifers that discharge into the ocean. IMS can help to search for the sources of pollution [Rodacy *et al.*, 1999]. A manually operated system that can detect 2,4,6-trinitrotoluene (TNT) in seawater at a concentration of 0.010 parts-per-trillion in less than five minutes was developed [Rodacy *et al.*, 2002]. Recently IMS was used to analyze triacetone triperoxide, ammonium nitrate, black powder, and smokeless powder and optimized analytical conditions were found [Colon *et al.*, 2002; McGann *et al.*, 2002].

The application of ion mobility spectrometry is not limited to the use of IMS instrument as standalone spectrometer. Coupling IMS with different sampling or analytical techniques (as a detector following chromatographic analysis or as a separator before a mass spectrometer) allows the solution of more complex analytical problems. Gas chromatography (GC) was studied to complete with its own chemical selection capability the advantages of IMS. GC-IMS was proposed as a hyphenated technique sufficiently flexible with respect to a broad range of chemical detection capabilities. The application of this dual technology can provide unique solutions in many operational environments. GC-IMS methodology sharply reduces competitive ionization and facilitates identification of mixture components, thereby enabling quantitation of volatile and semivolatile compounds over a broad range of concentrations in air. This detector configuration can

be customized to detect and identify explosives, International Civil Aviation Organization (ICAO) markers, and narcotics [DeBono *et al.*, 2002; Su and Babcock, 2002b; Buryakov *et al.*, 2001; Miller *et al.*, 2001; Haley and Romeskie, 1998; Dworzanski et al., 1994]. Recently ion mobility spectrometry has acquired prominent advances in sample introduction. The combination of IMS and electrospray ionization has expanded the application of IMS in detection of non-volatile and high molecular mass compounds. The analysis of explosives with IMS directly from aqueous solutions was shown using an electrospray ionization technique. The detection limits in the reference were in the range of 15-190 mg/l. A mixture of TNT, RDX and HMX was used to demonstrate the high separation potential of the IMS system. Analysis time for baseline separation of the three compounds was within 6.4 seconds [Asbury *et al.*, 2000].

6.2 Experimental

6.2.1 Chemicals and reagents

Nitroglycerine (NG) in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). Methanol RS for HPLC was Carlo Erba (Milano, Italy). The working solutions were prepared by diluting the standard solution with methanol at the following concentrations: 200 mg/l, 20 mg/l, 2 mg/l, 200 μg/l, 20 μg/l and 2 μg/l. The working and standard solutions were stored at –20°C.

6.2.2 Equipment

The IMS system used was an Ionscan Model 400 Barringer (Mississauga, Ontario, Canada). The instrument conditions were as follows: mode, explosives; desorber temperature, 223°C; inlet temperature, 238°C; drift tube temperature, 105°C; drift flow, 330 cm^3/min; sample flow, 355 cm^3/min; carrier gas, purified air; drift gas, dried air; reactant gas, hexachloroethane; internal

calibrant, 4-nitrobenzonitrile. The filter cartridges with Teflon membranes stock No. 11510 were from Barringer (Mississauga, Ontario, Canada).

6.2.3 IMS analysis

A filter cartridge with a Teflon membrane filter (**1**, for this number and the following see Fig. 7) was positioned in the holder of the calibrated IMS system [Barringer Instruments, 2000]. The cartridge was inserted by sliding the holder to the right and the analysis was automatically started. The sample was vaporized due to the high temperature of the oven (**2**) and the vapours were pushed into the inlet by the sample flow (**3**). The ^{63}Ni emitter ionized the analytes in the ionization chamber (**4**) and the ions travelled into the drift tube (**5**) in the presence of the counter current of the drift gas (**6** and **7**), before arriving to the electrode for detection. After sliding the cartridge holder back to the left, when the analysis was finished, the plasmagram of the blank analysis was examined to check the absence of peaks related to the presence of explosive compounds. The blank analysis of the membrane was repeated three times. 1 μl of methanol was then deposited on the same Teflon membrane using a syringe. After waiting 60 seconds for evaporation the cartridge was analyzed. Three blank analyses with methanol were performed.

An aliquot of 1 μl of methanolic solution of NG was then deposited on the Teflon membrane using a syringe. After waiting 60 seconds for solvent evaporation the cartridge was analyzed and the plasmagram was recorded. The blank analysis of the membrane was repeated three times after adding 1 μl of methanol. If the blank analyses did not produce any signal it was possible to make another analysis on the same cartridge, otherways the analyses of methanol were repeated to obtain three subsequent blank results. All the analyses of standard solutions were repeated three times. Samples from shooting tests, obtained as described in the previous chapter, were analyzed as well. For every sample from shooting tests a new cartridge and a new Teflon membrane filter were used after a blank analysis. All samples from shooting tests were analyzed twice.

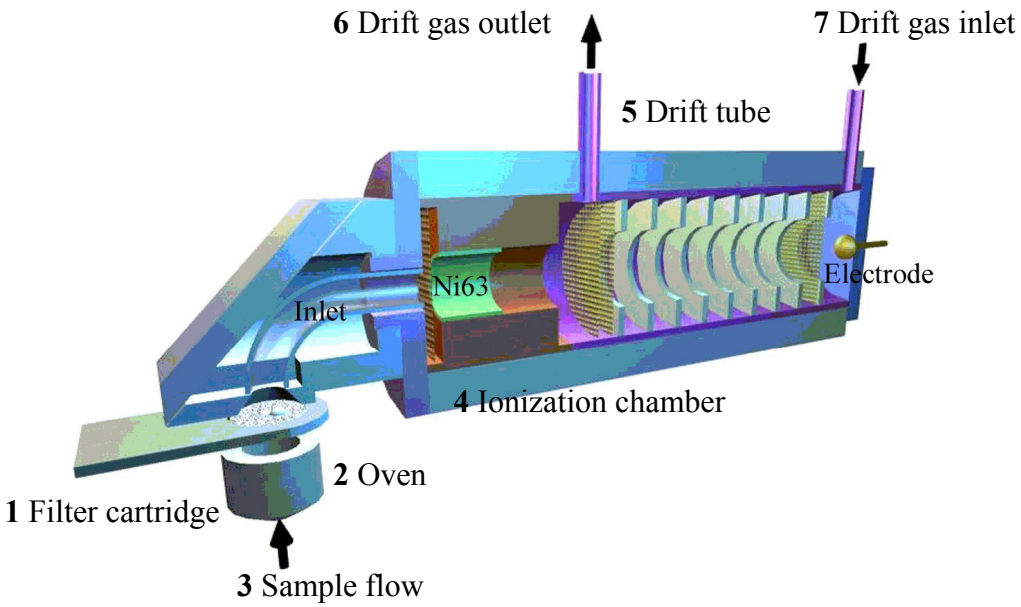


Fig. 7. Diagram of the Ion Mobility Spectrometry system used [Schelling, 2000].

6.3 Results and discussion

The Ionscan system adds together data from several scans, before further processing, to improve the signal-to-noise ratio. Then the detector carries out the data processing to identify target ions in the IMS spectrum. At the end of the analysis the detector recorded the peaks of the current of the electrode, the peak amplitude and the difference between the peak position found and the predicted position (called Delta) and carries out a series of calculations, called the "alarm algorithm", to determine if it should produce an alarm. Some compounds produce a variety of different ions which appear as different peaks in the spectrum. It is expected that the detector unit should display only one compound name to the user when it alarms. In order for the substance to be declared as detected the detector compare the signals found with the substance definition. Up to 10 channels may be used in a substance definition. Several segments are considered in every channel. A detection criterion (P=present, A=absent, O=optional) is assigned to each channel [Barringer Instruments, 2000]. In the present study the position of the three peaks of the nitroglycerine was always carefully examined, regardless of the instrumental alarm. Nitroglycerine produces three peaks, called NG-C, NG-N and NG/TNT (see Fig. 8). The highest peak from the analysis of a standard NG solution was NG-C, which is the peak of the adduct of NG with Cl^-, coming from the reactant gas. The NG-N peak is the peak of the adduct of NG with NO_3^-. The NG/TNT peak is the lowest from the analysis of a standard NG solution. Results of the analyses of NG standard solutions are in Table 12, results of the analysis of samples from shooting tests are in Table 13.

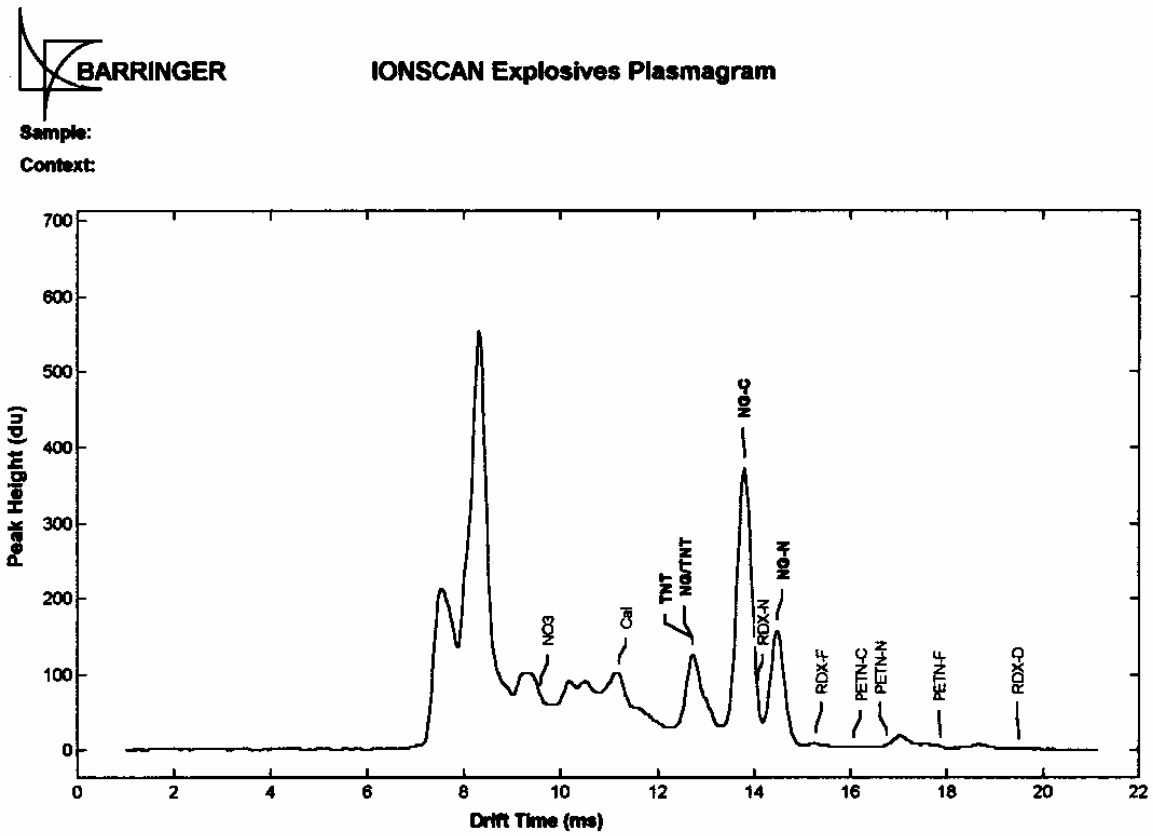
The analysis of the NG standard solution at the lowest concentrations (2 μg/l and 20 μg/l) in triplicate never activated any channel. The first activation of a channel related to NG was with the 200 μg/l standard solution. The NG-C channel was activated twice, at the first and at the second analysis, while the following third analysis of the same standard solution resulted in a negative result. The presence of a small peak close to the NG-N peak position did not activate the channel. The presence of peaks not able to activate any channel was noted in the previous analysis of blanks

and of more diluted standard NG solutions. The analysis of the NG standard solution at 2 mg/l in triplicate always activated both the NG-C and the NG-N channel and once the NG/TNT channel. The analysis of the NG standard solution at 20 mg/l and at 200 mg/l in triplicate always activated all the three NG channels. TNT and NO_3 channels were activated as well. In Table 12 are the results of the analyses of NG standard solutions with all the data related to the NG-C, NG-N and NG/TNT channels. The "Max Amplitude" is the highest amplitude reached in any segment of the channel detected. The "Cumulative Amplitude" is the sum of the amplitude of the peak for each segment the channel was detected in. The "Hits" indicate the number of segments in which the peak was detected in the channel. The arithmetic mean, the sample standard deviation (s) and the coefficient of variation (CV) [Skoog *et al.*, 1996][1] of "Max Amplitude" and "Cumulative Amplitude" were calculated. In Fig. 9 and 10 are shown the "Max Amplitude" and in Fig. 11 and 12 are shown the "Cumulative Amplitude" results of the analyses of NG standard solutions in graphic format.

In Table 13 are the results of some samples from shooting tests, obtained as described in the previous chapter[2], with the data related to the NG-C and NG-N channels. NG/TNT data are not reported in the table because only one sample gave a positive signal on this channel (V44-A: Max Amplitude = 87, Cumulative Amplitude= 314, Delta = -4, Hits = 4; V44-B: Max Amplitude = 124, Cumulative Amplitude= 523, Delta = 5, Hits = 5). **The Ionscan Model 400 showed its capability to detect NG traces in samples from clothes after shooting tests if the NG-C signal is taken into account.** The limit of detection (LOD) of the method, i.e. the minimum amount giving a positive result, increases if we need a signal both on the NG-C and on the NG-N channel for a positive result. Attention must be paid in the alarm of the instrument. The "alarm algorithm" was developed for airport security and not for organic GSR detection. The Ionscan Model 400 never gave an NG alarm on samples from shooting tests. Three samples gave an RDX alarm, five samples activated at least one RDX channel and one sample activated the NO_3 channel. **Eight samples from**

[1] For the 0,20 mg/l solution the distance from the arithmetic mean and the relative distance % from the mean are listed.
[2] Without using the internal standard.

clothes after shooting tests out of ten gave a positive result of the NG-C signal.



Name	DTime	Ko	CF	Max Amp	Cum Amp	Delta	Hits
Cal	11.191	1.6523	564				
NG-C	13.803	1.3394	1.00019	770	4826	0	11
NG-N	14.493	1.2766	1.00023	477	1989	-4	8
NG/TNT	12.720	1.4535	1.00013	292	1242	13	8
TNT	12.742	1.4510	1.00014	292	1242	-8	8
NO3	9.570	1.9325	0.99968	292	774	-12	3
PETN-C	16.067	1.1505	1.00029				
PETN-F	17.887	1.0334	1.00036				
PETN-N	16.769	1.1023	1.00032				
RDX-C	13.305	1.3895	1.00017				
RDX-D	19.485	0.9486	1.00040				
RDX-F	15.309	1.2075	1.00026				
RDX-N	14.069	1.3140	1.00021				

Fig. 8. IMS analysis of 1 μl of a solution of nitroglycerine 200 mg/l.

mg/l	NG-C				NG-N				NG/TNT			
	Max Amp	Cum Amp	Delta	Hits	Max Amp	Cum Amp	Delta	Hits	Max Amp	Cum Amp	Delta	Hits
0,20												
0,20	108	212	6	2								
0,20	381	825	4	3								
mean=m	**245**	**519**										
x-mean =d	137	307										
d*100/m	56	59										
2	487	1106	-3	3	242	490	-15	3	79	146	31	2
2	486	1485	4	4	235	602	-6	3				
2	508	1512	-6	4	245	590	-16	3				
mean	**494**	**1368**			**241**	**561**						
s	12	227			5	61						
CV	3	17			2	11						
20	681	2370	-2	6	429	1538	-4	5	286	843	12	5
20	639	2083	-9	5	544	1626	-9	5	331	871	6	4
20	673	2150	0	5	469	1503	-2	5	311	773	13	4
mean	**664**	**2201**			**481**	**1556**			**309**	**829**		
s	22	150			58	63			23	50		
CV	3	7			12	4			7	6		
200	774	5258	6	13	275	1564	0	9	262	1150	21	10
200	821	9189	1	14	425	4751	0	15	366	3853	9	14
200	770	4626	0	11	477	1989	-4	8	292	1242	-8	8
mean	**788**	**6358**			**392**	**2768**			**307**	**2082**		
s	28	2472			105	1730			54	1535		
CV	4	39			27	63			17	74		

Table 12. Results, arithmetic mean (m), sample standard deviation (s) and coefficient of variation (CV) of the analyses of NG standard solutions (in triplicate).

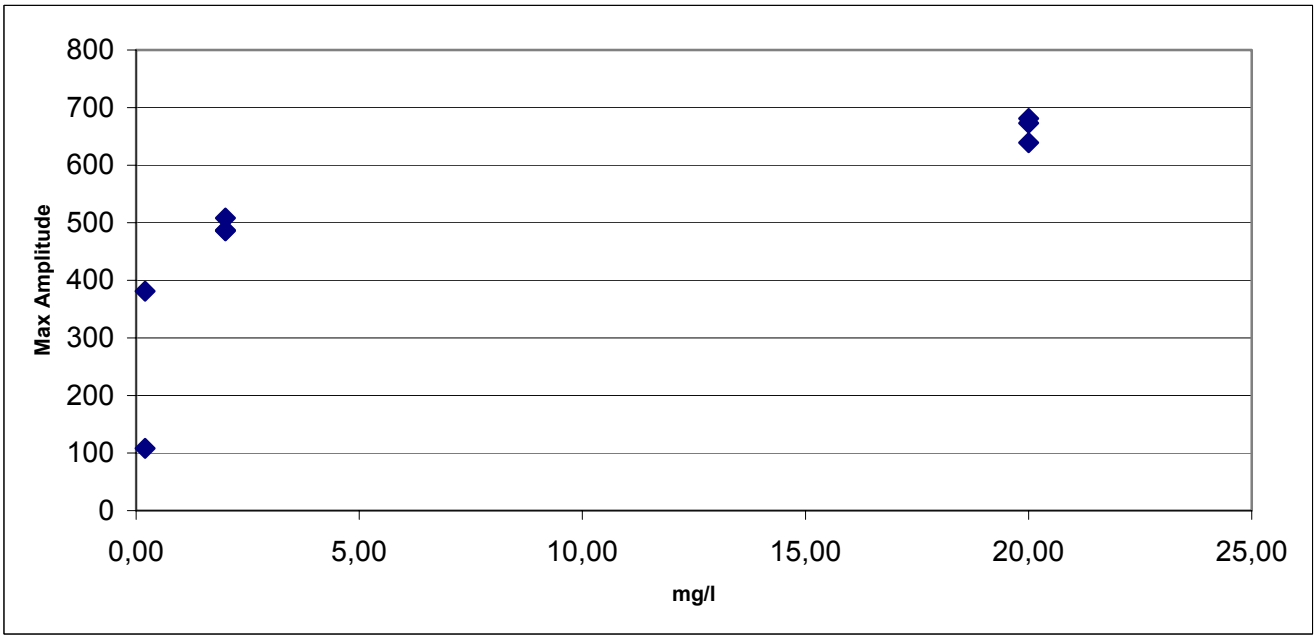


Fig. 9. ”Max Amplitude” results of the analyses of nitroglycerine standard solutions.

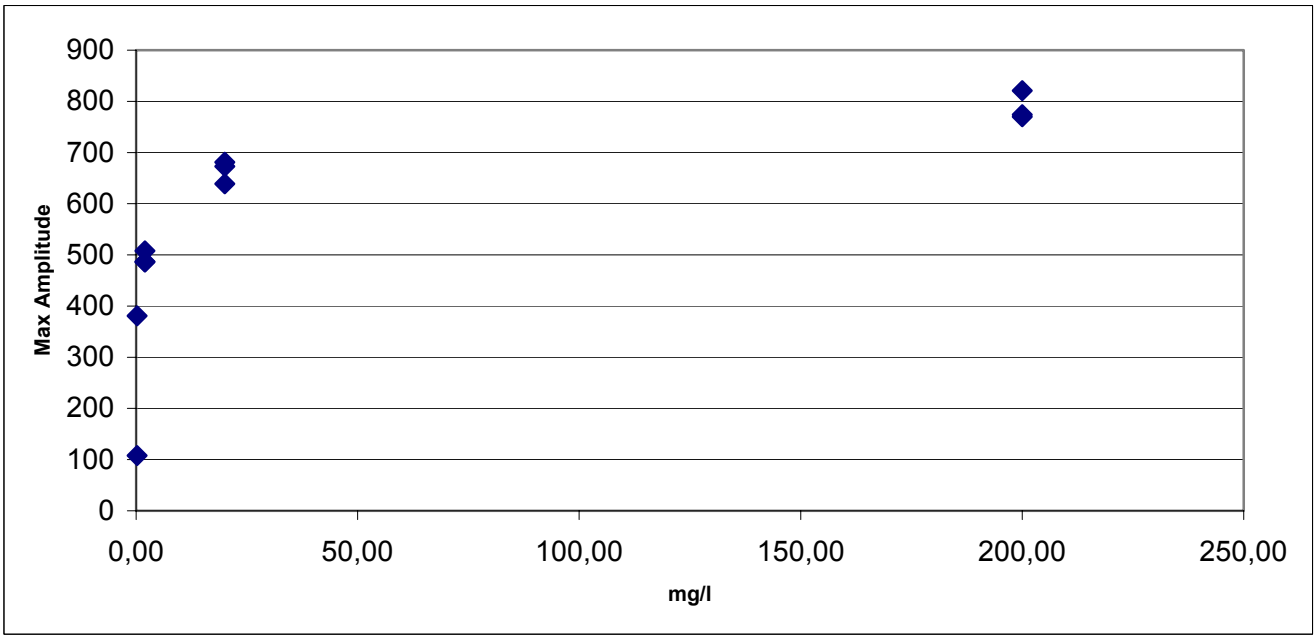


Fig. 10. ”Max Amplitude” results of the analyses of nitroglycerine standard solutions.

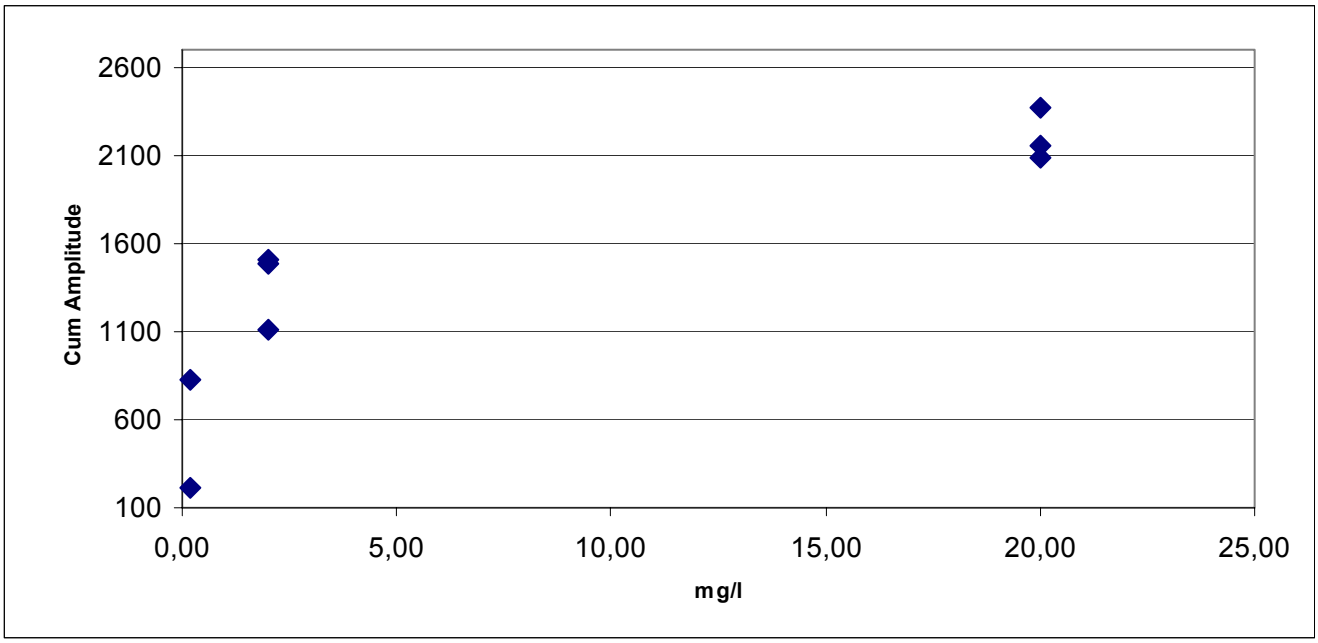


Fig. 11. ”Cum Amplitude” results of the analyses of nitroglycerine standard solutions.

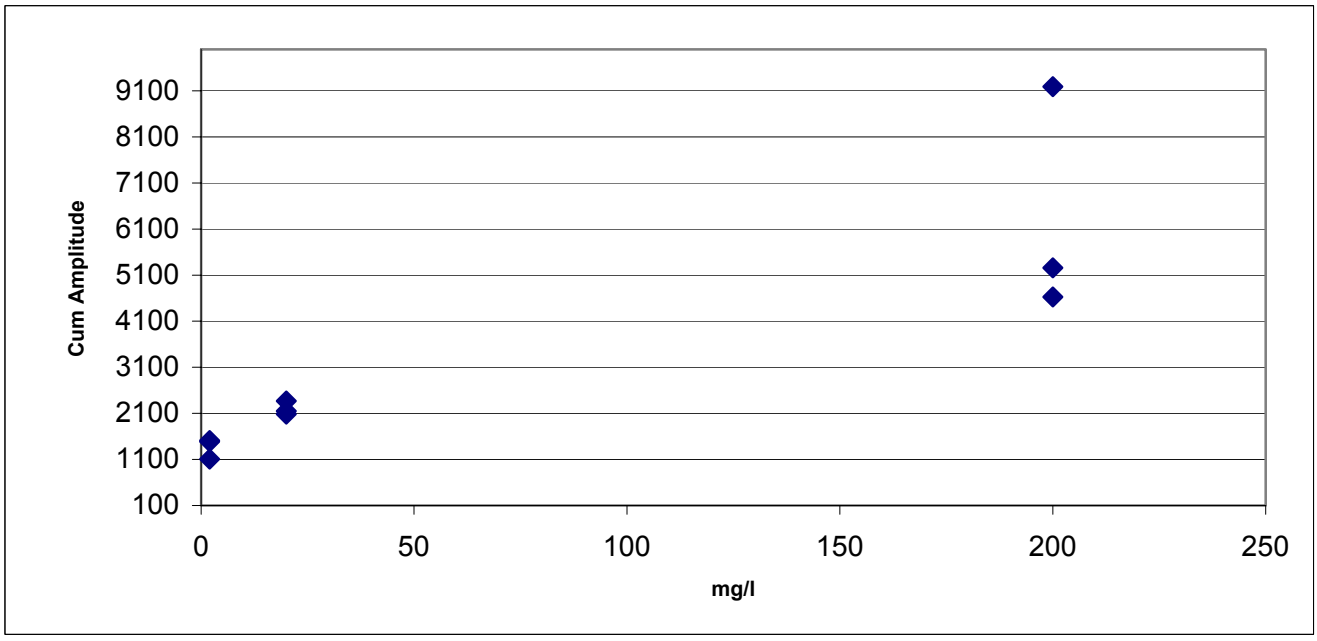


Fig. 12. ”Cum Amplitude” results of the analyses of nitroglycerine standard solutions.

sample	analysis	NG-C Max Amp	Cum Amp	Delta	Hits	NG-N Max Amp	Cum Amp	Delta	Hits
V41	A	194	1161	3	7				
	B	257	1075	-2	4				
V42	A	257	631	4	4				
	B	162	809	7	6				
V43	A								
	B								
V44	A	320	711	6	3	206	483	-5	3
	B	267	597	8	3	175	1575	-8	11
V45	A	214	1400	2	9	130	602	-23	5
	B	362	659	6	3				
V46	A	105	438	-23	5	309	632	-20	3
	B	143	1038	-22	8	159	736	-5	8
V47	A	120	510	-11	6				
	B	78	237	7	4				
V48	A	351	714	5	3				
	B	159	274	4	2				
V49	A								
	B								
V50	A	122	210	-6	2	143	365	-2	3
	B	71	170	-18	3	101	390	-2	5

Table 13. Results of the analyses of tests (A and B correspond to results of two analyses of the same solution).

VII.

Analysis with EGIS

7.1. Introduction

An EGIS detector is an analytical system for detection of explosives developed mainly for airport security activity. The EGIS was developed by Thermedics Detection, Inc. and it is based on high-speed gas chromatography combined with a chemiluminescence detector (TEA). It is a fast, sensitive and selective instrument used in both laboratory and field situations [Byall, 2001; Elias *et al.,* 1998; Bromberg *et al.,* 1998, Ornath *et al.,* 1998]. It is comparable to a conventional laboratory GC-TEA system (see annexes 5). The Thermedics EGIS is capable of analyzing samples in 18 seconds. It can be used in a layered security system for screening carry-on baggage, checked baggage, vehicles and for others applications [Fine and Wendel, 1994]. In a comparison of four explosives detection devices of use in airport security for checking of hand-held luggage the EGIS "was found superior" and was recommended by the German Bundeskriminalamt. The devices were compared based on operation, sampling method, detection sensitivity, susceptibility to false alarms (e.g., from common consumer products), and detection time [Hnatnicky, 1994]. Thermedics developed a walk-through portal sampling module based on a high speed GC-chemiluminescence explosives detection system as well. The exterior clothing of subjects is actively brushed and traces are entrained in an air stream and transported to the analytical system. The module provides automatic screening of passengers at rates of 10 per min. [Wendel *et al.*, 1997]. The ion mobility spectrometers and chemiluminescence detectors are the most common systems for field screening of explosive traces [Fetterolf, 2002; Miller *et al.*, 1998]. The use of EGIS can be taken into account for use in forensic laboratories. Its speed, sensitivity and selectivity can help to analyze samples collected from scenes [Thompson et al., 1999].

7.2 Experimental

7.2.1 Chemicals and reagents

Nitroglycerine (NG) in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). Methanol RS for HPLC was Carlo Erba (Milano, Italy). The working solutions were prepared by diluting the original standard solution with methanol at the following concentrations: 10 mg/l, 5 mg/l, 1 mg/l, 600 μg/l, 200 μg/l, 20 μg/l and 2 μg/l. The working and standard solutions were stored at –20°C.

7.2.2 Equipment

The EGIS system used was a Thermedics Detection, Inc. (Chelmsford, Massachusetts, USA). The instrument is equipped with a sampler for direct air and vacuum sampling. Conditions were as default. The system considers six channels to produce an alarm: EGDN, NG, PETN, RDX, DNT and TNT. When a chromatographic peak is present in the time window associated to a channel, an alarm is produced.

7.2.3 EGIS analysis

The vacuum sampler was analyzed and the chromatogram of the blank analysis was examined to check the absence of peaks related to the presence of explosive compounds. The blank analysis of the vacuum sampler was repeated three times. 1 μl of methanol was then deposited on the sampling coil of the vacuum sampler using a syringe. After waiting 60 seconds for evaporation, the sampler was positioned and the analysis was started. Three blank analyses with methanol were performed. An 1 μl aliquot of methanolic solution of NG was then deposited on the sampling coil using a syringe. After waiting 60 seconds for solvent evaporation the sampler was positioned, the analysis was started and the chromatogram was recorded.

The blank analysis of the membrane was repeated three times after adding 1 μl of methanol. If the

blank analyses did not produce any signal it was possible to make another analysis of a sample or standard solution, otherwise the analyses of methanol were repeated to obtain three subsequent blank results. All the analyses of standard solutions were repeated three times. Samples from shooting tests, obtained as described in chapter V, were analyzed as well. All samples from shooting tests were analyzed twice.

7.3 Results and discussion

The EGIS system is equipped with an operator friendly display for alarms and permits to store, to print and to examine the chromatograms for an in depth view of the analytical results. The print-out of the result includes the chromatogram and a list of the data related to the peaks detected. In the present study the position of the peak of the analysis of nitroglycerine was always carefully examined, regardless of the instrumental alarm. Nitroglycerine produces a peak which is considered for an alarm on channel 2 of the system (see Fig. 13). Results of the analyses of NG standard solutions are in Table 14, results of the analysis of samples from shooting tests are in Table 15.

The analysis of the NG standard solution at the lowest concentration (2 μg/l and 20 μg/l) in triplicate never gave any peak. The first peak related to NG was with the 200 μg/l standard solution. The EGIS gave a peak presumably due to NG every time the 200 μg/l standard solution was analyzed, giving a positive result (three analysis). In Table 14 are the results of the analyses of NG standard solutions with the arithmetic mean, the sample standard deviation (s) and the coefficient of variation (CV) [Skoog *et al.*, 1996] of the signal related to the NG peak.

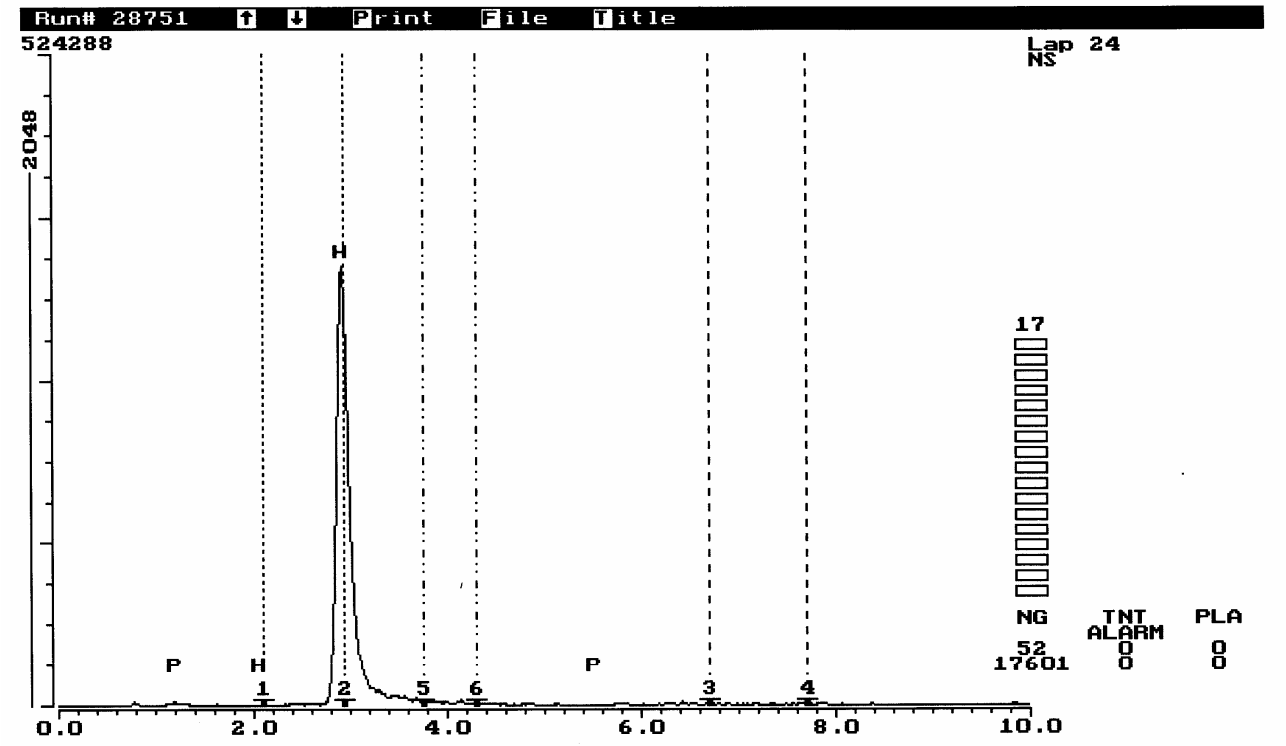


Fig. 13. EGIS analysis of 1 µl of a solution of nitroglycerine 10 mg/l.

	mg/l	**NG**	**mg/l**	**NG**	**mg/l**	**NG**	**mg/l**	**NG**	**mg/l**	**NG**
	0,20	91	0,60	1324	1,00	1950	5,00	7195	10,00	15510
	0,20	242	0,60	1430	1,00	2837	5,00	9675	10,00	17601
	0,20	127	0,60	1775	1,00	4428	5,00	11534	10,00	16853
mean		**153**		**1510**		**3072**		**9468**		**16655**
s		79		236		1256		2177		1060
CV		51		16		41		23		6

Table 14. Results, arithmetic mean (m), sample standard deviation (s) and coefficient of variation (CV) of the analyses of NG standard solutions (in triplicate).

In Fig. 14 and 15 are shown the results of the analyses of NG standard solutions in graphic format.

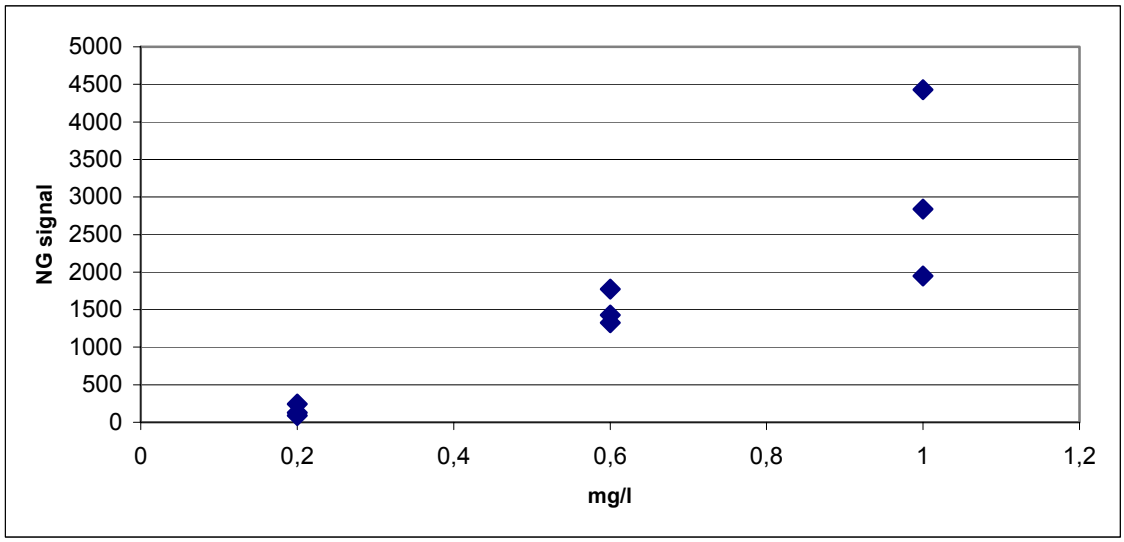


Fig. 14. EGIS results of the analyses of nitroglycerine standard solutions.

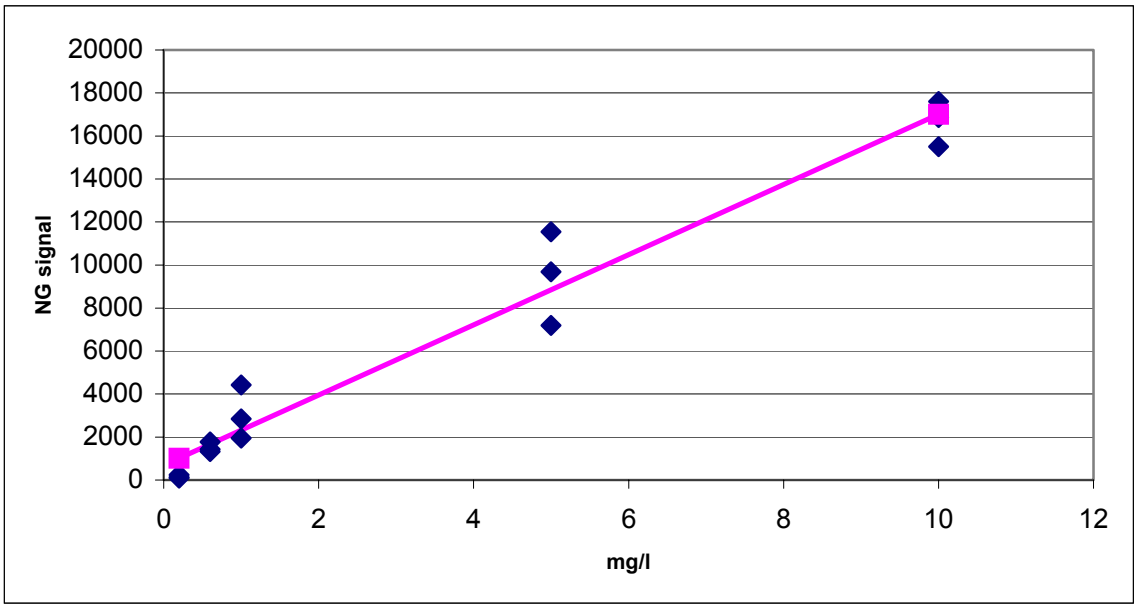


Fig. 15. EGIS results of the analyses of nitroglycerine standard solutions.

Analytical results were used to calculate the regression line shown in Fig. 15 by the method of least squares (y=1633x + 685, r=0.9822). In Table 15 are the results of some samples from shooting tests, obtained as described in chapter V[3], with the data related to the NG peak.

The EGIS showed its capability to detect NG traces in samples from clothes after shooting tests if the NG peak is taken into account. Nine samples out of ten gave a positive result of the NG signal.

[3] Without using the internal standard.

sample	NG signal
V41	5531
V42	19513
V43	2770
V44	17794
V45	5727
V46	1530
V47	0
V48	1018
V49	708
V50	555

Table 15. EGIS results of the analyses of the solutions from shooting tests.

VIII.

Analysis with HPLC-MS-MS

8.1. Introduction

HPLC is the method of choice for thermally labile and high-boiling-point compounds. In 1983 Lloyd introduced the pendant mercury drop electrode (PMDE) for HPLC explosive trace analysis [Lloyd, 1983]. More recently liquid chromatography mass spectrometry became the method of choice for the analysis of explosive traces [Yinon, 2001]. Casetta and Garofolo [1994] and Garofolo *et al.* [1996a] showed the capability of LC-MS-MS in the analysis of explosives. Preliminary results of the research presented in this chapter were showed at the 15th meeting of the International Association of Forensic Science, in Los Angeles [Romolo and Margot, 1999], where the applicability of HPLC-MS-MS to analyse organic gunshot residues from lead-free ammunition was demonstrated for the first time in samples from shooting tests.

8.2. Experimental

8.2.1. Chemicals and reagents

Nitroglycerine (NG) in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). Diphenylamine (DPA) 99% and ethylcentralite (EC) 99% were purchased from Sigma Aldrich S.r.l. (Milano, Italy). Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in acetonitrile solution (1 mg/ml) was purchased from Radian International (Austin, TX, USA). Isosorbide dinitrate (ISDN) containing 25% of lactose was from Menarini (Firenze, Italia) and kindly provided by Istituto Luso Farmaco S.p.A. (Milano, Italy). Ammonia, formic acid, acetic acid and ammonium acetate were Carlo Erba (Milano, Italy). Acetonitrile and methanol RS-Plus for HPLC were Carlo

Erba (Milano, Italy). Ultra pure water was prepared using the Milli-Q-plus water purification system (Millipore, Bedford, MA, USA) and used throughout the study. The working solutions were prepared by diluting standard solutions at higher concentration with acetonitrile. All the standard and working solutions were stored at –20°C. Diatomaceous earth was Hydromatrix from Dionex GmbH (Idstein, Germany). High-purity nitrogen was used as curtain and collision gases, high-purity air was used as nebulizer gas during mass spectrometric analyses.

8.2.2. Equipment

Between 1997 and 2001 HPLC analyses were carried out on a HPLC system PE LC pump 250 (Perkin Elmer, Norwalk, CT, USA), equipped with a Rheodyne 8125 (Cotati, CA, USA) injection valve (loop volume 5 µl). The LC pump was coupled with a tandem MS spectrometer PE-SCIEX API 300 (Toronto, Canada) with an atmospheric pressure ionization interface (API). Since 2002 were used a Series 200 LC Micro Pump equipped with a Series 200 Autosampler and a Series 200 Vacuum Degasser (Perkin Elmer, Norwalk, CT, USA). The LC column effluent was diverted to the TurboIonSpray source of a PE-SCIEX API 3000 tandem triple-quadrupole mass spectrometer (Toronto, Canada). High-purity nitrogen gas was used as curtain and collision gases; high-purity air was used as nebulizer and heater gases. A PE Diode Array Detector 235L (Perkin Elmer, Norwalk, CT, USA) was used to optimise the chromatographic conditions. The injection volume was 5 µl. The columns used were a SGE 250GL2-W5C18-R, length cm 25 x 2 mm i.d., packing Wakosil II 5C18 RS, 5 µm, from SGE Italia S.r.l. (Roma, Italy) and an X Terra 150 x 2.1 mm i.d. packed with C_{18} reversed phase 3.5 µm particles from Waters (Milford, MA, USA). An infusion system Pump 11 from Harvard Apparatus (Holliston, MA, USA) was used to optimize MS conditions.

8.3. Results

8.3.1. HPLC analysis

The HPLC analysis conditions were optimized with the PE LC pump 250, the SGE column and the PE Diode Array Detector 235L. The injection volume was 5 µl. The detector operated at fixed wavelength λ=210 nm. The analyses were made in isocratic mode and, after several tests with different solvent systems, the composition of the mobile phase chosen was acetonitrile/water (80:20 v/v). The flow rate of the mobile phase was 0.2 ml/min. The chromatographic conditions permitted a good separation of RDX (t_r=3.4[4]), NG (t_r=3.9[4]), EC (t_r=5.9[4]) and DPA (t_r=6.6[4]).

When RDX was substituted with ISDN the analysis was made with the X Terra column in isocratic mode and the composition of the mobile phase was acetonitrile/water (65:35 v/v). The chromatographic conditions permitted a good separation of ISDN (t_r=3.1[4]), NG (t_r=3.4[4]), DPA (t_r=5.2[4]) and EC (t_r=5.5[4]). **The aim of a short analysis time was obtained, resulting in the possibility to analyze a large number of sample and to fulfil the need of laboratories with a heavy workload.**

[4] Retention times in minutes.

8.3.2. MS analysis

Mass axis calibration of each mass-resolving quadrupole (Q1 and Q3) was obtained by infusion of a polypropylene glycol solution (PE-SCIEX) at 10 µl/min. Unit mass resolution was established and maintained in each mass-resolving quadrupole by setting a full width at half maximum (FWHM) of approximately 0.7 Da. The mass spectrometry data handling system used with the API 300 was the Masschrom1.1 software from PE-SCIEX. The experimental strategy included at this stage the choice of the buffer composition (anion and cation) and concentration to obtain the desired ionization of analytes in the MS source. So called "volatile buffers" are recommended for this purpose for long lasting optimal performance of the HPLC-MS system. Ammonium formate and ammonium acetate were preliminarily tested. Ammonium formate was prepared with ammonia and formic acid. Solutions for infusion tests were initially prepared with NG and RDX 5 mg/l in acetonitrile. The infusion flow was 5 µl/min. The spectrometer was the API 300. The single quadrupole mass spectrum analysis of the NG and RDX solutions in positive ion mode gave no significant results. In negative ion mode both solutions gave a base peak of 62, probably due to the nitrate ions. NG gave a weak molecular ion (39000[5]) at 227 and a weaker quasi-molecular ion $[M-H]^-$ at 226 (24000[5]) while RDX gave a weak molecular ion (80000[5]) at 221 and a stronger quasi-molecular peak $[M+NO_3]^-$ at 284 (320000[5]). The same NG and RDX solutions in acetonitrile were analyzed by infusion after adding aliquots of a water solution containing ammonium acetate and/or acetic acid. The intensity of the mass peaks measured in counts per seconds (cps) is shown in the following Table 16 for NG and in Table 17 for RDX.

[5] Intensity of the mass peak, counts per seconds [cps].

59 $CH_3CO_2^-$	**62** NO_3^-	**226** $[M-1]^-$	**227** M^-	**286** $[M+59]^-$	**289** $[M+62]^-$	
3.2×10^7	9.0×10^7	4.5×10^6	1.1×10^6	4.0×10^4	2.1×10^5	ammonium acetate 6.5 mM[6]
7.0×10^7	1.2×10^7	3.4×10^6	2.1×10^6	9.6×10^4	1.0×10^5	ammonium acetate 71 mM[7]
4.0×10^6	3.2×10^7	7.3×10^5	3.4×10^5	4.0×10^4	1.7×10^5	acetic acid 8.0 mM[6]
1.4×10^7	2.6×10^7	1.6×10^6	8.0×10^5	2.4×10^4	n.d.[8]	ammonium acetate 6.5 mM + acetic acid 8.0 mM[6]
3.9×10^7	3.6×10^7	3.6×10^6	1.8×10^6	3.4×10^4	n.d.[8]	ammonium acetate 71 mM + acetic acid 88 mM[7]

Table 16. Abundance of ions after nitroglycerine infusion [cps].

[6] Solvent system acetonitrile/water (950:50 v/v).
[7] Solvent system acetonitrile/water (900:100 v/v).
[8] Not detected.

59 $CH_3CO_2^-$	**62** NO_3^-	**221** $[M-1]^-$	**281** $[M+59]^-$	**284** $[M+62]^-$	
3.9×10^7	3.6×10^7	2.8×10^5	1.7×10^6	7.5×10^5	ammonium acetate 6.5 mM[9]
7.0×10^7	1.4×10^7	3.4×10^5	3.2×10^6	1.8×10^6	ammonium acetate 71 mM[10]
6.0×10^6	3.4×10^7	1.0×10^5	5.5×10^5	7.0×10^5	acetic acid 8.0 mM[9]
8.7×10^6	3.6×10^6	2.1×10^5	1.8×10^6	n.d.[11]	ammonium acetate 6.5 mM + acetic acid 8.0 mM[9]
4.8×10^7	n.d.[3]	3.5×10^5	4.0×10^6	n.d.[11]	ammonium acetate 71 mM + acetic acid 88 mM[10]

Table 17. Abundance of ions after RDX infusion [cps].

[9] Solvent system acetonitrile/water (950:50 v/v).
[10] Solvent system acetonitrile/water (900:100 v/v).
[11] Not detected.

These results were only preliminary. It was necessary to study the behaviour of the compounds of interest in solution closer to chromatographic conditions. Solutions of the four analyte of interest (NG, RDX, DPA, EC) 5 mg/l in acetonitrile/water (80:20 v/v) with ammonium formate or ammonium acetate at 6.5 mM were then prepared. The single quadrupole mass spectrum of the solution of NG in negative ion mode showed a quasi-molecular base peak $[M+HCOO]^-$ at 272 with formate buffer and $[M+CH_3COO]^-$ at 286 with acetate buffer. It was interesting to find a peak at 346 with acetate buffer, which could result from $[M+CH_3COO+ CH_3COOH]^-$ (see Fig. 16). The RDX solution in negative ion mode gave a quasi-molecular base peak $[M+HCOO]^-$ at 267 with formate buffer and $[M+CH_3COO]^-$ at 281 with acetate buffer. The analysis of NG and RDX solutions in positive ion mode gave no significant results. The analysis of DPA in positive ion mode gave a quasi-molecular base peak $[M+H]^+$ at 170 both with formate and with acetate buffer (see Fig. 17). The EC solution in positive ion mode gave a quasi-molecular base peak $[M+H]^+$ at 269 both with formate and with acetate buffer (see Fig. 18). In the analysis of DPA and EC solutions in negative ion mode it was not possible to find significant signals related to the molecules analyzed. The DPA strongly adsorbed on the silica capillary used to introduce the solution into the API source. For this reason, in the following steps of the method development, solutions in acetonitrile/water (80:20 v/v) with buffer 6.5 mM and 5% acetic acid were used. Ammonium acetate was preferred to ammonium formate because the latter did not give better results than the former, which was easier to prepare and because the production of $[M+45]^-$ adduct ions, giving a 45 fragment (formate ion), is close to the 46 fragment $[NO_2]^-$ produced by nitrocompounds.

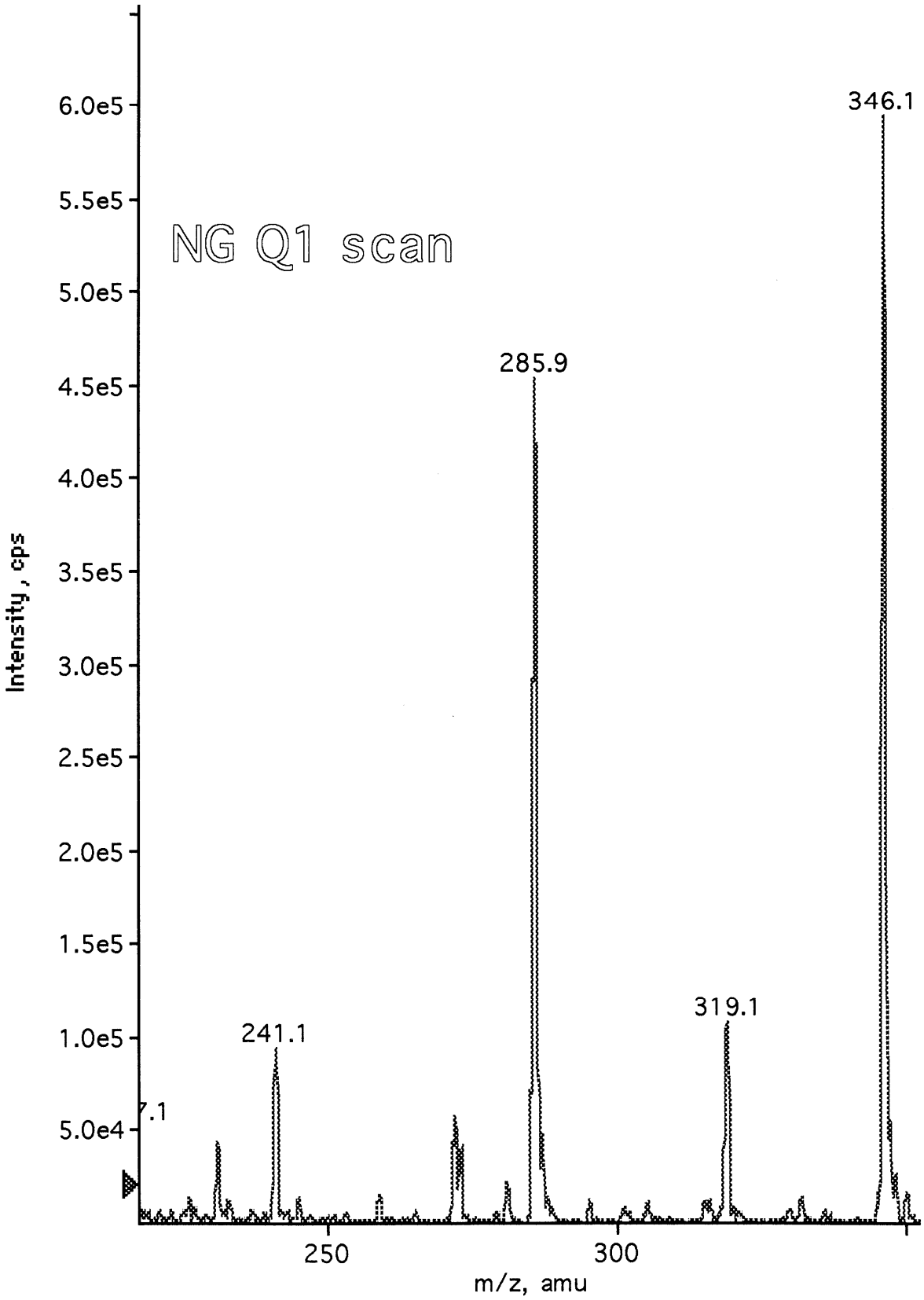


Fig. 16. MS analysis of a nitroglycerine solution with acetate buffer (negative ion mode).

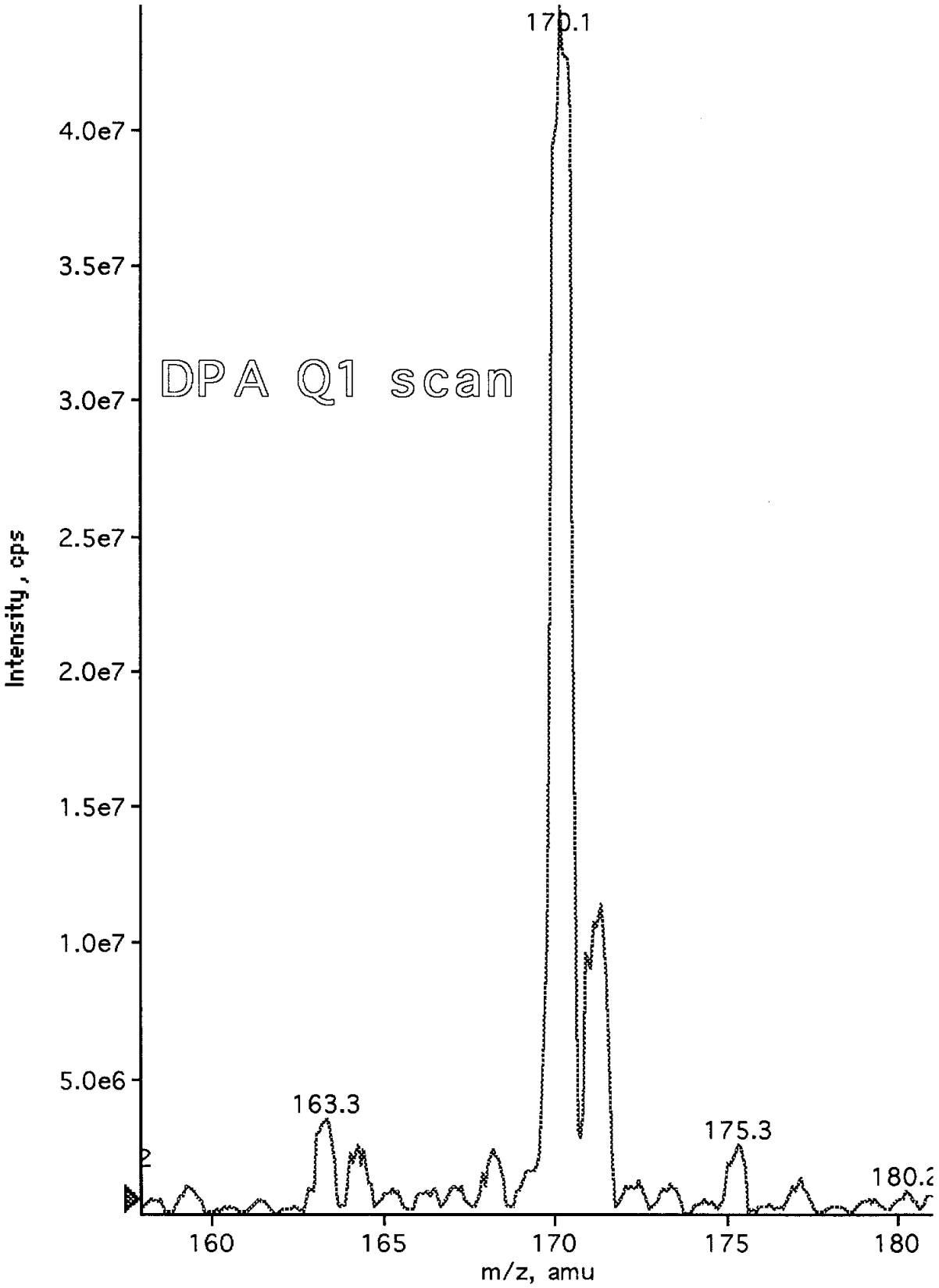


Fig. 17. MS analysis of a diphenylamine solution with acetate buffer (positive ion mode).

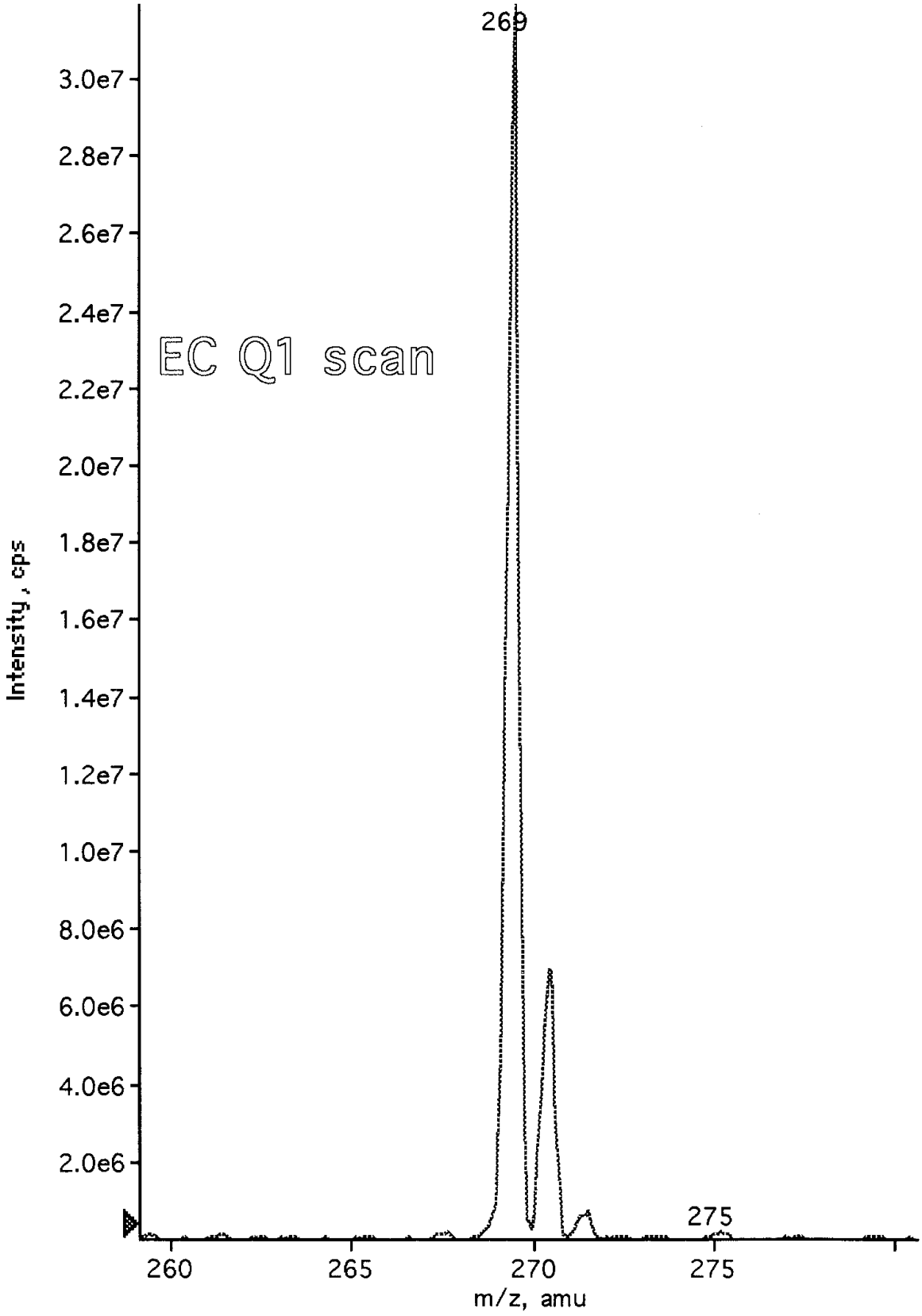


Fig. 18. MS analysis of an ethylcentralite solution with acetate buffer (positive ion mode).

The solutions of the four compounds of interest were then used to optimize the MS conditions of API 300 for analysis (state files). The parameters of the state files control the flow of the nebulizer and curtain gases in the source, the gas pressure in the collision cell and the potentials affecting the ion production and detection. The optimum value for each parameter was studied in order to obtain the highest signal from the quasi-molecular ion (286 for NG, 281 for RDX, 170 for DPA and 269 for EC) in the first quadrupole (Q1) and highest signal from the fragments produced in the collision cell and analyzed in the third quadrupole of the mass spectrometer.

The ion spray voltage was -4000 V (negative ion mode) for NG and RDX and + 4500 V (positive ion mode) for DPA and EC. The two fragmentation reactions producing the most intense signals were chosen for each quasi-molecular ion. For NG they were 286 $\rightarrow$ 62 and 286 $\rightarrow$ 46, for RDX 281 $\rightarrow$ 46 and 281 $\rightarrow$ 59, for DPA 170 $\rightarrow$ 93 and 170 $\rightarrow$ 65[12], for EC 269 $\rightarrow$ 120 and 269 $\rightarrow$ 148. The multi reaction monitoring (MRM) approach allowed the maximum sensitivity with acceptable selectivity (see chapter 9). The settings for the nebulizer and curtain gases were respectively 9 and 10, while the gas in the collision cell was set at 6. The effect of a critical parameter such as the collision energy is shown with EC. In Fig. 19 is the MS-MS spectrum of the ion 281 obtained with EC. In Fig. 20 is shown how the intensity of the signal from the EC fragments changes (y axis) with different collision energy values (on x axis) with the API 300 system.

[12] The transition 170 $\rightarrow$ 92 was ruled out because of excessive noise during the chromatographic analyses.

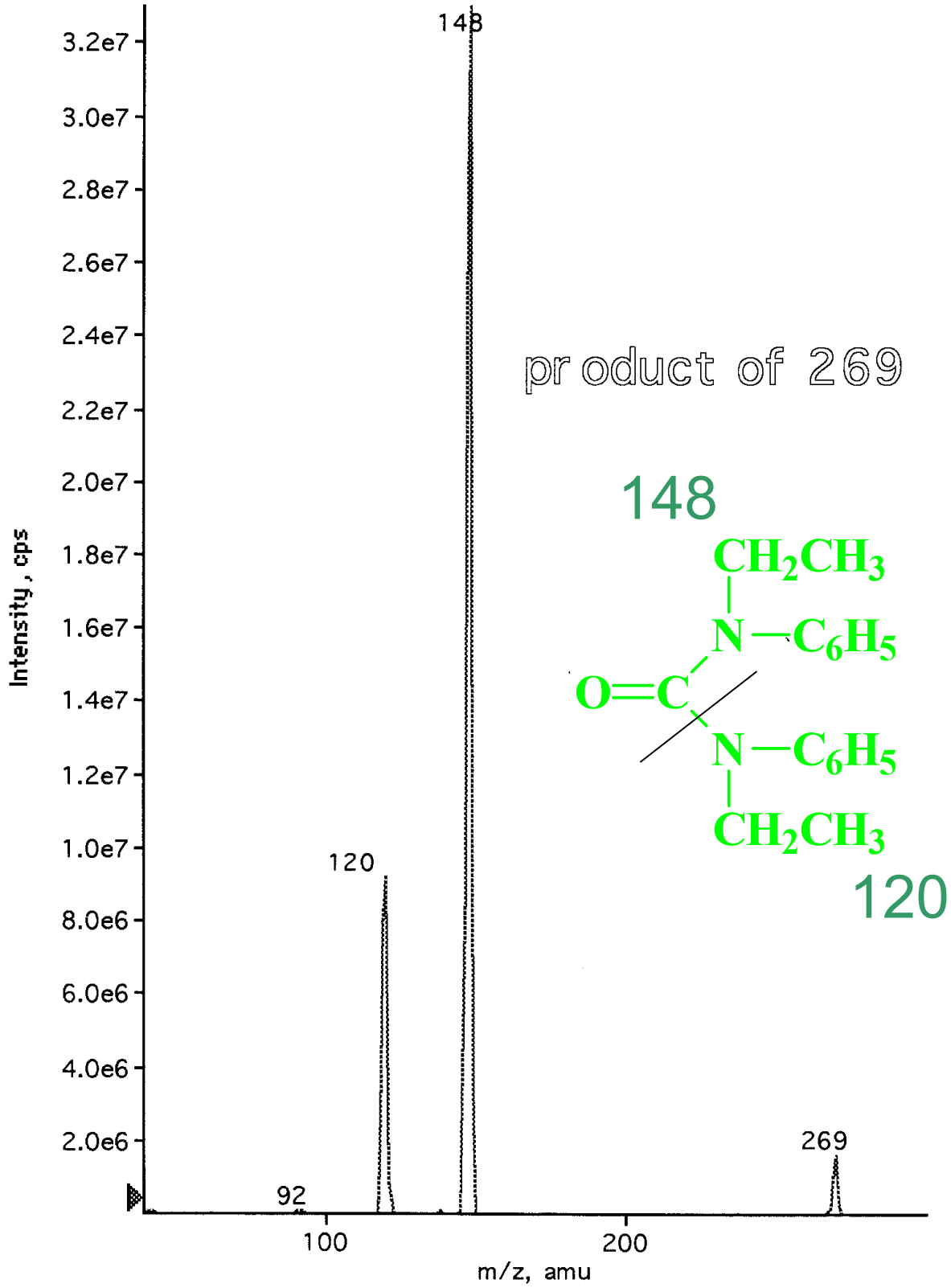


Fig. 19. MS-MS spectrum of the ion 281 of EC with acetate buffer (positive ion mode).

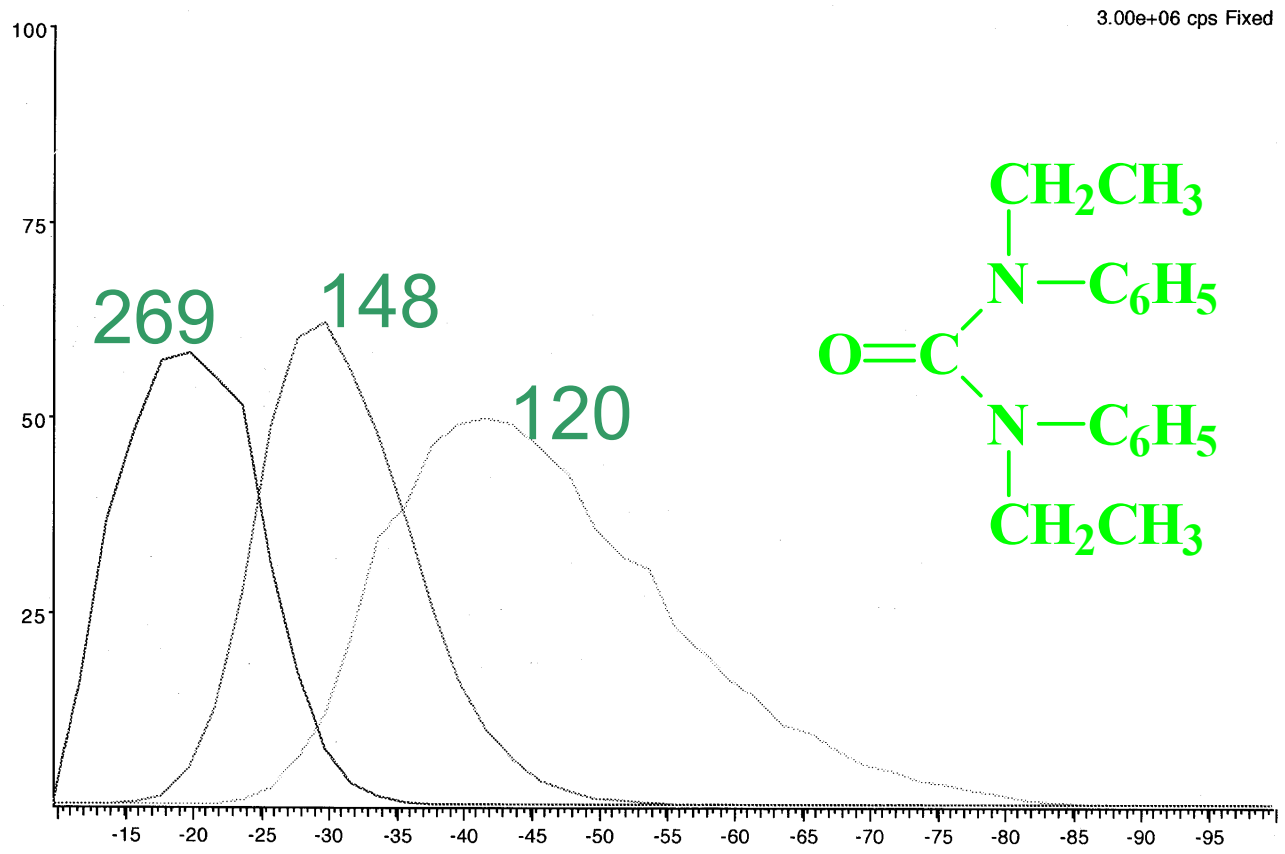


Fig. 20. Intensity [cps] of the signals from the EC fragments (y axis) vs. collision energy (x axis) [eV] with the API 300 system.

With the new API 3000 the optimization procedure was repeated. Mass axis calibration of each mass-resolving quadrupole Q1 and Q3 was obtained by infusion of a polypropylene glycol solution (PE-SCIEX) at 10 µl/min. Unit mass resolution was established and maintained in each mass-resolving quadrupole by setting a full width at half maximum (FWHM) of approximately 0.7 Da. The mass spectrometry data handling system used was the Analyst 1.2 software from PE-SCIEX.

The solutions of the four compounds of interest were then used to optimize the MS conditions of API 3000 for analysis (state files). In Table 18-19 are the state files with the optimized parameters for API 3000 and in Annexe 6 is the diagram of an API 3000. With the API 3000 the base peaks of NG, DPA and EC were confirmed at 286, 170 and 269 respectively (NG in negative ion mode, DPA and EC in positive ion mode). The experiment with API 3000 were conducted with solutions in acetonitrile/water (65:35 v/v) with buffer 5 mM due to a limited capability of the instrument to work with more concentrated buffers. The capillary used to introduce the solution into the source was made of silica in the API 300 and of polyetheretherketone (PEEK) in the API 3000. The problem of DPA adsorption on silica was avoided with PEEK. The internal standard used with API 3000 was ISDN. It showed a quasi-molecular peak at $[M+CH_3COO]^-$ at 295. The settings for the nebulizer and curtain gases were respectively 1.20 and 1.55 l/min, while the gas pressure in the collision cell was set at $3.8x10^{-3}$ Torr. The scan type was multi reaction monitoring (MRM). The Ion Spray was at –4500 V to detect ISDN and NG and +4500 V to detect DPA and EC. The transitions chosen for the analytes were 286 → 62 and 286 → 46 for NG, 295 → 59 and 295 → 46 for ISDN, 170 → 93 and 170 → 65[13] for DPA, 269 → 120 and 269 → 148 for EC.

[13] The transition 170 → 92 was ruled out because of excessive noise during the chromatographic analyses.

compound	**ISDN**	
MRM transitions	295 → 46	295 → 59
DP[a] [V]	-20	-20
FP[b] [V]	-115	-115
EP[c] [V]	-4	-4
CE[d] [eV]	-25	-15
CXP[e] [V]	-5	-10

compound	**NG**	
MRM transition	286 → 46	286 → 62
DP[a] [V]	-11	-11
FP[b] [V]	-95	-95
EP[c] [V]	-3	-3
CE[d] [eV]	-30	-25
CXP[e] [V]	-5	-10

Table 18. Experimental conditions[14] for the MRM determination of ISDN and NG with the API 3000 system (negative ion mode).

[14] [a]Declustering Potential; [b]Focusing Potential; [c]Entrance Potential;[d]Collision Energy; [e]Cell Exit Potential.

compound	**DPA**	
MRM transitions	170 → 93	170 → 65
DP[a] [V]	38	38
FP[b] [V]	180	180
EP[c] [V]	10	10
CE[d] [eV]	35	60
CXP[e] [V]	5	5

compound	**EC**	
MRM transition	269 → 148	269 → 120
DP[a] [V]	45	45
FP[b] [V]	250	250
EP[c] [V]	10	10
CE[d] [eV]	24	34
CXP[e] [V]	7	7

Table 19. Experimental conditions[15] for the MRM LC/MS determination of DPA and EC with the API 3000 system (positive ion mode).

[15] [a]Declustering Potential; [b]Focusing Potential; [c]Entrance Potential;[d]Collision Energy; [e]Cell Exit Potential.

8.3.3 Chromatography and mass spectrometry

The optimal buffer concentration was studied for NG and RDX in HPLC-MS-MS, monitoring the transition 286 → 62 for NG and 281 → 46 for RDX with the API 300 system. The flow was 200 µl/min, splitted ¼ before the API source. In Fig. 21 (pag. 87) are the chromatograms of the same solution of NG analyzed in the same MS conditions using acetonitrile/water (80:20 v/v) with different ammonium acetate concentration, from 1 mM a 10 mM. In Fig. 22 (pag. 88) are the chromatograms of the same solution of RDX analyzed in the same MS conditions using acetonitrile/water (80:20 v/v) with ammonium acetate concentration from 1 mM a 10 mM. In Fig. 23 (pag. 89) and 24 (pag. 90) are the chromatograms of the same solution of NG and RDX respectively, analyzed using ammonium acetate concentration from 20 mM a 200 mM. The buffer concentration of 10 mM was chosen because permitted to obtain the higher S/N value.

To determine the optimal buffer concentration with the API 3000, 5 µl of a solution containing NG 5 µg/ml, DPA 5 µg/ml and EC 0.2 µg/ml were injected in triplicate. The concentration of 1 mM was chosen after studying the S/N ratios measured with different buffer concentrations (see Table 20).

	NG	**DPA**	**EC**
0.5 mM	1	1	1
1 mM	2.8	0.9	1.1
2 mM	1.9	0.6	0.7
5 mM	2.1	0.4	0.6
10 mM	2.5	0.3	0.6

Table 20. Normalized S/N values calculated after analyses with different buffer concentration.

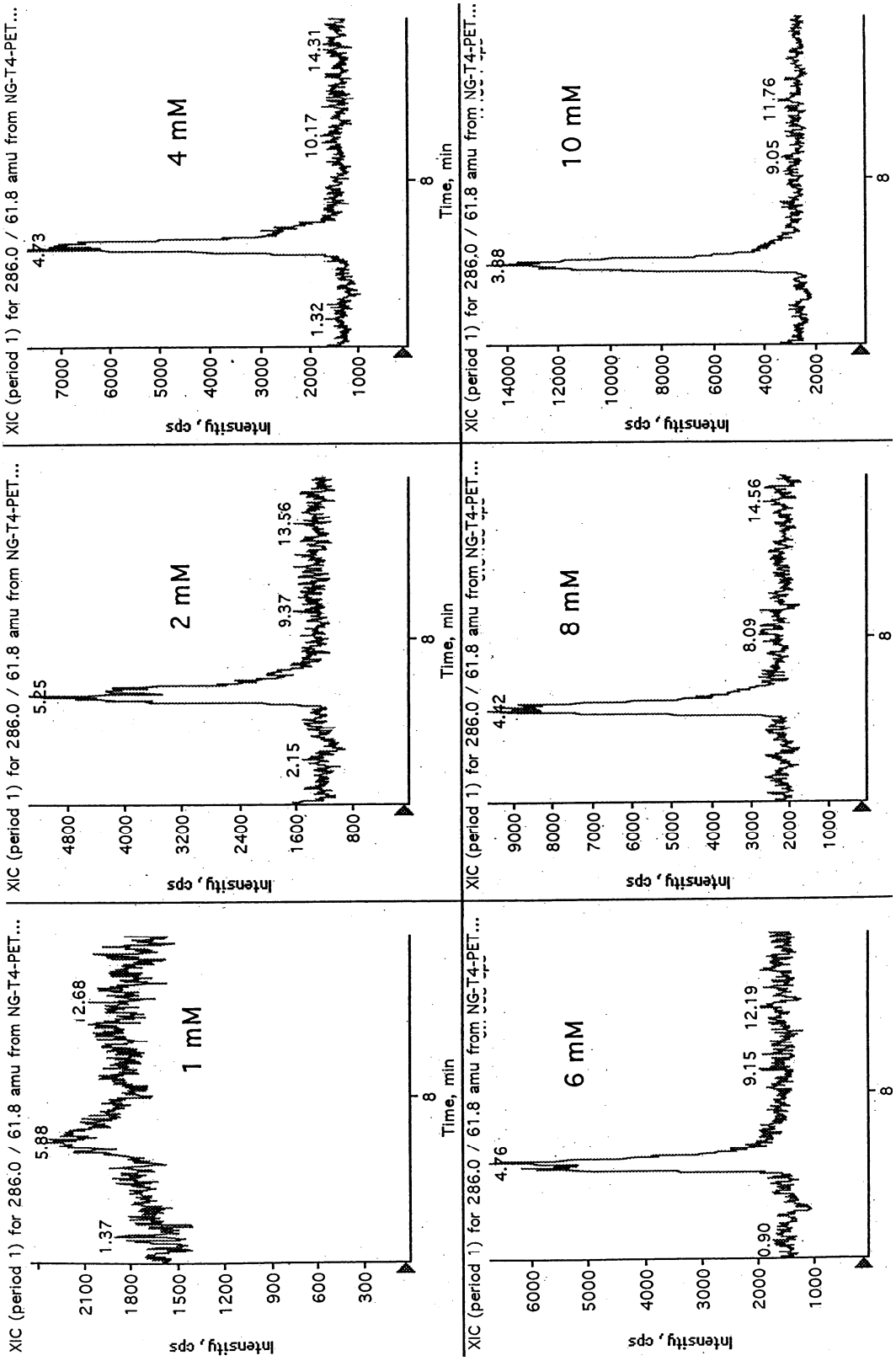


Fig. 21. Analysis of the same NG solution with different ammonium acetate concentration.

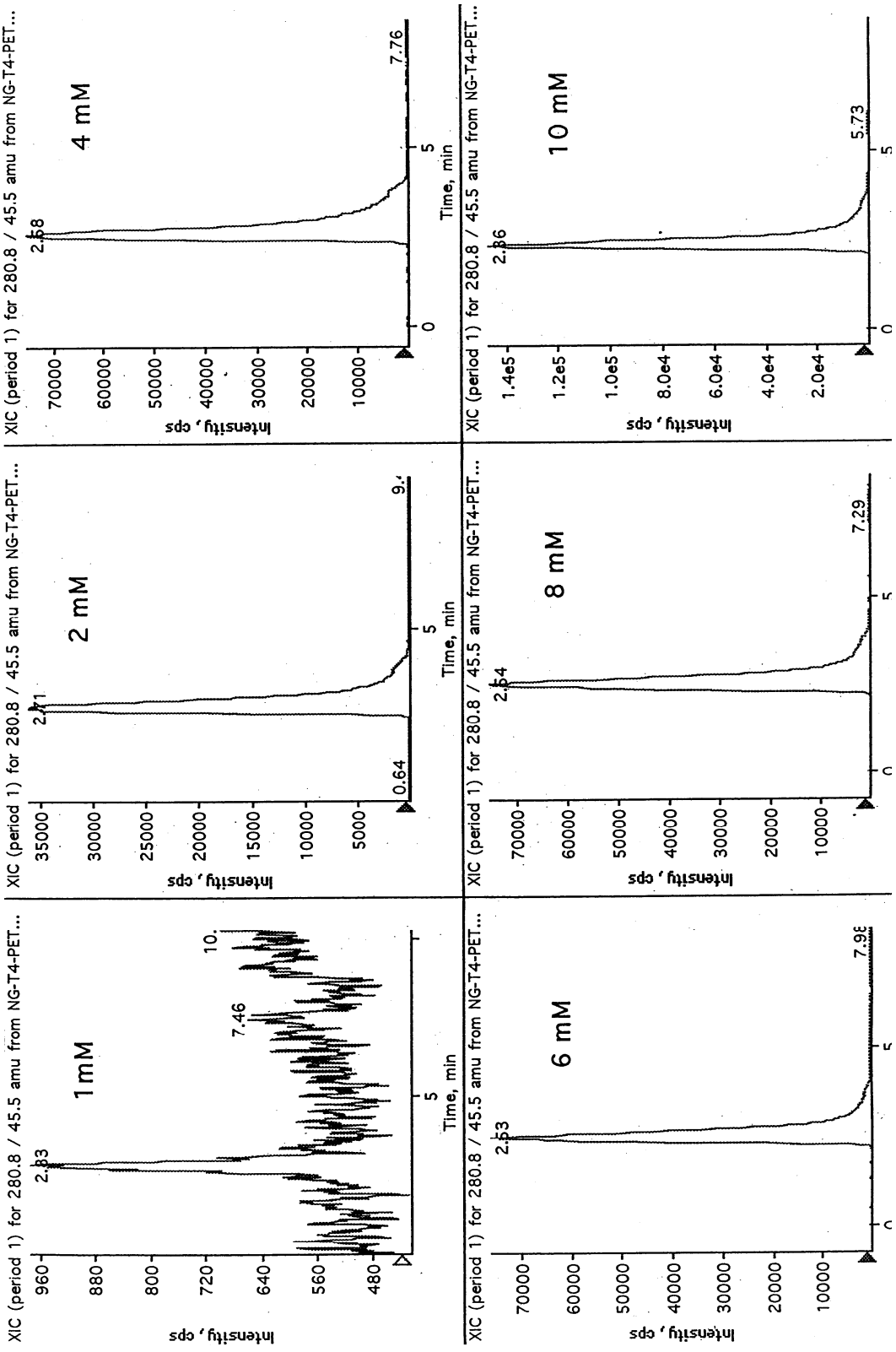


Fig. 22. Analysis of the same RDX solution with different ammonium acetate concentration.

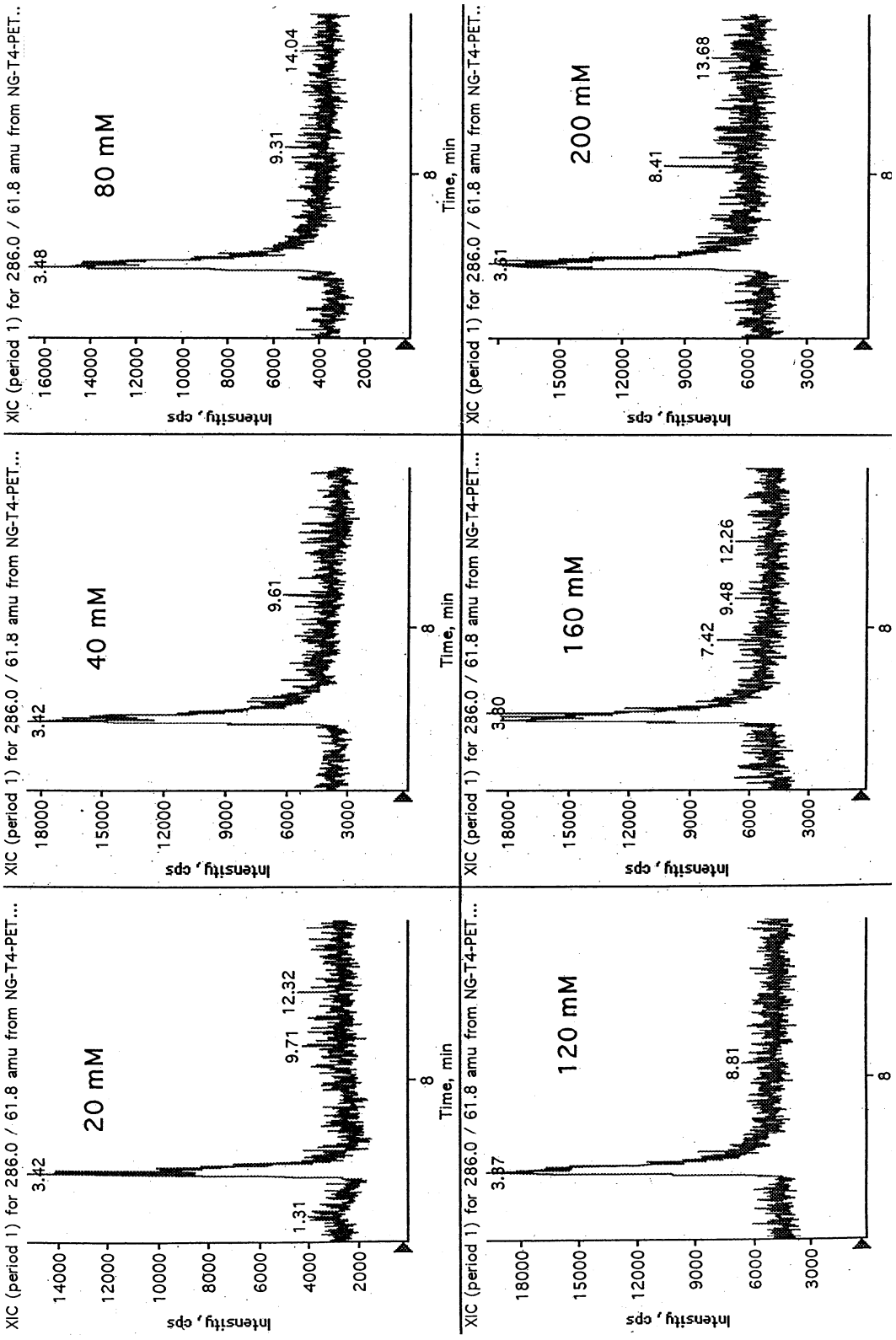


Fig. 23. Analysis of the same NG solution with different ammonium acetate concentration.

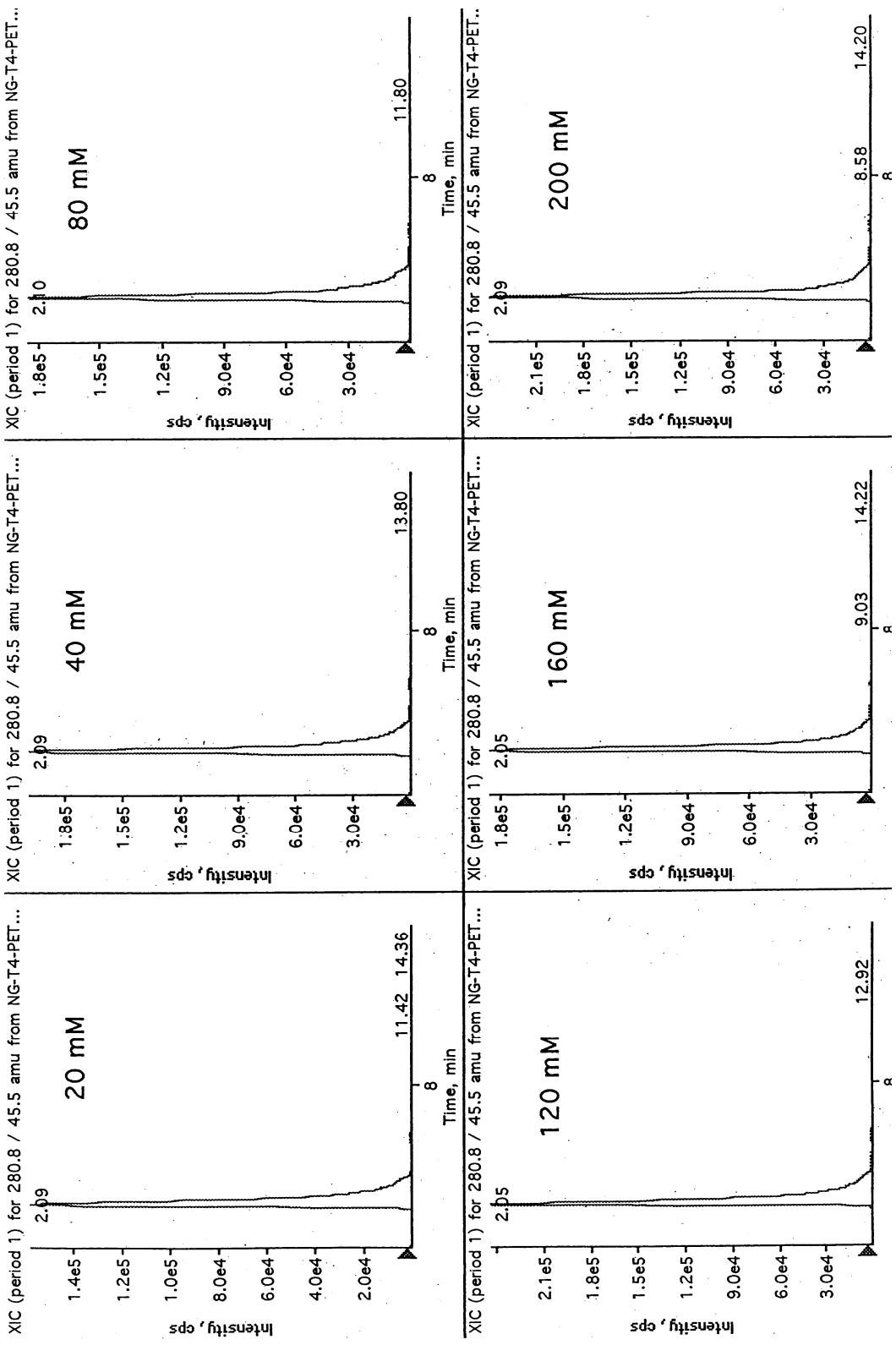


Fig. 24. Analysis of the same RDX solution with different ammonium acetate concentration.

8.3.4 Limits of detection

The HPLC–MS-MS analyses with the API 300 had to be operated in two different periods of the MS system during the chromatographic run. In the first half of the run (first period of five minutes) the MS was in negative ion mode, while it worked in positive ion mode in the second period (following five minutes). The limit of detection (LOD, calculated for a signal-to-noise ratio = 3:1) values were determined with the API 300 system analyzing a solution containing 200 µg/l of NG, 200 µg/l of RDX, 200 µg/l of EC and 10 mg/l DPA in triplicate. The LOD was 5 pg injected for RDX, 50 pg for NG, 5 pg for EC and 140 pg for DPA (injected volume = 5 µl), calculated on the less intense transition, assuming a linear extrapolation to the origin. The column was the SGE and the mobile phase was acetonitrile/water (80:20 v/v) with ammonium acetate 10 mM and 5% acetic acid. The flow was 200 µl/min, splitted ¼ before the API source.

With API 3000, the voltage of the Ion Spray could be alternated during the analysis, continuously cycling between –4500 V and +4500. For determination of the LOD, a solution containing 1 mg/l NG, 1 mg/l ISDN, 1 mg/l EC and 10 mg/l DPA was assayed in triplicate. The LOD with the API 3000 system was 200 pg injected for NG, 50 pg for ISDN, 60 pg for EC and 600 pg for DPA (injected volume = 5 µl), calculated on the less intense transition, assuming a linear extrapolation to the origin. The column was the X Terra and the mobile phase acetonitrile/water (65:35 v/v) with ammonium acetate 1.0 mM. The flow was 200 µl/min, splitted ¼ before the API source.

The TurboIonSpray was tested with API 3000, without splitting the HPLC column effluent, with the heater gas set at 8 l/min and the probe temperature maintained at 350 °C. The use of the heated source resulted in a better LOD only for DPA. In an experimental strategy when samples should be analyzed in both ways, it is necessary to take into account the minimum time of 30 min to be able to reach the temperature with the heated source. With the API 3000 it is possible to detect alternatively positive ions in one scan and negative ions in the subsequent scan or to detect negative ions in the

first half of the chromatographic run and positive ions in the final half. The latter approach gave worse LODs because of a higher noise.

The effect of the temperature on the analysis was studied with the API 3000 system, the X Terra column and the mobile phase acetonitrile/water (65:35 v/v) 1 mM ammonium acetate. Lowering the temperature to 10°C resulted in poor peak shape. The little difference between 20°C and 30°C suggested to use the room temperature T=23°C (see Table 21). Careful control of the temperature was critical for the reproducibility of the retention time of the analytes.

	S/N	
	T=20 °C	**T=30 °C**
NG	**19**	**22**
ISDN	**113**	**129**
CE	**46**	**52**
DPA	**73**	**73**

Table 21. S/N values calculated after analyses with different HPLC column temperature.

8.3.5. Linearity

Linearity was studied with both the API 300 and the API 3000 system. In the present work are reported only the results of the study with the latter system. Five calibration standard solutions were prepared with concentrations reported in Table 22 and analyzed with API 3000.

	A	**B**	**C**	**D**	**E**
NG	0.6	1.6	5	17	41
DPA	11	30	84	300	730
EC	0.033	0.90	2.6	9	22
ISDN	10	10	10	10	10

Table 22. Composition of calibration standards [mg/l].

On one hand the method showed a good linearity in the calibration range for NG both taking into account the ratio NG/ISDN (r=0.9995) and using the area of the NG peak alone (r=0.9979) without considering the internal standard. On the other hand the DPA and EC signals suffered an evident saturation effect. DPA/ISDN gave r=0.9639, DPA alone gave r=0.9566. EC/ISDN gave r=0.9829 and EC alone gave r=0.9798. Considering the three points at lower concentration DPA/ISDN gave r=0.9957, DPA alone gave r=0.9997, EC/ISDN gave r=0.9922 and EC alone gave r=0.9988. When the calibration standard solutions on NG were assayed twice during the same batch the method confirmed the good r values (r=0.9975 for NG/ISDN and r=0.9907 for NG alone). The area of the signal from the two transitions was used for calculations.

Other five calibration standard solutions were prepared with concentrations reported in Table 23 and analyzed.

	A	**B**	**C**	**D**	**E**
NG	0.5	1.5	2.5	5	10
DPA	3	5	7.5	15	30
EC	0.05	0.25	0.5	1	2
ISDN	10	10	10	10	10

Table 23. Composition of calibration standards [mg/l].

The method showed a good linearity in the new calibration range for the three compounds (NG, DPA and EC). The calibration standard solutions were assayed three times during the same batch. The area of the signal from the two transitions was used for calculations. In Table 24 are the data from the regression calculations based on analyte/ISDN ratio, whose graphic plots are in Fig. 25, 26 and 27. In Table 25 are the data from the regression calculations based only on the analyte results, whose graphic plots are in Fig. 28, 29 and 30.

	y=ax+b	r
NG	0.040359 x + 0.020305	0.9975
DPA	0.166697 x – 0.315829	0.9951
EC	18.74591 x – 0.561808	0.9947

Table 24. Regression parameters based on analyte/ISDN ratio.

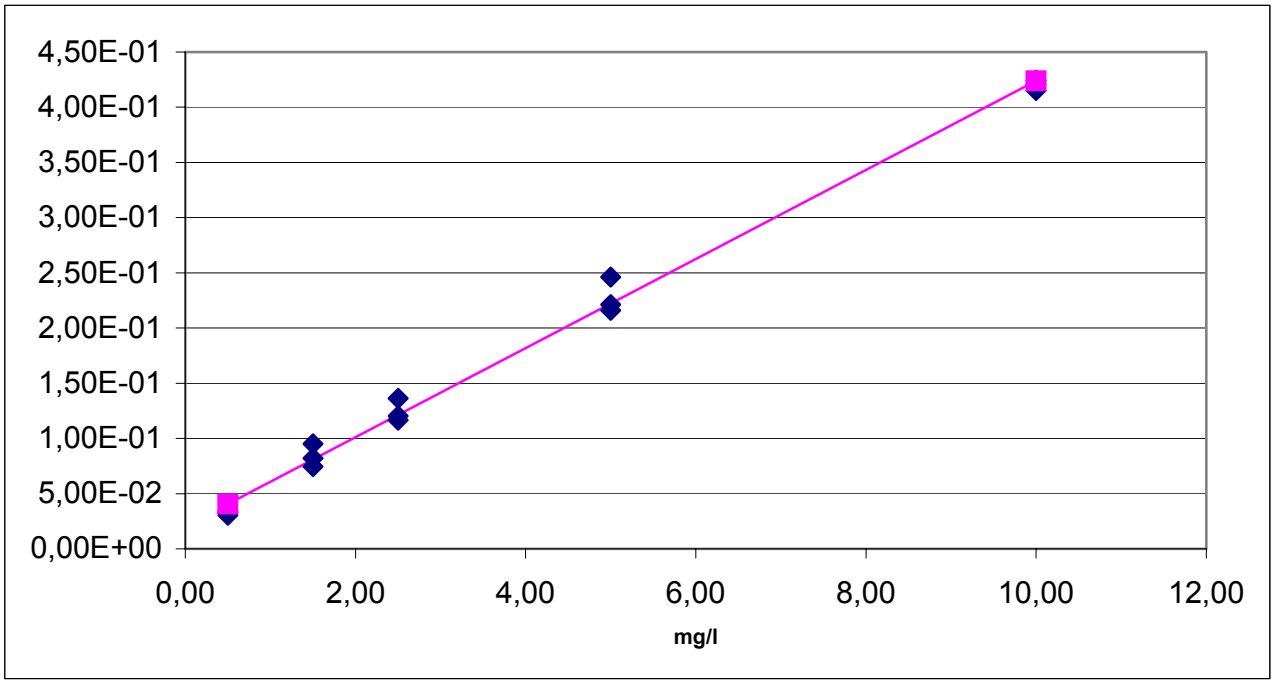


Fig. 25. Regression plot based on NG/ISDN ratio.

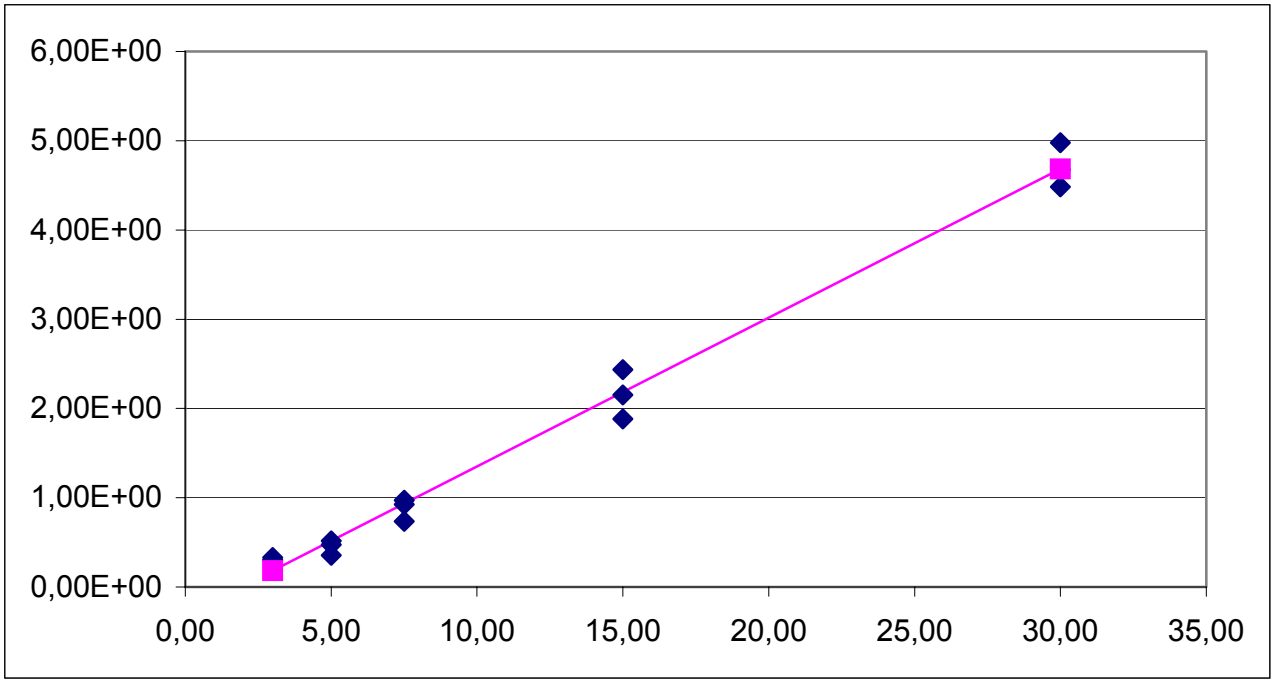


Fig. 26. Regression plot based on DPA/ISDN ratio.

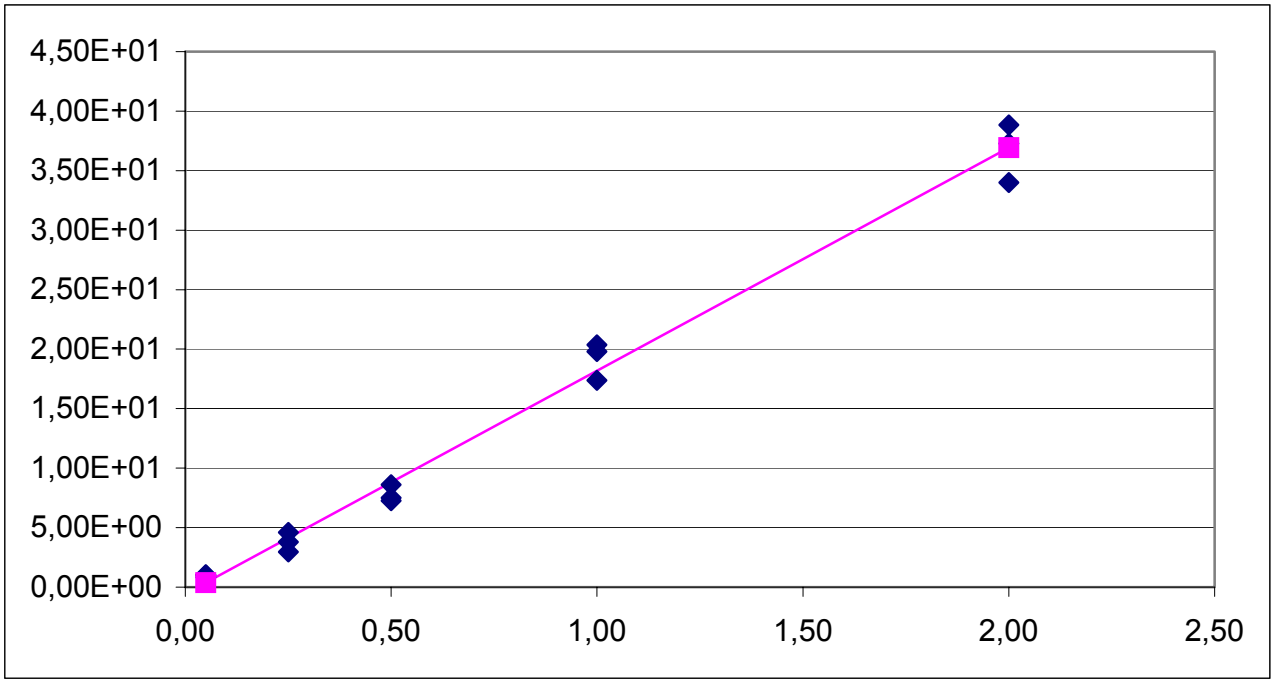


Fig. 27. Regression plot based on EC/ISDN ratio.

	y=ax+b	r
NG	10656 x + 11522	0.9851
DPA	41562 x – 30303	0.9958
EC	5945446 x - 111805	0.9974

Table 25. Regression parameters based on analyte peaks only.

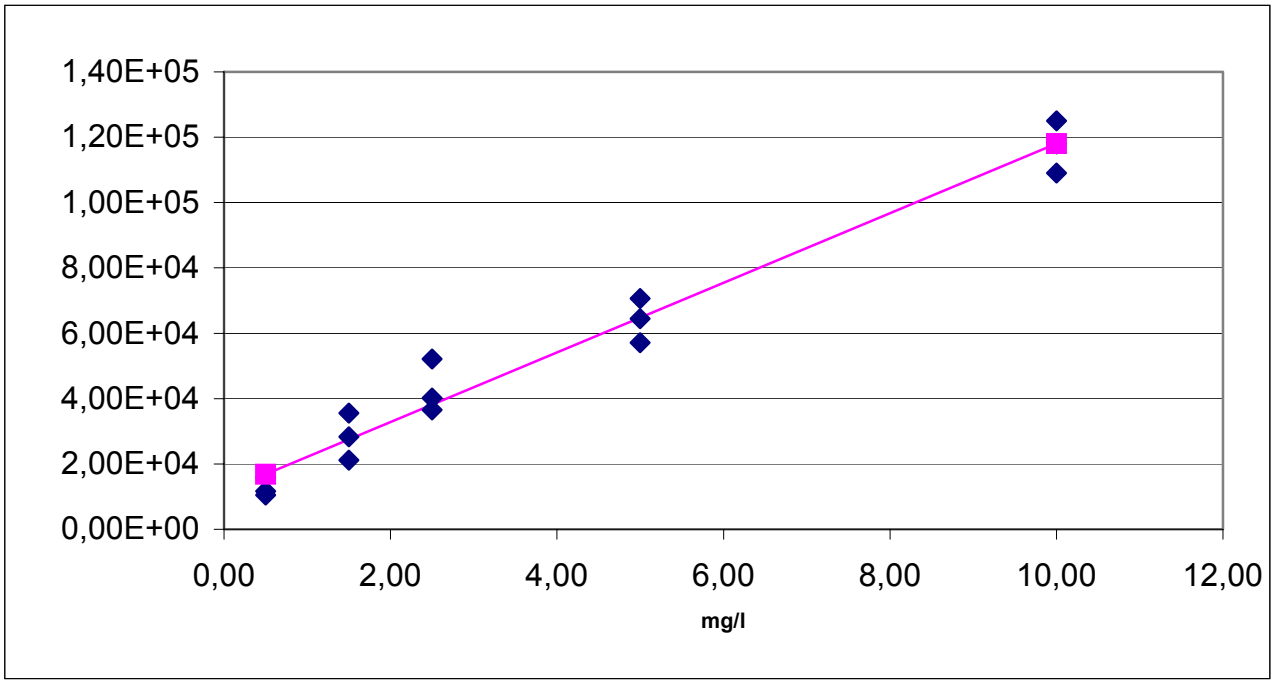


Fig. 28. Regression plot based on NG peak only.

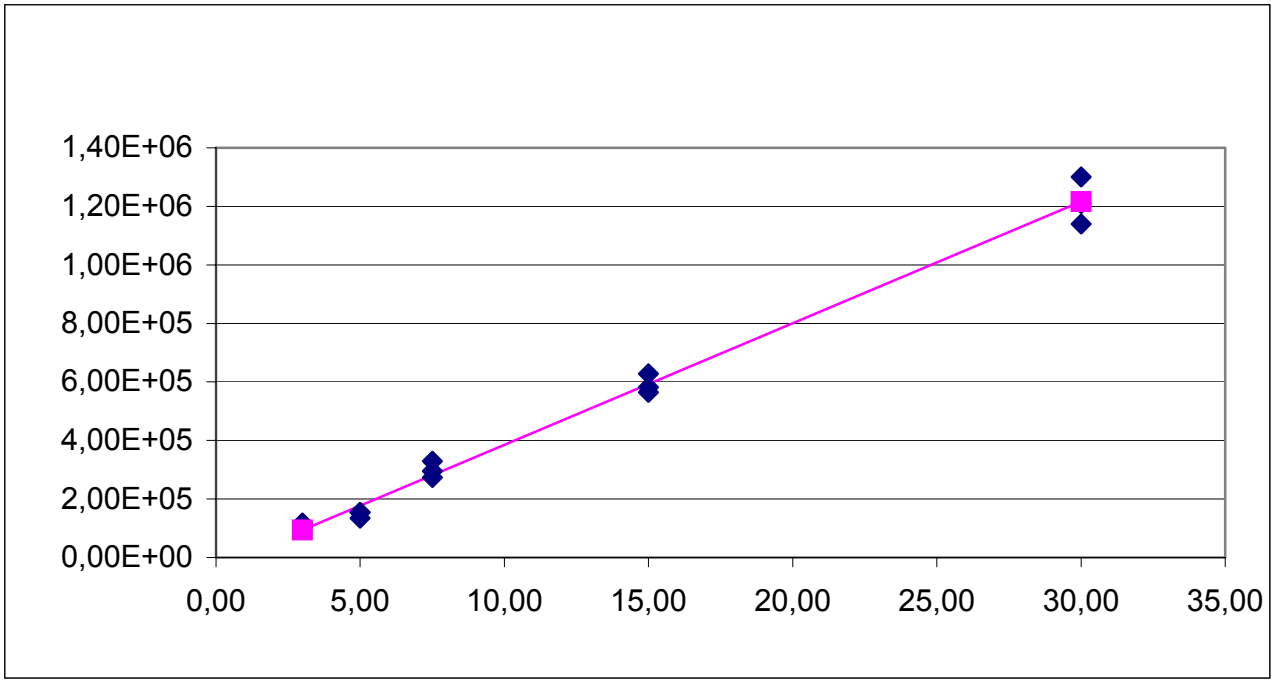


Fig. 29. Regression plot based on DPA peak only.

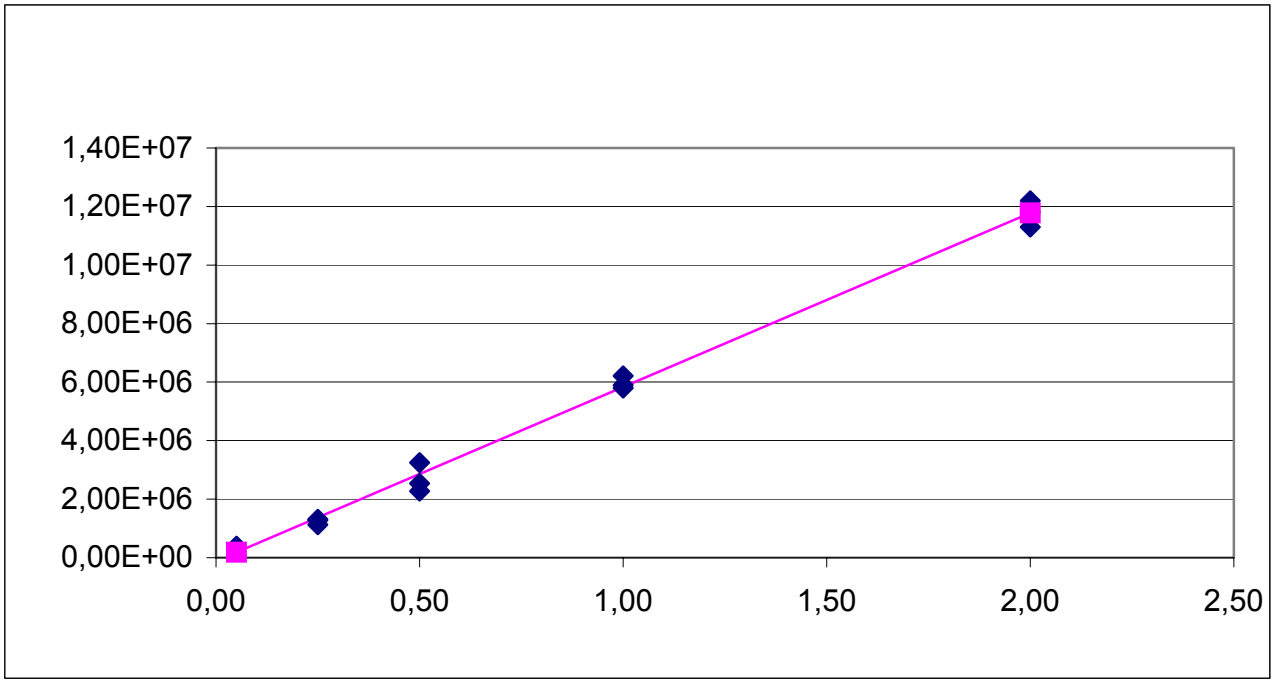


Fig. 30. Regression plot based on EC peak only.

8.3.6. Accuracy and precision.

Intra-day and inter-day precision and accuracy data are shown in Table 26 and 27. Accuracy was expressed as the percent of measured concentration relative to the known concentration of the solutions analyzed. Precision was expressed as the coefficient of variation (CV). Intra-day accuracy and precision were calculated from the analysis of three replicates with the solution in Table 23 within a single analytical batch. Inter-day accuracy and precision were determined for the same concentrations from three separate analytical sessions conducted on separate days within 3 months. The solutions were analyzed in triplicate during every batch. Both the analyte/ISDN ratio and the analyte were used for calculations. The results from the analyte/ISDN ratio were better and are shown in the following tables.

compound NG

	intra-day		inter-day	
target concentration	accuracy (%)	precision (CV)	accuracy (%)	precision (CV)
0.5 mg/l	94	6.1	107	13
1.5 mg/l	95	5.1	93	12
2.5 mg/l	105	4.5	104	7.6
5 mg/l	100	4.1	95	6.8
10 mg/l	103	3.7	101	5.9

Table 26. Intra-day and inter-day accuracy and precision.

compound DPA

	intra-day		inter-day	
target concentration	accuracy (%)	precision (CV)	accuracy (%)	precision (CV)
3 mg/l	92	6.5	108	9.9
5 mg/l	106	4.0	102	15.1
7.5 mg/l	105	4.9	94	12.8
15 mg/l	106	5.1	99	8.9
30 mg/l	94	3.0	112	3.3

compound EC

target concentration	accuracy (%)	precision (CV)	accuracy (%)	precision (CV)
0.05 mg/l	91	5.9	110	9.8
0.25 mg/l	92	4.2	104	15
0.5 mg/l	103	3.8	99	7.6
1 mg/l	100	4.7	95	7.1
2 mg/l	90	3.0	97	8.9

Table 27. Intra-day and inter-day accuracy and precision.

8.3.7. Recoveries

Several experiments were run in order to investigate the yield of extraction from swabs, from filters and from hands. The experiments were conducted following the procedures described in chapter V with slight modifications.

8.3.7.1. Recoveries from swabs

The procedure for the first experiment with swabs is in the following stepwise description.

1. Pipette 100 µl of a standard mixture in methanol containing NG (10 mg/l), DPA (20 mg/l) and EC (2.74 mg/l) onto three different swabs (A, B, C).
2. Wait ½ h leaving swabs at room temperature.
3. Extract as described in chapter V using 1 ml of methanol, without adding ISDN.
4. Measure the extract volume after the centrifugation.
5. Quantitate NG, DPA and EC by HPLC-MS-MS.

Results are in the following Table 28.

% recovery from cotton swabs

	A	B	C	**Mean**	**s**	**CV**
NG %	108	92	77	**92**	16	17
DPA %	84	112	97	**98**	14	14
EC %[16]	83	80	80	**81**	1.7	2.1

% recovery from Alco-Prep®

	A	B	C	**Mean**	**s**	**CV**
NG %	108	99	78	**95**	15	16
DPA %	103	96	81	**93**	11	12
EC %	87	90	93	**90**	3	3

Table 28. Results of recovery [%] tests after a single centrifugation step from three different swabs (A, B, C).

Another experiment was developed to determine if a single centrifugation step with 1 ml of methanol was better than three centrifugation steps with 0.5 ml aliquot methanol each. The procedure is in the following stepwise description.

1. Pipette 100 µl of a standard mixture in methanol containing NG (10 mg/l), DPA (20 mg/l) and EC (2.74 mg/l) onto two different swabs (A, B).
2. Wait ½ h leaving swabs at room temperature.
3. Extract as described in chapter V using the 1 ml of methanol, without adding ISDN.
4. Measure the extract volume after the centrifugation.

[16] Solutions were diluted to quantitate EC after determining NG and DPA.

5. Quantitate NG, DPA and EC by HPLC-MS-MS.
6. Pipette 100 µl of a standard mixture in methanol containing NG (10 mg/l), DPA (20 mg/l) and EC (2.74 mg/l) onto two different swabs (A, B).
7. Wait ½ h leaving swabs at room temperature.
6. Extract as described in chapter V using three aliquots of 0.5 ml of methanol each (1, 2 and 3), without adding ISDN.
7. Collect the three extracts of the three centrifugation steps separately.
8. Measure the extract volume.
9. Quantitate NG, DPA and EC by HPLC-MS-MS.

Results are in the following Table 29, 30 and 31.

	1.0 ml single centrifugation	0.5 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	90	68	12	0	80
NG B	89	79	9	0	88
DPA A	90	92	0	0	92
DPA B	85	79	0	0	79
EC A	86	55	4	0	59
EC B	85	58	2	0	60

Table 29. Results of recovery [%] tests following different centrifugation procedures with two different swabs (A, B) Alco-Prep®.

	1.0 ml single centrifugation	0.5 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	102	96	8	0	104
NG B	90	88	10	0	98
DPA A	92	100	0	0	100
DPA B	99	101	0	0	101
EC A	76	48	8	2	58
EC B	70	54	7	1	62

Table 30. Results of recovery [%] tests following different centrifugation procedures with two different swabs (A, B) Alco-Prep® squeezed before adding the standard mixture.

	1.0 ml single centrifugation	0.5 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	99	89	11	0	100
NG B	90	85	12	0	97
DPA A	100	96	6	0	102
DPA B	89	74	17	0	91
EC A	70	45	15	5	65
EC B	73	40	16	5	61

Table 31. Results of recovery [%] tests following different centrifugation procedures with two (A, B) different cotton swabs.

Another experiment was developed to determine the best solvent to extract the compounds of interest from the swabs. An aliquot of 100 µl of the same standard mixture in methanol of the previous experiment, containing NG (10 mg/l), DPA (20 mg/l) and EC (2.74 mg/l), were added onto two different swabs (A, B). The extraction was conducted using 1.0 ml of the solvent in a single centrifugation extraction procedure.

Results are in the following table 32.

	water	acetone	ethyl acetate	methanol[17]
NG A	76	98	86	102
NG B	71	90	94	90
DPA A	0	108	44	92
DPA B	0	98	38	99
EC A	32	66	66	76
EC B	35	66	61	70

Table 32. Results of recovery [%] tests using different solvents with two different swabs (A, B) Alco-Prep® squeezed before adding the standard mixture.

[17] Values from Table 30.

The last experiment with swabs allowed determining the recoveries at lower concentrations. An aliquot of 50 µl of a standard mixture in methanol, containing NG (5 mg/l), DPA (10 mg/l) and EC (1.37 mg/l), were added onto two different swabs (A, B). The extraction was conducted using 1.0 ml of methanol in a single centrifugation extraction procedure.

Results are in the following Table 33.

	Cotton swab	Alco-Prep®
NG A	107	97
NG B	105	106
DPA A	93	81
DPA B	80	105
EC A	68	72
EC B	67	68

Table 33. Results of recovery [%] tests from two different cotton swabs (A, B) and two different swabs (A, B) Alco-Prep® squeezed before adding the standard mixture.

8.3.7.2. *Recoveries from filters*

The procedure for the first experiment with filters is in the following stepwise description.

1. Pipette 50 µl of a standard mixture (see Table 34) onto three different filters (A, B, C).
2. Wait ½ h leaving filters at room temperature.
3. Extract as described in chapter V using 1 ml of methanol, without adding ISDN.
4. Measure the extract volume after the centrifugation.
5. Quantitate NG, DPA and EC by HPLC-MS-MS.

Results are in the following table 35.

	C low	**C medium**	**C high**
NG	20	40	160
DPA	30	60	240
EC	5	10	40

Table 34. Composition of solutions for recovery tests [mg/l].

	% recovery from filters C low					
	A	B	C	**Mean**	**s**	**CV**
NG %	83	65	88	**79**	12	15
DPA %	84	73	64	**74**	10	14
EC %	70	84	66	**73**	9	13

	% recovery from filters C medium					
	A	B	C	**Mean**	**s**	**CV**
NG %	93	68	90	**84**	14	16
DPA %	89	62	70	**74**	14	19
EC %	86	74	87	**82**	7	9

	% recovery from filters C high					
	A	B	C	**Mean**	**s**	**CV**
NG %	77	95	90	**87**	9	11
DPA %	69	91	75	**78**	11	15
EC %	69	71	87	**76**	10	13

Table 35. Results of recovery [%] tests from three different filters (A, B, C) at three different concentrations.

Another experiment was developed to determine if a single centrifugation step with 1 ml of methanol was enough to obtain a complete recovery from filters. The procedure is in the following stepwise description.

1. Pipette 50 µl of a standard mixture (see Table 34) onto two different filters (A, B).
2. Wait ½ h leaving filters at room temperature.
3. Extract as described in chapter V using a first aliquot of 1 ml of methanol (1) and two following aliquots o 0.5 ml of methanol each (2 and 3), without adding ISDN.
4. Collect the three extracts of the three centrifugation steps separately.
5. Measure the extract volume.
6. Quantitate NG, DPA and EC by HPLC-MS-MS.

Results are in the following table 36, 37 and 38.

	1 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	82	3	0	85
NG B	78	0	0	78
DPA A	67	0	0	67
DPA B	85	0	0	85
EC A	70	2	2	74
EC B	66	3	2	71

Table 36. Results of recovery [%] tests following different centrifugation procedures with two different filters (A, B) at low concentration.

	1 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	85	3	0	88
NG B	80	1	0	81
DPA A	72	0	0	72
DPA B	77	0	0	77
EC A	70	2	1	73
EC B	88	1	2	91

Table 37. Results of recovery [%] tests following different centrifugation procedures with two different filters (A, B) at medium concentration.

	1 ml first centrifugation (1)	0.5 ml second centrifugation (2)	0.5 ml third centrifugation (3)	(1)+(2)+(3)
NG A	89	1	0	90
NG B	85	0	0	85
DPA A	96	0	0	96
DPA B	74	0	0	74
EC A	65	1	0	66
EC B	70	1	1	72

Table 38. Results of recovery [%] tests following different centrifugation procedures with two different filters (A, B) at high concentration.

8.3.7.3. Recovery from hands

The procedure for the first experiment of recovery from hands is in the following stepwise description.

1. Pipette 50 µl of a standard mixture in methanol containing NG (10 mg/l), DPA (20 mg/ml) and EC (2.47 mg/ml) onto the hands of six volunteers (A, B, C, D, E, F), who immediately rubbed their hands to distribute the solution on both hands.
2. Wait ½ h without washing hands.
3. Sample the two hands with two different swabs (left, right).
4. Extract and analyze following the procedure in Chapter 5.
5. Analyze by HPLC-MS-MS.

Results are in the following table 39 and 40.

		NG	**DPA**	**EC**
Test A	left hand	2.5	2.5	3.1
	right hand	3.0	3.9	2.7
	total	**5.5**	**6.4**	**5.8**
Test B	left hand	3.1	3.4	2.2
	right hand	3.0	2.7	2.1
	total	**6.1**	**6.1**	**4.3**
Test C	left hand	2.2	2.0	1.9
	right hand	3.0	3.1	2.3
	total	**5.2**	**5.1**	**4.2**

Table 39. Results of recovery [%] tests from hands of three volunteers with Alco-Prep®.

		NG	DPA	EC
Test D	left hand	1.4	1.2	1.7
	right hand	1.4	1.9	1.6
	total	**2.8**	**3.1**	**3.3**
Test E	left hand	3.3	4.8	3.1
	right hand	2.7	3.6	2.9
	total	**6.0**	**8.4**	**6.0**
Test F	left hand	2.2	3.0	2.2
	right hand	1.5	2.5	0.9
	total	**3.7**	**5.5**	**3.1**

Table 40. Results of recovery [%] tests from hands of three volunteers with cotton swabs.

The procedure for the second experiment of recovery from hands is in the following stepwise description.

1. Put mg 20 of diatomaceous earth on a glass plate.
2. Pipette 50 µl of a standard mixture in methanol containing NG (10 mg/ml), DPA (20 mg/ml) and EC (2.47 mg/ml) onto the diatomaceous earth.
3. Wait ½ h leaving diatomaceous earth at room temperature.
4. Distribute the earth onto the hands of three volunteers (G, H, I), who immediately rubbed their hands to distribute it on both hands.
5. Wait ½ h without washing hands.
6. Sample the two hands with two different Alco-Prep®.

7. Extract and analyze following the procedure in Chapter 5.

8. Analyze by HPLC-MS-MS.

Results are in the following table 41.

		NG	**DPA**	**EC**
Test G	left hand	3.2	7.9	1.9
	right hand	3.3	7.7	2.2
	total	**6.5**	**15.6**	**4.1**
Test H	left hand	4.9	8.3	2.9
	right hand	3.1	8.5	2.2
	total	**8.0**	**16.8**	**5.1**
Test I	left hand	3.9	8.3	2.0
	right hand	5.4	8.2	2.2
	total	**9.3**	**16.5**	**4.2**

Table 41 Results of recovery [%] tests from hands of three volunteers with Alco-Prep®.

8.3.8. Analysis of samples from shooting tests

The vials were placed in the autosampler tray of the HPLC-MS-MS in the order of the analysis form (See chapter V). Five calibrant solutions were analyzed before samples and repeated at the end of the batch. Every analysis was preceded and followed by a blank analysis. Results are in the following pages (Table 42 and 43). Not only samples from shooting tests were analyzed. Twenty blank samples from clothes of volunteers were taken and analyzed. It was never possible to find NG, DPA or EC in these blank samples. Swabs from hands of ten volunteers (twenty samples) were taken and analyzed too. In one of these sample was found 0.85 μg of DPA.

In the following tables, results above LOD but below LOQ are considered positive. For signals below the LOD is reported n.d. for "not detected" in the following tables. LOQ values are calculated as three times the LOD values. The chromatogram of the analysis of a standard solution with API 3000 is in Fig. 31.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE	NG µg/filter	EC ng/filter	DPA µg/filter
V1[18]	Colt	Win .38	1	laboratory coat	0.18	17	n.d.
V2[18]	Colt	Win .38	1	woollen jacket	0.45	59	n.d.
V3[18]	Colt	Win .38	1	sweatshirt	0.25	83	n.d.
V4[18]	Colt	Win .38	1	cotton shirt	0.12	23	n.d.
V6[18]	Colt	Win .38	4	cotton shirt	0.6	370	n.d.
V7[18]	Colt	Win .38	4	cotton shirt	0.2	561	n.d.
V8[18]	Colt	Win .38	4	cotton shirt	0.1	89	n.d.
V9[18]	Colt	Win .38	4	cotton shirt	1.9	50	n.d.
V10[18]	Colt	Win .38	4	cotton shirt	0.2	87	n.d.
V11[18]	Colt	Win .38	4	cotton shirt	1.0	17	n.d.
V12[18]	Colt	Win .38	4	cotton shirt	0.6	21	n.d.
V13[18]	Colt	Win .38	4	cotton shirt	1.2	133	n.d.
V14[18]	Colt	Win .38	4	cotton shirt	4.2	69	n.d.

Table 42. HPLC-MS-MS results of samples from clothes.

[18] Analysis with API 300.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE	NG µg/filter	EC ng/filter	DPA µg/filter
V15[18]	Colt	Win .38	4	cotton shirt	1.2	354	n.d.
V16[18]	Colt	Win .38	4	cotton shirt	8.6	95	n.d.
V17[18]	Colt	Win .38	4	cotton shirt	2.7	55	n.d.
V18[18]	Colt	Win .38	4	cotton shirt	n.d.	n.d.	n.d.
V19[18]	Colt	Win .38	4	cotton shirt	4.2	84	n.d.
V20[18]	Colt	Win .38	4	cotton shirt	23	139	n.d.
V21[18]	Colt	Win .38	4	cotton shirt	3.1	54	n.d.
V22[18]	Colt	Win .38	4	cotton shirt	71	35	n.d.
V23[18]	Colt	Win .38	4	cotton shirt	4.0	63	n.d.
V24[18]	Colt	Win .38	4	cotton shirt	36	121	n.d.
V25[18]	Colt	Win .38	4	cotton shirt	1.2	51	n.d.
V26	SIG Sauer	Win 9 mm	4	cotton shirt	14	n.d.	1.1
V27	SIG Sauer	Win 9 mm	4	cotton shirt	8.0	n.d.	3.8

Table 42. HPLC-MS-MS results of samples from clothes.

[18] Analysis with API 300.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE	NG µg/filter	EC ng/filter	DPA µg/filter
V28	SIG Sauer	Win 9 mm	4	cotton shirt	8.1	n.d.	0.6
V29	SIG Sauer	Win 9 mm	4	cotton shirt	4.1	n.d.	n.d.
V30	SIG Sauer	Win 9 mm	4	cotton shirt	n.d.	150	0.8
V31	SIG Sauer	Win 9 mm	4	cotton shirt	n.d.	310	0.7
V32	SIG Sauer	Win 9 mm	4	cotton shirt	0.8	300	n.d.
V33	SIG Sauer	Win 9 mm	4	cotton shirt	n.d.	n.d.	n.d.
V34	SIG Sauer	Win 9 mm	4	cotton shirt	0.07[19]	140	1.1
V35	SIG Sauer	Win 9 mm	4	cotton shirt	n.d.	58	n.d.
V36	SIG Sauer	GECO 9 mm	4	cotton shirt	n.d.	60	n.d.
V37	SIG Sauer	GECO 9 mm	4	cotton shirt	7.5	n.d.	n.d.
V38	SIG Sauer	GECO 9 mm	4	cotton shirt	3.5	n.d.	n.d.
V39	SIG Sauer	GECO 9 mm	4	cotton shirt	17	n.d.	0.5
V40	SIG Sauer	GECO 9 mm	4	cotton shirt	2.5	n.d.	n.d.

Table 42. HPLC-MS-MS results of samples from clothes.

[19] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE	NG µg/filter	EC ng/filter	DPA µg/filter
V41	SIG Sauer	GECO 9 mm	3	cotton shirt	4.8	n.d.	n.d.
V42	SIG Sauer	GECO 9 mm	3	cotton shirt	17	n.d.	0.8
V43	SIG Sauer	GECO 9 mm	3	cotton shirt	1.3	n.d.	n.d.
V44	SIG Sauer	GECO 9 mm	3	cotton shirt	16	n.d.	0.7
V45	SIG Sauer	GECO 9 mm	3	cotton shirt	5.1	n.d.	n.d.
V46	SIG Sauer	GECO 9 mm	2	cotton shirt	0.9	n.d.	0.4
V47	SIG Sauer	GECO 9 mm	2	cotton shirt	0.1[19]	n.d.	n.d.
V48	SIG Sauer	GECO 9 mm	2	cotton shirt	0.6	n.d.	n.d.
V49	SIG Sauer	GECO 9 mm	2	cotton shirt	0.8	n.d.	n.d.
V50	SIG Sauer	GECO 9 mm	2	cotton shirt	0.9	n.d.	n.d.
V51	SIG Sauer	GECO 9 mm	1	cotton shirt	n.d.	n.d.	n.d.
V52	SIG Sauer	GECO 9 mm	1	cotton shirt	0.4	n.d.	n.d.
V53	SIG Sauer	GECO 9 mm	1	cotton shirt	0.1[19]	n.d.	n.d.

Table 42. HPLC-MS-MS results of samples from clothes.

[19] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	SAMPLE	NG µg/filter	EC ng/filter	DPA µg/filter
V54	SIG Sauer	GECO 9 mm	1	cotton shirt	0.2	n.d.	n.d.
V55	SIG Sauer	GECO 9 mm	1	cotton shirt	0.5	n.d.	n.d.

Table 42. HPLC-MS-MS results of samples from clothes.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	TIME minutes	NG µg/swab	EC ng/swab	DPA µg/swab
S5D[20]	Colt	Win .38	6	0	0.024[21]	13	n.d.
S5S[20]	Colt	Win .38	6	0	n.d.	n.d.	n.d.
S6D	Colt	Win .38	4	0	0.31	n.d.	n.d.
S6S	Colt	Win .38	4	0	n.d.	n.d.	n.d.
S36D	SIG Sauer	GECO 9 mm	4	0	n.d.	60	0.64
S36S	SIG Sauer	GECO 9 mm	4	0	n.d.	n.d.	n.d.
S37D	SIG Sauer	GECO 9 mm	4	0	0.046[21]	n.d.	n.d.
S37S	SIG Sauer	GECO 9 mm	4	0	n.d.	n.d.	n.d.
S38D	SIG Sauer	GECO 9 mm	4	0	0.077[21]	n.d.	1.09
S38S	SIG Sauer	GECO 9 mm	4	0	n.d.	n.d.	1.44
S39D	SIG Sauer	GECO 9 mm	4	0	0.26	n.d.	n.d.
S39S	SIG Sauer	GECO 9 mm	4	0	n.d.	n.d.	n.d.
S40D	SIG Sauer	GECO 9 mm	4	0	0.56	n.d.	0.76

Table 43. HPLC-MS-MS results of samples from hands.

[20] Analysis with API 300.
[21] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	TIME minutes	NG µg/swab	EC ng/swab	DPA µg/swab
S40S	SIG Sauer	GECO 9 mm	4	0	2.35	n.d.	n.d.
S41D	SIG Sauer	GECO 9 mm	3	0	0.27	69	1.5
S41S	SIG Sauer	GECO 9 mm	3	0	n.d.	n.d.	1.4
S42D	SIG Sauer	GECO 9 mm	3	0	0.063[21]	n.d.	0.79
S42S	SIG Sauer	GECO 9 mm	3	0	n.d.	n.d.	n.d.
S43D	SIG Sauer	GECO 9 mm	3	0	n.d.	n.d.	n.d.
S43S	SIG Sauer	GECO 9 mm	3	0	0.56	56	n.d.
S44D	SIG Sauer	GECO 9 mm	3	0	3.35	n.d.	n.d.
S44S	SIG Sauer	GECO 9 mm	3	0	2.14	n.d.	n.d.
S45D	SIG Sauer	GECO 9 mm	3	0	n.d.	n.d.	n.d.
S45S	SIG Sauer	GECO 9 mm	3	0	0.80	n.d.	n.d.
S46D	SIG Sauer	GECO 9 mm	2	0	0.10[21]	n.d.	n.d.
S46S	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	0.90

Table 43. HPLC-MS-MS results of samples from hands.

[21] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	TIME minutes	NG µg/swab	EC ng/swab	DPA µg/swab
S47D	SIG Sauer	GECO 9 mm	2	0	0.040[21]	n.d.	n.d.
S47S	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	1.08
S48D	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	n.d.
S48S	SIG Sauer	GECO 9 mm	2	0	0.175	n.d.	n.d.
S49D	SIG Sauer	GECO 9 mm	2	0	n.d.	33[21]	n.d.
S49S	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	n.d.
S50D	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	n.d.
S50S	SIG Sauer	GECO 9 mm	2	0	n.d.	n.d.	n.d.
S51D	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	1.1
S51S	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S52D	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S52S	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S53D	SIG Sauer	GECO 9 mm	1	0	0.060[21]	n.d.	0.58

Table 43. HPLC-MS-MS results of samples from hands.

[21] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	TIME minutes	NG µg/swab	EC ng/swab	DPA µg/swab
S53S	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S54D	SIG Sauer	GECO 9 mm	1	0	0.072[21]	98	1.14
S54S	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S55D	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S55S	SIG Sauer	GECO 9 mm	1	0	n.d.	n.d.	n.d.
S56D	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.
S56S	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.
S57D	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.
S57S	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.
S58D	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	0.95
S58S	SIG Sauer	GECO 9 mm	3	60	n.d.	18[21]	1.07
S59D	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.
S59S	SIG Sauer	GECO 9 mm	3	60	n.d.	n.d.	n.d.

Table 43. HPLC-MS-MS results of samples from hands.

[21] Below LOQ but above LOD is considered a positive result.

CODE	WEAPON	CARTRIDGE	NUMBER OF SHOTS	TIME minutes	NG µg/swab	EC ng/swab	DPA µg/swab
S60D	SIG Sauer	GECO 9 mm	1	60	n.d.	n.d.	n.d.
S60S	SIG Sauer	GECO 9 mm	1	60	n.d.	n.d.	n.d.

Table 43. HPLC-MS-MS results of samples from hands.

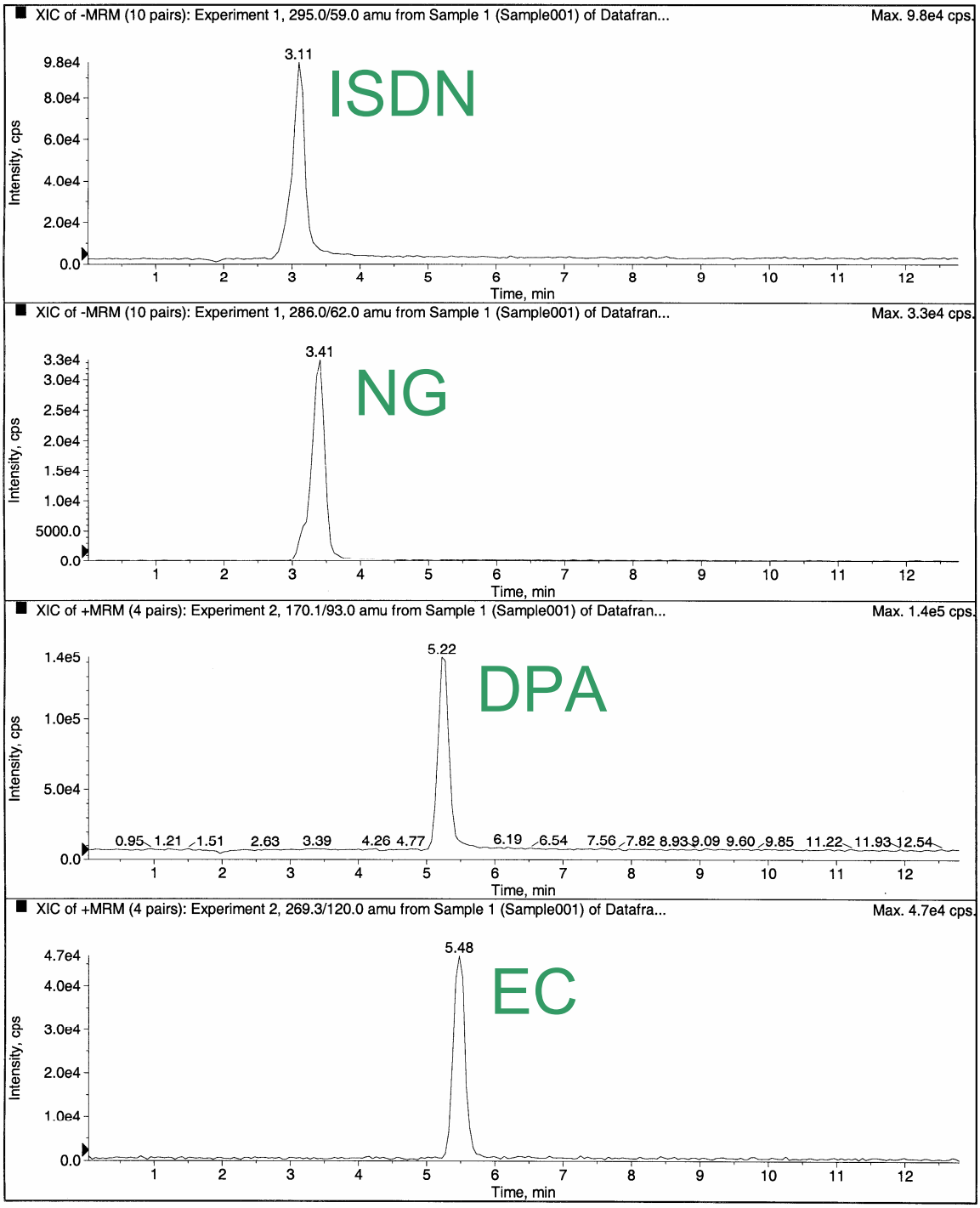


Fig. 31. HPLC-MS-MS analysis of isosorbide dinitrate (ISDN), nitroglycerine (NG), diphenylamine (DPA) and ethylcentralite (EC).

8.3.9 Analysis of possible sources of DPA

To complete the research some samples from possible sources of DPA not related to explosives or weapons were analyzed. DPA is known to be used in the production of rubber and in the post harvest treatment of fruits. Several plastic and rubber objects and fruits were tested by swabbing their surfaces with Alco-Prep®. DPA was found only on some apples and on some tyres (see Table 44).

Sample	**Test A**	**Test B**
Apple Smith	1.4	0.37
Apple Marlene	< LOQ	< LOQ
Apple Granny Smith	0.2	0.31
Apple Stark	0.29	0.32
Tyre Pirelli	1.4	1.2
Tyre Michelin	0.68	0.74

Table 44. DPA [µg] found on fruits and tyres after swabbing with Alco-Prep®.

8.4. Discussion

The results of the experimental work with HPLC-MS-MS were taken into account to develop the sample procedures described in Chapter V. First of all the use of the internal standard was introduced in the procedure not because of a dramatic effect on linearity, accuracy and precision but to give a control parameter for the analysis by HPLC-MS-MS with the autosampler. Sometimes the formation of bubbles in the autosampler could result in a chromatogram without peaks, which could be misinterpreted as a negative result. Another aspect to consider about the use of the internal standard is the variability of the solvent volume recovered after extraction of swabs or filters. The quantitation of the analytes present in the extracts is possible only if the volume of the solvent recovered is measured. The measure of the volume of extracts after centrifugation introduces a further step in the procedure, increasing the risk of contamination of samples.

The two swabbing system studied did not show great differences in the extraction of the analytes and in the efficiency to collect the compounds of interest from hands. Alco-Prep® swabs were not used for ion mobility spectrometry and EGIS analysis. They are not suitable for GC analysis due to the presence of water.

Acetone, ethyl acetate, methanol and water were tested for the extraction step from swabs. The recovery data for acetone and methanol were similar but methanol was preferred because the acetone is expected to extract more interferents. Acetone can dissolve nitrocellulose from smokeless powder particles, which would be deposited on the sampling coil of the EGIS, rapidly affecting the sensitivity of the system, or would precipitate in the HPLC column.

The results of recoveries from spiked hands were quite poor compared to the result of 67% for EC by Meng and Caddy [1996] but close to the value reported by Mach *et al.* [1978], who found 6,5% for DPA. Recoveries from spiked swabs were good, always above 89% for NG while Twibell et al. [1984] determined a recovery for NG of 63 % after the first spin and of 70.5 % after the third spin by centrifuging the swab.

The single centrifugation procedure was found to be effective both with swabs and with filters, permitting faster analysis with less manipulation of samples.

The HPLC-MS-MS showed adequacy to analyze NG, EC and DPA traces in samples from shooting tests. The aim of a short analysis time was also reached, resulting in the possibility to analyze a large number of sample and to fulfil the need of laboratories with a heavy workload.

IX.

Discussion

The experimental work presented in the past chapters showed the possibility to detect organic GSR in samples from shooting tests using an ion mobility spectrometry apparatus, an EGIS system and an HPLC equipped with an API ion source and a triple-quadrupole mass spectrometer detector (HPLC-MS-MS). The results obtained lead to a discussion that may be grouped in two main subjects: a first group of considerations are related to the chemical interpretation of the analytical results, the second concerns the forensic significance of the analytical information.

9.1 Chemical interpretation of the analytical results

The possibility of identification of nitroglycerine with ion mobility spectrometry or with EGIS is the first area of concern. The number of techniques required for the identification of a compound is a fundamental problem for the chemical interpretation of analytical information. The European Commission Decision of 12th August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results [European Communities, 2002], although applied to residues in products of animal origin for public health, can be analogically examined to find useful points of discussion. In the document, screening methods “are used to detect the presence of a substance… These methods have the capability for a high sample throughput and are used to sift large numbers of samples”. If the screening method gives a positive result, this result shall be tested by a confirmatory method. A confirmatory method is a method that provides full or complementary information enabling the substance to be individualized and if necessary quantified at the level of interest.

The present research demonstrated that Ionscan and EGIS are very effective tools for screening samples but the results need a confirmatory analysis. A positive result with Ionscan or EGIS is indicative for the presence of explosive traces but need confirmation. A "Workshop on explosives trace analysis methods" was held at the Forensic Explosives Laboratory (UK) in April 1999 to compare methods and experiences of laboratories [Phillips and Hiley, 1999]. During the meeting Dr. Zitrin, from the Israel National Police, explained that the question of which and how many techniques are required for confirmation of identification of an explosives trace is complicated and there is no single answer. Dr. Zitrin considered that a good chromatographic technique followed by detection with the TEA should be sufficient for a positive identification. Prof. Caddy explained that "the minimum requirements for a positive identification is a separation technique combined with two detectors, based on different principles, or alternatively two separation techniques with one specific detection method". The Forensic Explosive Laboratory adopted gas chromatography with thermal energy analyzer, using three different columns. No compounds interfering with an explosive in all the three systems to give rise to a false identification was found. It was accepted that a single analysis is insufficient but that the number required depends on how orthogonal the techniques are. The Forensic Science Service Laboratory in Birmingham and the Northern Ireland Forensic Science Laboratory used HPLC with pendant drop mercury electrode plus GC with thermal energy analyzer [King, 1995; Speers *et al.*, 1994].

9.1.1 Diagnostic sensitivity of Ionscan and EGIS

One scientific study on the reliability of one of the most commonly used portable ion mobility spectrometry instruments, the Ionscan, showed 14 of 139 (10%) innocuous substances tested caused false positives when detecting controlled substances. In another study evaluating the utility of the Ionscan for the detection of trace explosive, evidence demonstrated the instrument registered a positive response on 12 of 17 (71%) post-blast fragments from improvised explosive devices

[Furton and Meyers, 2001]. Ion mobility spectrometry units typically produce 2-3% false positives when operating at marginal sensitivities [Thermo]. False alarm rates depend on the exact number of compounds detected and location of the testing, since contamination will vary with geography. EGIS gives typically less than 0.5% false alarms (from 0.1 - 0.5%) for a standard set of explosives Mix 5 which includes RDX, TNT, NG, DNT, PETN. Some locations certainly are in the 1-2% range as we see close analogs to these compounds come through frequently [McBee, 2004].

In the present study the use of diagnostic sensitivity and specificity was preferred. Diagnostic sensitivity and specificity are used to evaluate the performance of diagnostic screening tests [Hino *et al.*, 2003; Loong, 2003; Ferrara *et al.*, 1994; Spiehler *et al.*, 1988]. Diagnostic sensitivity and specificity are calculated using the following formulas where TP are the number of true positive results, FP are the number of false positive results, TN are the number of true negative results and FN are the number of false negative results.

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

The use of the term “specificity” should not be promoted, following the IUPAC recommendations [Vessman *et al.*, 2001], because is considered as an absolute term, and thus cannot be graded. “Selectivity” is the recommended term in analytical chemistry to express the extent to which a particular method can be used to determine analytes under given conditions and will be used in the following discussion.

$$Selectivity = \frac{TN}{TN + FP}$$

It is possible to use the formula of "sensitivity" with the results of the samples analyzed using Ionscan, EGIS and HPLC-MS-MS. In samples V41 to V50 HPLC-MS-MS always identified NG (see Table 42, pag. 121) and we can consider these results as true positives. With data in Table 13 (page 62) it is possible to calculate a sensitivity of 0.4 considering a positive signal both for NG-C and for NG-N (samples V44, V45, V46 and V50). When only the NG-C signal of Ionscan is considered for a positive result (single channel approach) the sensitivity is 0.8. Of course these values are only indicative, due to the limited number of tests, because an accurate determination of sensitivity and selectivity of Ionscan and EGIS was beyond the scope of this research. However, despite the limitation of the study, the sensitivity of the Ionscan following the single channel approach is expected to be higher than considering a positive signal both for NG-C and for NG-N (double channel approach). It was shown in Table 12 (page 59) that the limit of detection (LOD) of the Ionscan, e.g. the minimum amount giving a positive result, increased with the double channel approach.

With data in Table 15 (page 69) it is possible to calculate a sensitivity of 0.9 for the EGIS. It was already shown that with EGIS a solution of NG 0.2 mg/l gave three positive results in three different analysis (see Table 14 page 67) while the same solution gave only two positive results in three different analysis with the Ionscan following the single channel approach.

A possible explanation of the false negative results obtained by Ionscan is the "matrix effect" which is the influence on the response from compounds that are in the sample with the analyte and may compete in the ionisation process, due to their electron affinities.

An explanation of the false negative result obtained by EGIS could be that the amount of NG was too little. The result by HPLC-MS-MS above the LOD (40 pg/µl) but below the LOQ (120 pg/µl) for NG supports this hypothesis and raises some doubts about the possibility of such a little amount of NG to give a positive result with Ionscan. Analysis to study the selectivity of Ionscan and EGIS were not conducted. EGIS was used in a "population study", carried out to find the background

levels of explosive traces aboard commercial passenger aircrafts, to screen samples (swabs wetted with isopropanol and water). Approximately 14% of over 2100 swabs were positive for at least one explosive after the screenings by EGIS but presumptive analyses were never confirmed [Stabler *et al.*, 1999].

The study of the scientific literature and the data obtained in this study does not allow considering that both a positive Ionscan and a positive EGIS analysis reach the minimum requirements for a positive identification of NG, needing further analysis for confirmation. Despite the limitations of Ionscan and EGIS in forensic analysis it is important to conclude this part of the discussion pointing out that their use is fundamental for police activity such as security strategies or intelligence activity. When the lives of innocent people may depend upon Ionscan or EGIS results, it is wise to take into account such data in term of possible identification to avoid any risk.

9.1.2 Analysis with HPLC-MS-MS

Hyphenated techniques based on chromatography and mass spectrometry are the preferred methods to make confirmatory analysis, according to the Commission Decision [2002], where methods based on chromatographic analysis without the use of spectrometric detection are not considered suitable on their own for use as confirmatory methods. If a single technique lacks sufficient selectivity, the desired selectivity shall be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and detection techniques. The Commission Decision describes different requirements for confirmatory methods. There is a group A of substances, whose confirmation can only be made using liquid-chromatography or gas-chromatography with mass-spectrometric detection or with IR spectrometric detection, and a group B of substances with less strict requirements (e.g. GC-electron capture detection, only if two columns of different polarity are used).

The Commission Decision [2002] includes performance criteria both regarding chromatographic separation and concerning the mass spectrometric analysis. For liquid chromatography analysis a tolerance of ±2.5% in the ratio of the chromatographic retention time of the analyte to that of the internal standard is accepted. When mass fragments are measured using other than full-scan techniques, a system of identification points is described. Tables 45 and 46 show the number of identification points that each of the basic mass spectrometric techniques can earn.

MS technique	Identification points earned per ion
Low resolution (LR) mass spectrometry (MS)	1.0
LR-MS^n precursor ion	1.0
LR-MS^n transition products	1.5
High resolution (HR) mass spectrometry	2.0
HR- MS^n precursor ion	2.0
HR-MS^n transition products	2.5

Table 45 Relationship between a range of classes of mass fragment and identification points [Commission Decision, 2002].

Technique(s)	Number of ions	Identification points
GC-MS (EI or CI)	n	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI) 2 derivatives	2 (derivative A) + 2 (derivative B)	4
LC-MS	n	n
GC-MS-MS	1 precursor and 2 products	4
LC-MS-MS	1 precursor and 2 products	4
GC-MS-MS	2 precursor ions, each with 1 product	5
LC-MS-MS	2 precursor ions, each with 1 product	5
LC-MS-MS-MS	1 precursor, 1 product and 2 products of the last transition	5,5
HRMS	n	2n
GC-MS and LC-MS	2 + 2	4
GC-MS and HRMS	2 + 1	4

Table 46 Examples of the number of identification points [Commission Decision, 2002] earned for a range of techniques and combinations thereof (n = an integer).

The maximum number of points of identification required in the Commission Decision [2002] is 4. However, in order to qualify for the identification points required a minimum of at least one ion ratio shall be measured and all relevant measured ion ratios shall meet the criteria in table 47.

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS^n LC-MS, LC-MS^n (relative)
> 50 %	± 10 %	± 20 %
20 % – 50 %	± 15 %	± 25 %
10 % – 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

Table 47 Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques [Commission Decision, 2002].

The stricter criteria described in the Commission Decision were chosen and respected in the identification of NG, DPA and EC (4 identification points for each molecule) during the experimental work described in chapter VIII.

The acceptance of such criteria for forensic chemical analysis deserves a debate to obtain a wide consensus in the community of experts. In the present discussion they are used as an index to compare the performances of different analytical procedures. HPLC with pendant drop mercury electrode plus GC with thermal energy analyzer are used in the Forensic Science Service Laboratory

in Birmingham and in the Northern Ireland Forensic Science Laboratory to reach a positive identification of explosive traces [King, 1995, Speers, *et al.*, 1994; Wallace and McKeown, 1993]. Gas chromatography with thermal energy analyzer, using three different columns, is adopted in the Forensic Explosive Laboratory [Phillips and Hiley, 1999]. All these procedures are considered to have enough selectivity for forensic purposes. They do not appear to have a higher selectivity to identify traces of nitroglycerine in samples for forensic purposes than the HPLC-MS-MS method described in the present research.

The HPLC-MS-MS procedure has the strong benefit that not only NG, but EC and DPA can be considered individualised too, using the Commission Decision criteria, while gas chromatography with thermal energy analyzer is not capable of detecting EC and DPA.

Wu *et al.* [2001, 1999] published two papers about the utilization of MS-MS method in detection of GSR. They described a method where only methylcentralite (MC), a stabilizer having a structure similar to ethylcentralite, was detected following a single transitions 241 $\rightarrow$ 134. The samples from shooting tests "were injected into the tandem MS instrument", apparently avoiding the HPLC separation (the peak of a GSR sample had a time of 1.17 min., much shorter than the retention time of MC in the standard mixture, 22.3 min.). For these reasons the procedure by Wu *et al.* may be considered as only a screening method, needing further confirmation and limited to a single compound while the method presented in this research can be considered an identification procedure, whose results could be used without further analysis for three compounds.

9.2 Forensic significance of the analytical results

Detection and identification of GSR, both organic and inorganic, can help indicate whether or not a suspect fired a gun. From a general point of view the role of evidence is to help assess whether or not a particular individual is associated with a particular criminal activity. Talking about inorganic GSR particles it is necessary to specify that chemical analysis can identify traces from discharging a cartridge but cannot distinguish if the traces were deposited on the hands or clothes of the shooter, if they were deposited on the hands or clothes of someone close to the shooter or if there was a transfer from a surface rich of GSR (firearm, cartridge case, bullet hole,…) to the hands or clothes of someone [Romolo and Margot, 2001].

When we study the organic GSR the most important compound is NG. The finding of NG in the sample from the clothes or from the hands of a suspect can be related not only to different activities but also to different sources [Cook *et al.*, 1998]. Explosives, such as double base propellants or dynamites [Twibell *et al.*, 1982], and cardioactive drugs contain NG [Lloyd, 1983c]. Activities, which can be inferred after finding NG, are being in proximity of an exploding charge containing NG, touching a source of NG (primary transfer) or touching a surface where NG is present (secondary or subsequent transfer). The present research confirmed the results of Leggett and Lott [1989] that tyres and apples can be sources of DPA. They searched other possible sources of compounds having the same analytical behaviour of propellant stabilizers. Several fruits and all the rubber products tested showed peaks at the same retention time as diphenylamine after swabbing with cotton moistened with a solution of 2.5% glycerol in methanol. Neither the rubber nor the plastic products tested gave positive results for ethylcentralite, except a “Pentel” white pencil eraser. Hence a transfer from such sources of DPA may be reasonable after finding DPA in a sample [Dahl and Lott, 1987].

Currently, only limited data are available to assess the likelihood that a suspect might have become contaminated with NG without being involved in criminal activities. Maybe the most important

"population study" is the survey published in 1996 by Crowson *et al.*, carried out to determine the background levels of explosive residues, including NG, in public places. Samples were taken from 25 taxis (124 samples), ten buses (87 samples), 2 airports (16 and 18 samples), passengers from 2 aircrafts (7 and 8 samples), underground trains in 2 depots (44 samples), 4 underground stations (33 samples), 9 police stations (87 samples from different areas), 21 police vehicles (129 samples) 23 people working in police stations as civilian personnel (48 samples from hands). In 337 samples from public areas NG was never detected, while RDX was found in 3 samples from taxis and in 1 from an airport. NG traces were found in 5 police stations out of 9 (7 positive samples for NG out of 87, 1 positive sample for RDX) and in 8 police cars out of 21 but no explosive traces were found in any of the samples taken from the civilian personnel working at the various police stations. The data allow the inference that the probability of picking up NG traces onto the hands by entry into a police station is low [Crowson *et al.*, 1996]. The results of another "population study", conducted to find out the background levels of explosive traces aboard commercial passenger aircraft, were presented at the 15th meeting of the International Association of Forensic Science, held in Los Angeles. During the study over 2100 samples (swabs from aircraft lavatories, seating areas, overhead baggage compartments, cargo containers, and cargo holds) were analyzed and explosive compounds were never identified [Stabler *et al.*, 1999].

In 2001 Northrop studied 100 individuals from the general population "to test for the presence of any interfering compounds" and to establish whether any of the characteristic organic gunpowder components (COGC) could be found in samples from hands of people who had not recently fired a handgun. He reported that no reference had been "found in the literature to the examination of the general population for characteristic organic gunpowder components (COGC)." NG, DPA and EC were never found in samples taken from 100 volunteers representing a variety of occupation. Samples were collected by adhesive film lifting and analyzed by micellar electrokinetic capillary electrophoresis [Northrop, 2001a].

"Target studies" on a positive population (i.e. involved in shooting) were conducted by Jane *et al.* [1983], Lloyd [1986a], Dahl *et al.* [1987], Northrop and Mac Crehan [1992], Meng and Caddy [1994, 1995, 1996], King [1995], Northrop [2001b], Zeichner *et al.* [2003].

In the present research "target studies" about negative and positive groups were carried out. Tests were conducted in well-defined situations, trying to understand the forensic meaning of chemical information.

9.2.1 Interpretation of results from clothes in a forensic perspective

Samples from clothes of twenty volunteers, not related with the use of firearms, explosives or NG containing drugs were taken and analyzed, without finding NG, DPA or EC in a "target study" on a negative group.

The tests from V1 to V4 were realized to know whether the analytical procedure was able to identify traces of organic gunshot residue after shooting lead-free ammunition or not. After shooting one round of Winchester .38 Special Super-X® Super Unleaded cartridges, containing both NG and EC, with a revolver Colt Detective Special .38 Special it was possible to identify traces of NG and EC in samples from different clothes. Three results were below the limit of quantitation (LOQ) but above the limit of detection (LOD) and such results do indicate the presence of NG in the samples, regardless to the possibility to have a quantitative result. Hence the tests from V1 to V4 demonstrated the transfer of traces on the shooter's clothes and the capability of the procedure to obtain a positive result.

A second group of shooting tests (from V6 to V25) was realized as a model system. They were carried out in well-defined situations to study how the analytical information obtained by HPLC-MS-MS can help infer the involvement of a subject in a shooting. The model system was used to calculate the sensitivity and the selectivity of the method. All the positive results were considered true positive (TP), in the sense of being produced after analysis of clothes worn during shooting

tests. It is necessary to stress that the results of such calculations cannot be applied to the general population. Despite the limitations, the study of this approach using a model system can help the development of a more complex procedures to evaluate data, using diagnostic sensitivity and specificity/selectivity, well known in clinical chemistry and forensic toxicology but never used in GSR analysis. During the tests four rounds of Winchester .38 Special Super-X® Super Unleaded cartridges, containing both NG and EC, were shot with a Colt Detective Special .38 Special revolver. The shooters always used two hands and wore cotton shirts made of the same garment. Each shirt worn by the shooter during the shooting was taken, stored in nylon bags before sampling to avoid NG loss and sampled by vacuum lifting within one week after the shooting test. Both NG and EC were found in 19 samples out of 20. The mean value of NG was 8.7 μg and the standard deviation was 17 μg. The mean value of EC was 129 ng and the standard deviation was 143 ng (for the 19 positive samples).

Sensitivity and selectivity were calculated using the formulas of page 134. The number of true positive results (TP) used for calculation was 19, which is the number of tests where both NG and EC were found. The number of false negative results (FN) was 1, in the sense of being produced after analysis of clothes worn during a shooting test. The number of false positive results (FP) was 0 after the twenty blank samples of the "target study" on the negative group and the number of true negative results (TN) was 20. Resulted sensitivity was 0.95 and resulted selectivity was 1.

In a third group of shooting tests (from V26 to V35) four rounds of Winchester 9 mm Luger Super-X® Super Unleaded, containing both NG and DPA with traces of EC, were shot with a SIG Sauer P226 9 mm Luger pistol. Shooting, sampling and analysis were as previously described. NG was found in 6 samples out of 10 (for positive results: mean = 5.9 μg, standard deviation = 5.3 μg). EC was found in 5 samples out of 10 (mean = 192 ng, standard deviation = 110 ng). DPA was found in 6 samples out of 10 (for positive results: mean = 1.4 μg, standard deviation = 1.2 μg). Only in one test NG, DPA and EC were found. If we consider that a positive result is when all the three

compounds were found, the sensitivity calculated is 0.1. If we consider that positive is only when NG was found, the calculated sensitivity is 0.6. If we consider the result as positive when at least one of the three compounds was found, the calculated sensitivity is 0.9. These results are coherent considering the very complex and highly irreproducible parameters involved in a shooting case.

In a fourth group of shooting tests (from V36 to V40) four rounds of GECO SX 9 mm Luger, containing both NG and DPA, were shot with a SIG Sauer P226 9 mm Luger pistol. Shooting, sampling and analysis were as previously described. NG was found in 4 samples out of 5 (for positive results: mean = 7.6 μg, standard deviation = 6.6 μg), DPA was found in 1 sample while EC was found in another sample out of 5. Sensitivity calculations have already shown their limits with the results of the previous tests and will not be used for these and the followings results. The phenomenon of shooting appears to be too complex to find an help by calculating sensitivity and selectivity using such a small number of tests. In the subsequent groups of shooting tests the effect of the number of shots was studied. The same SIG Sauer P226 9 mm Luger pistol was used with the GECO SX 9 mm Luger cartridges, containing both NG and DPA. Shooting three rounds (tests from V41 to V45) and two rounds (tests from V46 to V50) the NG was found in five tests out of five. With only one round (tests from V51 to V55) the NG was found in four tests out of five, DPA was found only in two samples after shooting three rounds and in one sample after shooting two rounds. EC was never detected. The mean value of NG was 9 μg after three rounds, 0.7 after two rounds and 0.3 in the four positive tests after one round.

Quantitative results obtained can be considered in agreement with the data about NG on clothes already published by Jane *et al.*, who found up to 11 μg of NG [1983]. Lower values were found by Lloyd [1986a], in the range of 38 to 780 ng, and by King [1995], between 4 and 266 ng.

The analysis of 54 samples from shooting tests demonstrated the transfer of organic GSR (NG, EC and DPA) onto clothes worn by the shooter and the capability of the procedure to obtain a positive result. Two different weapons and three different cartridges were used and the

effect of shooting one, two, three or four rounds was studied too. It is important to point out that sometimes only one of the compounds contained in the cartridge was found. More data on clothes are necessary to attempt a calculation of the probability of finding NG, EC and DPA (separately and together) on clothes after shooting.

The "target study" on the twenty volunteers of the negative group permits to infer that the probability of having NG, EC and DPA on clothes is low but the calculation of such probability needs further data. It was not possible to find other target studies on clothes of negative groups in the scientific literature and more research is necessary to calculate the probability of finding traces of one or more of the studied compounds (NG, DPA, EC) on garments of people not related to criminal activity.

Despite not being possible yet to make statistical calculations, the possibility to identify both organic and inorganic GSR in samples from clothes was demonstrated in the present research. Speers *et al.* [1994] described how inorganic GSR particles to be examined by SEM-EDX can be extracted from the filter after the extraction of organic GSR. For Zeichner *et al.* [2003] the garment can be first sampled by tape lifting for inorganic GSR particles, then vacuum lifting can be used for collection of organic GSR. **The identification of at least one organic compound from the smokeless powder used in the criminal activity under investigation could give strength to the particles found by SEM, following a "case specific" or "case-by-case" approach.** Following this approach the traces found on a specific surface (clothes) is compared with those of the crime under investigation whenever possible (residues in the barrel, in the case, on the victim,…), considering together information related to the particles detected by SEM-EDX and HPLC-MS-MS results [Romolo and Margot, 2001]. The importance of such approach is more evident considering that in GSR analysis "uniqueness" of particles has been extensively used without any statistical background although Stoney clearly pointed out that it is not possible to reach "uniqueness" through statistics [1991].

If the procedure described in this study were adopted in forensic science laboratories, the collection of the data useful for statistical interpretation of this kind of evidence would be soon achieved. The adoption of such a procedure would have very limited influence on expenses in the laboratories where an SEM and an HPLC-MS-MS are already in use. Coordinating the activity of the GSR unit and of the explosive trace detection unit with slight changes in the procedures for examining garments would give benefits in a short period of time. Advantages would not only be "tactical" on single cases, where particles of poor evidential value are found, but strategic too, giving the opportunity to collect data and develop models allowing a Bayesian approach in the interpretation of GSR, such as have been developed in other trace evidence material (fibres, glass, etc.). In the interpretation of GSR findings in linking a suspect and a crime following Bayes' rule it is important to compare two probabilities, the first being, for example, that of the evidence if the suspect shot in a specific situation, the second being that of the evidence if the suspect was not involved in this shooting. More studies about statistical interpretation of GSR are desirable to allow the calculation of the strength of such kind of evidence (probability of the evidence supposing the first hypothesis, probability of the evidence given the alternative explanation, likelihood ratio) [Aitken, 1995; Robertson and Vignaux, 1995; Aitken and Stoney; 1991]

9.2.2 Interpretation of results from hands in a forensic perspective

In a "target study" on a negative group, swabs from hands of ten volunteers (twenty samples) were taken and analyzed. In one of these samples DPA was found (0.85 μg). This result is not surprising due to the results obtained during the present research analyzing swabs from tyres and apples and the studies by Leggett and Lott [1989]. A transfer from sources of DPA different from smokeless powders, such as a tyre, can be inferred after finding DPA in a sample from hands [Dahl and Lott, 1987].

In the present research 22 shooting tests (44 swabs) were conducted to study the transfer of organic GSR (NG, EC and DPA) onto the hands of the shooter and 5 shooting tests (10 swabs) were realized to study the persistence of organic GSR on hands.

The test S5 was realized to know if the analytical procedure was able to identify traces of organic gunshot residue from lead-free ammunition. After shooting six rounds of Winchester .38 Special Super-X® Super Unleaded cartridges, containing both NG and EC, with a revolver Colt Detective Special .38 Special, held by two hands, NG and EC were found only in the sample from the right hand (**S5D**), while the sample from the left hand (**S5S**) was negative. In the tables 42 and 43 with the analytical results the first letter of the code is "V" if the sample was obtained by vacuum lifting and "S" if it corresponded to a swab. For swabs there is a second letter at the end of the code: "D" for right and "S" for left. The hand holding the weapon during the shooting test is in **bold** type.

In test S6, five rounds of the same cartridges were shot with the same revolver, giving only a positive result for NG in the sample from the right hand (**S6D**), while the sample from the left hand (**S5S**) was negative.

In a group of shooting tests (from S36 to S40) four rounds of GECO SX 9 mm Luger, containing both NG and DPA, were shot with a SIG Sauer P226 9 mm Luger pistol. NG was found at least in the sample from one hand in four tests out of five. Only in one test, with the weapon held using both hands, the two swabs gave a positive result for NG. In two swabs DPA was found with NG, in other two swabs DPA was found without NG. EC was found in one swab.

In the following group of shooting tests (from S41 to S45) three rounds of the same ammunition were shot with the same pistol. NG was found at least in the sample from one hand in all the five tests. Only in one test, with the weapon held using both hands, the two swabs gave a positive result for NG. In two swabs DPA was found with NG, in another swab DPA was found without NG. EC was found in two swabs.

In another group of shooting tests (from S46 to S50) two rounds of the same cartridges were shot with the same pistol. NG was found at least in the sample from one hand in three tests out of five. No shooting test permitted the identification of NG for both hands. In two swabs DPA was found, always without NG. One swab showed the presence of EC.

In the group of shooting tests where only one round of GECO SX 9 were shot, using the SIG Sauer P226 pistol, NG was found at least in the sample from one hand in two tests out of five. In two swabs DPA was found with NG, in another swab DPA was found without NG. EC was found in one swab.

Jane *et al.* found up to 2 μg of NG on hands but could not detect DPA [1983]. Lloyd found NG on hands (between 25 and 825 ng) in handswabs taken after six single-round firings and found 8 ng of DPA in the swab from the firing hand shortly after shooting four .22 rounds [1986a]. Lower values, between 5 and 17 ng, were reported by King for swabs from GSR cases [1995] with a limit of detection corresponding to around 1 ng. Dahl *et al.* found DPA and/or EC in swabs from 19 tests out of 20, after shooting one round using different cartridges [1987]. Northrop and Mac Crehan analyzed tape lift samples after 9 firing tests and detected NG in 8 cases, DPA in 3 cases and EC in 3 cases [1992]. Meng and Caddy detected between 0.6 and 4 ng of EC in cotton swabs sampled from the shooting hand. The time lapse after firing and before sampling was between 0 and 120 minutes. The detection limit was 200 pg per sample [1995, 1996].

The results obtained are not surprising due to very complex and highly irreproducible parameters involved in a shooting case. It is possible to make a few comments about the effect of the number of rounds shot. In the tests with GECO SX 9 the results were positive for NG at least in the sample from one hand only in two out of five cases after one round, in three tests out of five after two rounds and in four tests out of five when four rounds were shot. But with three rounds NG was found at least in the sample from one hand in all the five tests. The interpretation of the results

related to stabilizers is even more complex and it is possible that EC results are caused by contamination of the powder, of the weapon, of the shooter or of the sample.

The last group of 5 shooting tests (10 swabs) was realized to study the persistence of organic GSR on hands. The volunteers were sampled one hour after shooting and, while waiting, went back to their normal office activity (mainly writing and reading) but did not wash their hands. In shooting tests from S56 to S59 three rounds of GECO SX 9 were used. NG was never found and DPA was found in two swabs, once with EC. In test S60 only one round was shot and the analysis of the swabs taken one hour after shooting were negative for NG, DPA and EC.

There are several explanations for these results. The high vapour pressure of NG can cause a rapid loss of this compound but transdermal absorption through the skin and hydrolysis are other possible explanations [Lloyd, 1986b]. Another possible mechanism to explain the negative results for the three compounds is the possibility of the larger particles from the propulsive charge dropping from hands during normal activity. These particles were clearly visible on hands immediately after shooting and on swabs taken without delay but were never seen on hands one hour after the shooting test.

It seems very difficult that the low diagnostic sensitivity of NG results after one hour can suggest a change in the procedure to laboratories using SEM on samples taken from hands by tape lifting. Another drawback is the low diagnostic sensitivity of EC and DPA results. Good results were published by Northrop [2001b] using an adhesive lift method, followed by capillary electrophoresis and scanning electron microscopy. From the analytical point of view, further work in optimization of the sampling system, in the extraction procedure and in the concentration step should result in better recovery from hands and in a higher diagnostic sensitivity of the procedure on a more general perspective.

X.

Conclusions

The increasing number of cartridges with lead-free primers on the market is a critical problem in forensic science and it is expected to become more serious in the next years. Analytical results obtained by SEM-EDX apparatus are not particularly indicative with particles from lead-free primers unlike those with particles containing lead, antimony and barium. In this research the new contributions of analytical chemistry to solve this typical forensic problem were explored.

1. The smokeless powders obtained from commercial lead-free cartridges were analyzed using gas chromatography with mass spectrometric detection (GC-MS). NG, DPA and EC were chosen as target molecules, to be detected in samples from clothes and from hands of shooters.
2. Two different sampling procedures were developed, one to collect organic GSR from skin surfaces (swabbing) and another to sample garments (vacuum lifting).
3. An IMS apparatus (Ionscan Model 400) and the EGIS system developed by Thermedics Inc. (USA) showed their capability to detect NG traces in samples from clothes after shooting tests.
4. The possibility to identify NG, DPA and EC in a single run in samples from garments and from hands after shooting tests by an HPLC apparatus equipped with an API ion source and a triple-quadrupole mass spectrometer detector (HPLC-MS-MS) was demonstrated.

The two main chemical issues concerning the analytical procedures developed were discussed.

A) NG can be detected using Ionscan or EGIS but their results must be confirmed by another analysis, to provide unequivocal identification.

B) This can be achieved using the HPLC-MS-MS method which gives full information, thus enabling NG, DPA and EC to be unequivocally determined in a single run. The analysis permits to earn 4 points of identification for the substances, according the criteria set by the European Commission with respect to the performance of analytical methods and the interpretation of results.

Experimental data from shooting tests conducted in well-defined situations were discussed from a forensic perspective.

There are laboratories where analysis of NG in swabs obtained from hands is already routinely processed. The adoption of the procedure developed here for HPLC-MS-MS by these laboratories could help get more information (DPA, EC) about shooting cases but the low diagnostic sensitivity of NG results after one hour would suggest that the introduction of the swabbing method in laboratories where SEM is used on samples taken from hands by tape lifting should be avoided.

Vacuum lifting from clothes gave more useful and unequivocal results, because organic GSR could be determined while leaving the possibility to make SEM analysis with only slight changes in the procedures to examine clothes. Such method could be adopted with little economical impact in the laboratories where an SEM and an HPLC-MS-MS are already in use. The identification of at least one organic compound from the smokeless powder used in the criminal activities under investigation could give strength to any further particle found by SEM, to support the hypothesis of a specific source and activity.

It is possible to conclude that the detection of NG using Ionscan or EGIS and the identification of NG, DPA and EC in samples from clothes following the methods developed in the present research allows the collection of more information about cases involving the use of firearms. Moreover such information may be successfully used not only in forensic cases involving lead-free ammunition,

but also in any shooting case to determine the proximity of a subject to a discharging firearm and/or the contact with a surface exposed to GSR (firearm, spent cartridge case, etc.). In forensic laboratories where both the inorganic (SEM) and the organic approach are followed the number of cases positive for GSR is expected to increase significantly.

As a direct result of the present research, studies in this field should focus on the collection of data and the development of models allowing a Bayesian approach in the interpretation of GSR, similar to what has been developed with other trace evidence material such as fibres or glass fragments. Currently, only limited data is available to assess the likelihood that a suspect might have become contaminated with both inorganic and organic GSR without being involved in criminal activities. The probability of finding traces of one or two of the studied compounds (NG, DPA, EC) on garments of people not related to crimes, the rates of deposition, transfer and persistence of NG, DPA and EC need further analysis. This information is critical for any satisfactory interpretation and there is definitely a need for more research to allow an accurate interpretation. But in the meantime, this should not slow down the introduction of new methods in a field where "uniqueness" of GSR particles has been extensively used without an in depth study of the statistical interpretation of SEM analysis.

Annexe 1 Historical introduction on firearms ammunition

The use of the earliest type of firearms is difficult to establish with any degree of certainty. In Europe it is generally believed that Roger Bacon was the first person to mention and record the formulation of true black powder, an intimate mixture of saltpetre, sulphur and charcoal, in a document written in 1242 [Gallusser *et al.*, 2002; Myatt, 1982]. The first weapons using gunpowder produced during the fourteenth century in Europe were cannons and hand-cannons: simple tubes closed at one end except for a small hole for lighting the gunpowder [Warlow, 1996]. In the beginning a particular kind of cannon called "bombard" was used in sieges: in 1324 Charles of Valois utilized this kind of weapon against English during the siege of Réole [Cadiou et Richard, 1976].

In 1346 Edward III defeated the much greater French army of Philip III of Valois in the battle of Crecy-en-Ponthieu using some "bombards" in open fields for the first time in Europe [Harding, 1980], as reported in Cronica by Giovanni Villani (1276? – 1348).

Handguns were, in the beginning, small cannons of wrought-iron or bronze, in which black powder and various projectiles, often irregularly shaped stone balls, were loaded into the barrel from the muzzle end. A small hole at the breech end of the barrel, the "touch-hole", was provided with a pan into which a "priming charge" of powder was placed. On igniting this priming charge, either with a hot iron or a lighted match, fire flashed through the touch hole and into the main powder charge to discharge the weapon [Lowry, 1986].

By the 15th century ammunition had become fairly standardized and consisted of black powder propellant, followed by some wadding, a spherical lead ball and further wadding to retain all in place. Towards the end of 16th century the bullet was tied into the top of a small paper bag, containing the powder charge, resulting in the first "self-contained" cartridge.

It is generally accepted that Alexander John Forsyth, a Scottish Minister, revolutionized the ignition of gun-powder by using a percussion primer composition, which exploded on being struck, producing a flash strong enough to ignite the main charge of powder in the barrel. With this invention a separate priming powder and sparking system (matchlock, wheel lock or flintlock) was no longer required and the basis for a self-contained cartridge was laid. Most writers believed that Forsyth's priming mixture was based on mercury fulminate but there is some respected opinion suggesting that his primer was based on potassium chlorate [Wallace, 1990].

Introduced to the UK at the Great Exhibition in 1851 by Lefaucheux, the **pinfire** weapons used a self-contained cartridge in which the propellant, missile and primer were all held together in a brass case. In this system the percussion cup was inside the cartridge case while a pin, which rested on the percussion cup, protruded through the side of the cartridge case and, driven by the weapon's hammer, caused the priming compound to detonate and so ignite the main propellant charge. The really great advance of the pinfire system was, however, not just the concept of a self-contained cartridge, but "obturation": the ability of the cartridge case under pressure to swell and so seal the chamber, preventing the rearward escape of gases.

In 1855 Smith and Wesson manufactured the first revolver to fire **rimfire** cartridges, initially developed by Flobert, a Paris gunsmith, who had working examples of it as early as 1847. The rimfire cartridge is a thin-walled cartridge with a hollow flanged rim. Into this rim is spun a small quantity of a priming compound, which explodes because of crushing the rim with the firing pin. Pinfire was at its most popular between 1890 and 1910 and was available until 1940, but only rimfire has survived and this only with the .22 calibre.

Daws introduced the **centre fire** system in 1861 this was the great milestone in weapon and ammunition development: the principles are still the same and are utilized in every type of weapon from the smallest handgun to some of the largest artillery pieces. In this system only the primer cup needed to be soft enough to be crushed by the firing pin, the cartridge case could thus be made of a

more substantial material which would act as a gas seal for much higher pressures than could be obtained with rimfire ammunition [Heard, 1997].

In modern ammunition there are basically three ways in which this is achieved: Boxer, Berdan or battery cup priming system. The "Berdan primer" was designed in 1867 by Colonel Berdan of the US Army Ordnance Department. In this system the "anvil", the surface for the priming compound to be crushed against by the impact of the firing pin, is actually part of the cartridge case. Around the anvil are a number of flash holes to permit the ignition of the propellant. In "Boxer primer", developed in 1866 by Colonel Boxer of the Laboratory at the Royal Woolwich Arsenal, England, the anvil is a small bent disc of steel which fits into the cup, making the primer completely self contained. Boxer-primed ammunition can be reloaded, substituting the fired cup, and is almost exclusively used in commercial ammunition. The "battery cup" system is a self contained primer with a second cup that provides a rigid support for the primer cup and anvil.

The modern cartridge cases manufactured in the Western world are generally made of brass with a 75:25 copper/zinc alloy. In ammunition from countries of the former "eastern block" and from China the cartridge cases are made of steel, coated with copper in China and with a heavy coat of lacquer elsewhere to prevent rusting. Aluminium can be used too, as shown in Fig. 1 (cartridge examined during the present research).

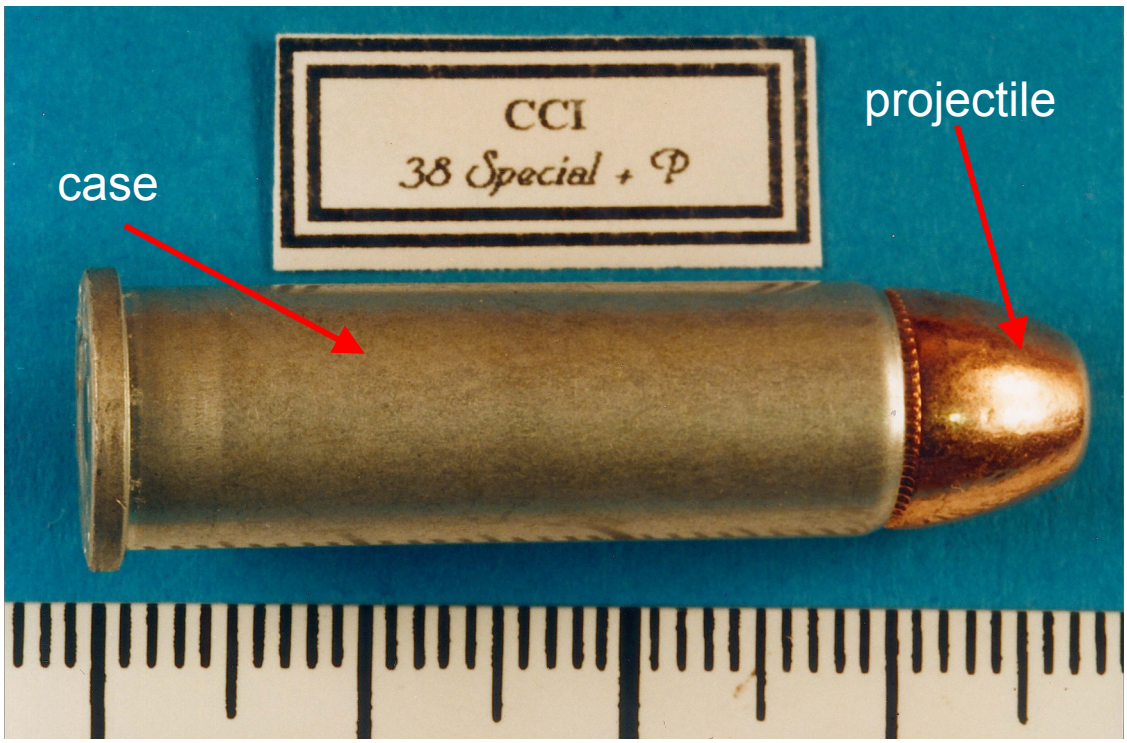


Fig. 1. CCI .38 Special + P cartridge with Blazer® primer.

The primer cup of the CCI .38 Special is shown in Fig. 2.

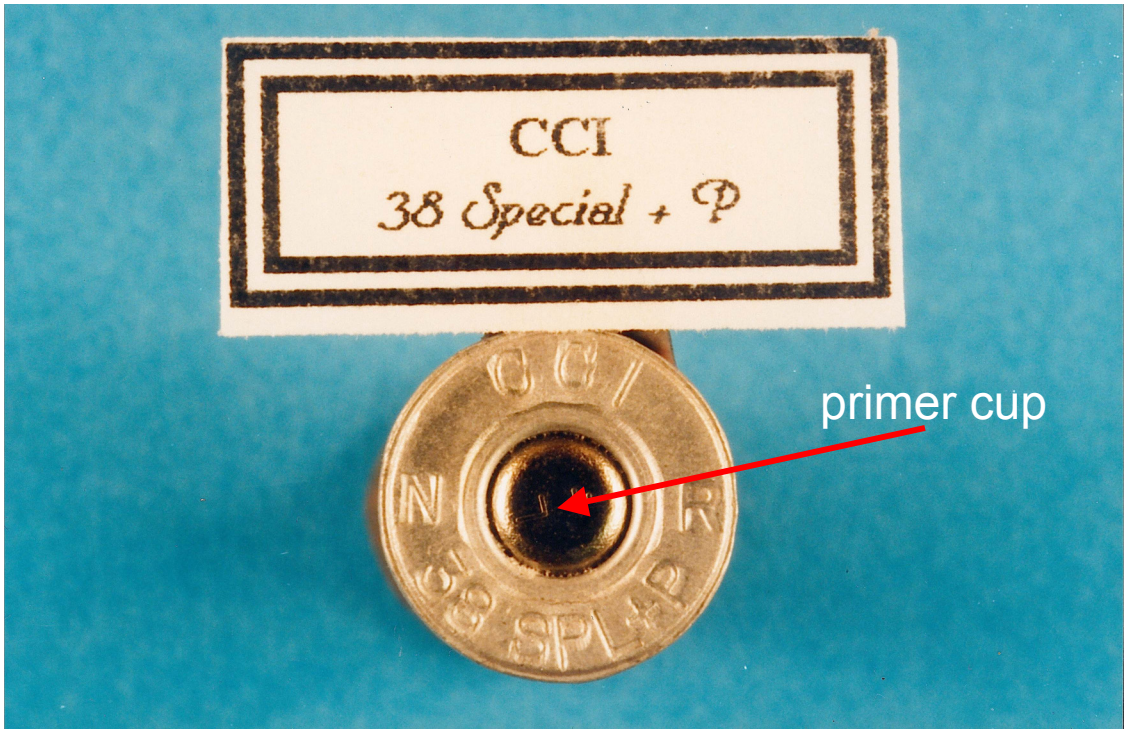


Fig. 2. CCI .38 Special + P cartridge with Blazer® primer.

After opening the CCI cartridge it is possible to see the smokeless powder, the primer cup and the anvil, as shown in Fig. 3.

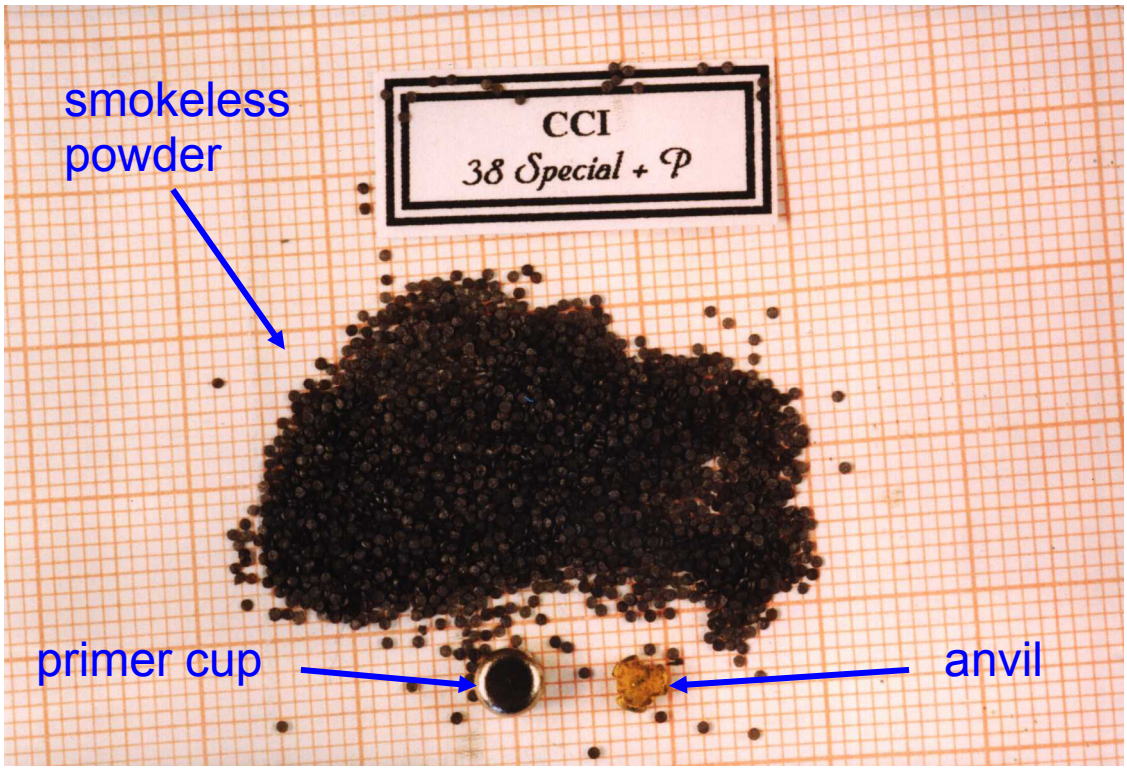


Fig. 3. Primer cup, anvil and smokeless powder contained in the CCI .38 Special + P cartridge shown in Fig. 1 and 2.

The primer cup and the anvil are shown in Fig. 4 and 5. In Fig. 4 the surface of the primer cup hit by the firing pin is shown. In Fig. 5 it is possible to see the internal surfaces of the cup and of the anvil where is possible to see the primer (yellow).

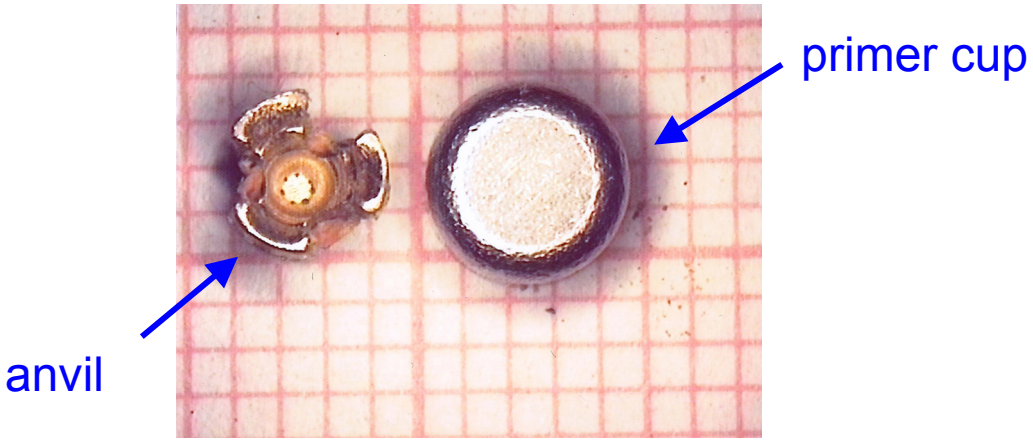


Fig. 4. Primer cup and anvil of the CCI .38 Special + P cartridge shown in Fig. 1 and 2.

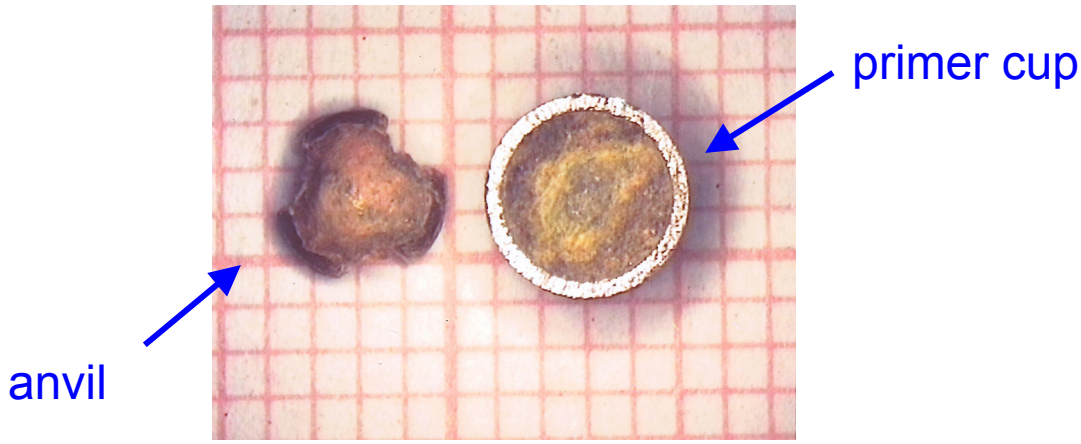


Fig. 5. Primer cup and anvil of the CCI .38 Special + P cartridge shown in Fig. 1 and 2.

In small arms ammunition bullets can be jacketed or unjacketed. The former type is commonly made with lead, alloyed with varying quantities of antimony to give it hardness and tin to assist the moulding process (for cast bullets). Jacketed bullets have a plain lead core covered with a thin layer of a much harder material. This can be a copper/zinc alloy, a copper/nickel alloy or plain steel coated with either a copper wash or a thick coat of lacquer to prevent corrosion.

A different way of producing jacketed bullet is to coat plain lead bullets with a thick layer of nylon or with a wash of copper [Thornton, 1994; Wallace, 1990].

To reduce lead contamination in ranges, a particular kind of ammunition was manufactured with a lead-free primer and a bullet with a thick coating of copper, electro-deposited over a plain lead core. To prevent a discharge of lead in fact, it is important, but not completely sufficient, to use a lead-free primer. The lead core of the bullet itself must be totally enclosed. This kind of bullet, where the coating extends over the whole surface of the bullet, is called Totally Metal Jacketed (TMJ). The bullets where the lead base is uncoated are known as Full Metal Jacket (FMJ) [Gallusser *et al.*, 2002]. Lead free projectiles can be manufactured to avoid any environmental hazard [Enlow *et al.*, 2002; Cesaroni, 2001; Schirneker, 1987]. In Fig. 6 and 7 the Totally Metal Jacketed bullet of a CCI .38 Special + P is shown.

Fig. 6. Totally Metal Jacketed bullet of the CCI .38 Special + P cartridge shown in Fig. 1 and 2.

Fig. 7. Totally Metal Jacketed bullet of the CCI .38 Special + P cartridge shown in Fig. 1 and 2.

Annexe 2 The explosives in ammunition

A. Explosions and explosives

A1. Definitions

Explosives are substances, both compounds and mixtures "which are in a metastable state and are capable, for this reason, of undergoing a rapid chemical reaction without the participation of external reactants such as atmospheric oxygen" [Meyer, 2002]. The explosive reaction needs an initial energetic contribution to be initiated.

An **explosion** is a very fast chemical reaction, producing a great amount of energy and gases at high pressure and temperature, which propagates as a shock wave. There are two kinds of explosions: detonation and deflagration. Detonation propagates above the sonic velocity of the material, deflagration has subsonic speed.

The chemical reactions involved in explosions are **redox**. Oxygen is generally the oxidizing species and can be present in an explosive either in a highly oxidized ion, like nitrate in black powder, or as a nitrogroup in an organic molecule. In black powder the reducing agents are carbon and sulphur. They constitute the explosive mixture with potassium nitrate; in molecules containing nitrogroups the oxygen oxidizes other atoms of the explosive compound (H to H_2O and C to CO_2).

Nitration is the easiest way for a chemist to introduce oxygen atoms in a fuel molecule to transform it in an explosive compound: glycerine can be transformed into nitroglycerine, toluene into trinitrotoluene or cellulose into nitrocellulose. The explosives can be divided into six groups from a chemical point of view: nitro compounds (dinitrotoluene, trinitrotoluene), nitric esters (nitroglycerine, nitrocellulose, pentrite), nitramines (RDX, HMX), derivatives of chloric and

perchloric acids and a last group of various compounds capable of producing an explosion such as fulminates or peroxides [Urbansky, 1964].

A2. Characteristics of explosives

The initial energetic contribution can be supplied to the explosive either as mechanical energy or as thermal energy. From a practical point of view an explosion can be caused by a mechanical shock, an explosive shock, heat, friction or sparks.

The most important characteristics of explosives are the sensitivity, the strength and the stability. The **sensitivity** of an explosive is defined by the intensity of a particular kind of stimulus which is necessary to start the explosive reaction: for example the shock sensitivity is measured by the minimal kinetic energy of a falling weight capable of causing the explosion of a sample; the heat sensitivity is measured by the temperature of the explosion; the friction sensitivity by a weight pushing down a moving pistil in a mortar containing an explosive sample. The Trauzl method is the best-known way of determining the **strength** of an explosive [Meyer, 2002]. It is performed by measuring the volume of the cavity produced by a charge put in an hole of a lead block. The **stability** of an explosive is the capability of maintaining its properties during storage in normal conditions: an explosive is chemically stable if the molecule doesn't spontaneously decompose. Nitrocellulose progressively loses NO_2 groups as nitrogen oxides, the reaction is self-catalyzed, and the evolving heat can cause an explosion. In producing smokeless powders, some substances capable of trapping nitrogen oxides are added (e.g. diphenylamine): the chemical composition varies during the time but the behaviour of the explosive is stable (ballistic stability).

A3. Factors influencing an explosion

The behaviour of an explosive is not only due to the chemical properties of the compound or of the mixture but is influenced by several factors. The main factors influencing an explosion are the **type and amount of energy** provided to the explosive, the confinement of the explosive and its density. If the stimulus to the explosive is inadequate, the reaction that starts can be incomplete or slower than an explosion (e.g. combustion or decomposition). The **confinement** is the resistance of the medium surrounding the explosive charge. The same explosive can burn unconfined, it deflagrates if wrapped in paper and detonates in a hole drilled in the rock. The **density** of a charge determines the speed of reaction: the same explosive can deflagrate in a loose form and detonate in a compressed form.

There are different ways for classifying explosives, according to their chemical structure or their properties for example. In the production of modern ammunition for small arms two kinds of explosives are used: an initiating explosive and a propellant. Initiating explosives start the explosive reaction on being struck and cause the explosion of propellants.

B. Initiating explosives and primers

B1. Initiating explosives

Complex explosive salts such as "fulminating gold" were investigated by Basilius Valentinus and van Drebble in the early 1600s [Bydal, 1990]. Chemist van Drebble also investigated mercuric fulminate, but its first preparation was published in 1690 by Kunkel [Urbansky, 1964]. Mercury fulminate is one of the most important initiating explosive in the history of primers. Other important compounds in this class are lead styphnate, tetracene and diazodinitrophenol (DDNP). Lead styphnate, which caused many fatalities in handling dry salt because of its sensitivity to static electricity, was patented for use in primers in 1914 by von Herz. Tetracene, first synthesized by Hofman and Roth in 1910, was patented for use in primer composition in 1921 by Rathsberg. Diazodinitrophenol (DDNP) was the first diazocompound discovered by Griess in 1858 and was patented in 1922 for use in primers and detonators by Dehn [Bydal, 1990].

Initiating or primary explosives are compounds used to initiate a main charge of secondary explosive by a shock wave. Their sensitivity to mechanical shock and friction is the most important characteristic of this kind of explosives. The initiating explosives are readily ignited by direct contact with flame or electrical sparks: the decomposition temperature is 142 °C for DDNP, 146 °C for tetracene and 252 °C for lead styphnate [Kaiser and Ticmanis, 1995]. The shock sensitivity of initiating explosives is between 0.1 and 0.5 kgm and their friction sensitivity ranges from 0.2 to 20 N. Initiating explosives detonate and produce a relatively slow detonation wave (around 5000 m/s compared to secondary explosives: 7000 m/s of TNT, 7700 m/s of NG or around 8500 m/s of PETN and RDX). Their strength is not very high, furthermore their chemical stability is very good. These characteristics combine ideally to start an “explosive chain” in an easy and reproducible way, minimizing the risk of manipulating dangerous material. Used in small quantity, they are found in

primers, military detonators and industrial blasting caps. All these devices are stored and transported separately from the secondary explosives [Quinchon, 1987]. The weight of explosive in the most common blasting caps (called N° 8) is 1.5 g and the amount of primer composition in a cartridge can range in fact from as low as 0.013 g to as high as 0.325 g, depending on the calibre and type of ammunition [Wallace, 1990].

Primers have other civil and military applications than for firearms ammunition, this includes blank cartridges, flare tripwires, mortars, pyrotechnic cartridges, hand grenades, ejector seat mechanisms and other jettison devices.

B2. Primer mixtures: from the beginning to corrosive-free

It is generally accepted that modern primers evolved from the percussion primer composition developed by Reverend Alexander Forsyth. Most writers believed that Forsyth's priming mixture was based on mercury fulminate but there is some respected opinion suggesting that his primer was based on potassium chlorate [Wallace, 1990].

The percussion cap was claimed by many inventors but it is probably attributable to Joshua Shaw, who began making mixtures of mercury fulminate, potassium chlorate and powdered glass held in a iron metal container in 1814 [Heard, 1997]. Copper percussion cups have been manufactured in the USA since 1816. First priming mixtures may not have contained mercury fulminate, which was not widely used until 1831 [Wallace, 1990].

The usual mixture for rifle ammunition in 1878, the time of the highest development of black powder, is the primer of Frankford Arsenal and consisted of mercury fulminate 35%, potassium chlorate 15%, black powder 40%, glass powder 5% with 5% gum arabic as binding agent [Sellier, 1991].

The changeover from black powder to smokeless powders started in the late 1800's. Smokeless powders were harder to ignite and required larger priming loads. The large volume of ash left in the barrel by black powder diluted the free mercury and the potassium chloride produced by primer explosion, limiting chemical attack to the cartridge case and to the barrel. The brass cartridge cases, the most expensive parts of the ammunition, became brittle and useless because of the formation of an amalgam of copper and zinc with mercury [Matty, 1987]. Potassium chloride left in the barrel attracted water and promoted rusting. It took a relatively long time to understand problems arising from use of smokeless powders. How far the priming mixtures were based on experience and not on chemical considerations is shown by the history of composition "No 42" from Frankford Arsenal. It was introduced as early as 1910 by the military and consisted of potassium chlorate 47%, antimony sulphide 31% and sulphur 22%. In 1917 millions of rounds failed to fire because the raw material decomposed after being mixed [Sellier, 1991]. The primer composition was changed to potassium chlorate 53%, lead thiocyanate 25%, antimony sulphide 12% and TNT 5% and this mixture was used by the USA military under the designation FA70 in pistol cartridges and rifle ammunition right up to the 1950s. This mixture permitted cartridge reloading without solving the problem of rusting inside the barrel, but was capable of being stored for almost unlimited periods.

The first corrosion free priming composition was brought onto the market by RWS (Rheinisch-Westphalische Sprengstoffwerke) as priming composition in 1901. It consisted of mercury fulminate 39%, barium nitrate 27% and TNT 7% [Bydal, 1990].

In 1928 RWS developed the first practical non-corrosive, non-mercuric (NCNM) primer, called SINOXID. Since then the mixture lead styphnate with tetracene as sensitizer began replacing the former primers based on mercury fulminate and potassium chlorate. By the 1950s the NCNM primer had shown such good stability that all US military small arms primers were converted to that type.

The ideal priming composition [Hagel and Redecker, 1986] would consist of a cheap, readily available, relatively safe to handle, simple chemical compound of uniform granulation. In practice no single chemical compound meets all requirements of an ideal primer. For this reason a primer is a mixture containing not only the explosive compound, called **initiator**, but different substances with specific functions. The **sensitizer** helps the ignition process of the initiator, when the firing pin hits the primer. Ground glass, tetracene and pentrite are used as sensitizers. The **fuel** helps sustain the flame and insure adequate time to light the powder. The fuel of highest interest in GSR tests is antimony sulphide. Powdered aluminium, calcium silicide, nitrocellulose, carbon black and lead thiocyanate can also be used as fuels. The **oxidizer** supplies an oxygen source for both the fuel and the initiator. Potassium chlorate, used in the past, and barium nitrate, common in actual primers, are typical oxidizers. Other substances can be added to the mixture like gum arabic or polyvinyl acetate as **binding agent** or a dye to aid in the production. In some cases a single additive may serve different purpose, e. g. antimony sulphide acts as a fuel as well as sensitizing the mixture to friction and gum arabic acts as a fuel and a binding agent.

The sensitivity of priming composition varies, but that of an individual composition can be varied to a certain extent by careful control of the granulation of each of the ingredients. This is sometimes more important than the proportions of the ingredients.

The rate of burning, the volume of gases, the weight of solid particles produced and the duration of the flame are the major properties with respect to the efficient functioning of a priming composition. For a typical priming composition of 0.15 g the volume of gas at 0° C and 760 mm Hg pressure (Standard Temperature and Pressure or STP) is in the order of 1.5 cm^3. Flame bursts from various primers were found to have effective duration varying from 400 to 750 microseconds and total duration varying from 650 to 1500 microseconds [Wallace, 1990].

B3. Lead-free primers

In firing practice, mainly in indoor shooting ranges, considerable amounts of toxic substances are produced by SINOXID primers and ammunition with not totally metal jacketed lead bullets. Inhaling these gases increases the level of lead in the blood and is a health hazard to the shooters. Different studies have been done in Canada, Germany and Great Britain about lead concentration in shooters' blood: people shooting indoor are exposed to levels exceeding the maximum allowed by health regulations, which vary from country to country. For the Occupational Safety and Health Administration (OSHA) the Permissible Exposure Limit (PEL) is 50 micrograms of lead per cubic meter of air, averaged over an 8-hour workday [OSHA].

Sellier [1991] reports that McNutt (Winchester) replaced lead styphnate with diazodinitrophenol (DDNP) in a U.S. patent in 1935. In 1954 Kenney (Remington) replaced lead compounds with a ferric styphnate and ferric hypophosphite double salt [Bydal, 1990].

In 1982 Hagel and Redecker patented a primer used for the manufacturing of a new ammunition, developed to minimize airborne lead levels and possibly other metallic residue such as barium and antimony [Hagel and Redecker, 1982]. SINTOX ammunition has a totally metal jacketed bullet and a new primer composition. Gunaratnam and Himberg [1994] reported the composition for a typical Sintox primer in the following table.

SINTOX primer composition reported by Guratnam and Himberg [1994]		
initiator	15%	diazodinitrophenol (DDNP)
sensitizer	3%	tetracene
fuel	5%	titanium metal powder 40 micron size
oxidizer	50%	zinc peroxide
secondary explosive	27%	nitrocellulose

Recently, different manufacturers introduced forms of lead-free ammunition: in 1994 Blount Incorporated of Lewiston (USA), Olin/Winchester in East Alton, Illinois (USA) and Federal Cartridge Company of Anoka, Minnesota (USA) [Haag, 1995].

Harris [1995] reported composition in the following table for the CCI lead-free cartridge produced by Blount Incorporated.

SINTOX primer composition reported by Harris [1995]	
initiator	diazodinitrophenol (DDNP)
sensitizer	tetracene
fuel	smokeless powder
oxidizer	strontium nitrate

Lichtenberg [1990] reports that the primer compounds of the CCI ammunition are manganese (IV) oxide, zinc peroxide, aluminium and the organic compound dinol (diazodinitrophenol).

In 1996, other manufacturers producing lead-free cartridges in Europe were Fiocchi (I), Fabrique Fédérale de Munitions (CH), Hirtenberg and Lapua. For Lichtenberg [1991] the primer of the Fiocchi ammunition contained commonly used compounds, only dinol replaced lead based compounds. Little work on lead free ammunition has been published so far: not all the products commercially available have been analyzed and results are sometimes contradictory [Herdener, 1996].

C. Propellants

C1. Black powder and smokeless powders

Propellants are explosive materials, solid or liquid, with a low rate of reaction. They burn smoothly at a uniform rate after ignition independently from interactions with the atmosphere. Ideally a propellant would be a single, solid, non-toxic chemical compound that is stable, easy to store, easy to ignite, compact, easy and safe to prepare. Furthermore, it should be smoke-free and without solid residue through combustion, i. e. it should be completely converted into gases and energy.

Originally, black powder was the only explosive known and was used both as initiator and propellant. Black powder is a mechanical mixture of charcoal, saltpetre (potassium nitrate) and sulphur in a typical proportion of 15/75/10. It is not very powerful, one gram produces 718.1 calories and 271.3 cc of permanent gas measured at STP. A simple calculation indicates that the explosion produces a temperature of about 2880 K. The combustion also produces 44% of its original weight as hot gases and 56% as solid residue [Wallace, 1990].

However, black powder is still currently used for specialized purpose in signal flares, blank rounds, safety fuzes and in many other ammunition components designed for large calibre guns.

Following the invention of nitrocellulose by Schonbein in 1846 attention was rapidly drawn to its explosive properties [Quinchon and Tranchant, 1987]. The work of Paul Vieille on the gelatinization of nitrocellulose by the solvent mixture ether-alcohol opened the way to the propellant which has exclusively been used for a long time in conventional military weapons: smokeless (or, more accurately, low-smoke) powder.

With reference to actual composition, it is possible to distinguish between single-base powders (e.g., nitrocellulose powder), double-base powders (e.g., nitrocellulose + nitroglycerine powder)

and triple-base powders (e.g., nitrocellulose + nitroglycerine or diglycol dinitrate + nitroguanidine powders).

The first shot of "powder B", the single-base propulsive powder produced with the method discovered by Vieille, was carried out using a 75 mm gun on the 23rd of December 1884. A few years later, in 1887, Alfred Nobel discovered the gelatinization of nitrocellulose by nitroglycerine and invented double-base powders [Quinchon and Tranchant, 1987].

Cellulose is a common polymeric compound in nature. Its molecular formula $(C_6H_{10}O_5)_n$ has been established for a long time. Around 1930, Haworth showed that a cellulose molecule is formed from glucose units in β-glucopyranose structure linked by acetal bonds between carbons 1 and 4. The number of these units, or degree of polymerization, is around 5000 and the resulting macromolecule is linear and rigid, because of numerous intramolecular hydrogen bonds. Cellulose is insoluble in water and difficult to hydrolyze. It reacts with a mixture of nitric and sulphuric acid producing nitrocellulose in a reversible esterification under thermodynamic control.

Cellulose contains three hydroxyl groups per unit and it is theoretically possible to obtain, for each glucose unit, the mononitrate derivative (6.76% nitrogen content), the dinitrate (11.12% nitrogen content) and the trinitrate (14.14% nitrogen content) derivative. The real nitrogen content results from the statistical distribution of the nitrogroups on the different glucose units.

The nitrogen content determines in part the energetic properties of the product, since the more attached nitrogen there is, the more oxygen is available to oxidize the reductive elements of the molecule.

Nitrocelluloses chosen for military use are the most highly nitrated, because of the high energy properties required. Two major types are distinguished for smokeless powder production, since their invention by Vieille, they are:

CP1 Cotton Powder insoluble in ether-alcohol mixtures, with a nitrogen content higher than 13.1% (up to 13.5%).

CP2 Cotton Powder soluble in ether-alcohol mixtures, with a nitrogen content generally between 12.3% and 12.8, more often between 12.6% and 12.8%.

The American nomenclature is:

Grade A "pyrocellulose" type I 12.60 +/- 0.10% N,

type II 12.60 +/- 0.15% N.

Grade B "guncotton" greater than 13.35% N.

Generally nitrocellulose for civilian use is solubilized in an organic solvent which, on evaporation, will deposit a film. Thus the nitrocelluloses chosen are those more soluble in common solvents (Azotic Cottons in French terminology, "pyroxylin", "collodion cotton" or "soluble guncotton" in American nomenclature). These nitrocelluloses, used for paints, varnishes, protective coatings etc., have a percentage of nitrogen of 12.5% [Dillon, 1991]. A case in particular is that of pyroxylin for dynamites or CA2, where an optimal number of hydroxyl groups is conserved in the molecule to give gelatinization by nitroglycerine. Nitroglycerine is used in double base powders as a high energy plasticizer that increases the performance of the propellant.

C2. Stabilizers

The main problem encountered when using nitrocellulose based products is the acid-catalyzed decomposition of the organic nitrates. This phenomenon influences both the ballistic properties of the powder and its safe storage. The strength of a powder decreases with its nitrogen content, resulting in poorer ballistic properties.

Stabilizers are generally defined as compounds that, when added in small amounts to other chemical compounds or mixtures, impart stability to the latter.

In propellant chemistry the stabilizers employed are compounds which, owing to their chemical structure, prevent the acid-catalyzed decomposition of nitrocellulose, nitroglycerine and similar nitric acid esters.

They exert their stabilizing effect by binding decomposition products, such as the free acids and nitrous gases; the stabilizers themselves are converted into relatively stable compounds at the same time. Neither stabilizers nor their secondary products are reactive with nitroglycerine or nitrocellulose [Stine, 1991].

The origin of using stabilizers in smokeless powders goes back to the invention of powder B by Paul Vieille, who first recommended amyl alcohol, but abandoned it after 1905 for diphenylamine, which was considered a better stabilizer.

Nowadays compounds used as stabilizers are mostly substitution products of urea and diphenylamine. Readily oxidizable compounds such as higher alcohols, camphor, hydrocarbons with double bonds (vaselines) may also be employed. For such compounds to be effective, their homogeneous incorporation into the powder must be easy, they must not be too volatile and must not leach out in the presence of water. Many stabilizers also display plasticizing (gelatinizing) properties; accordingly, they have both a stabilizing effect and, in the manufacture of powders, a softening effect.

Pure stabilizers include diphenylamine and akardite I (as diphenylurea). Stabilizers with a gelatinizing effect include: centralite I (diethyldiphenylurea), centralite II (dimethyldiphenylurea), centralite III (methylethyldiphenylurea), akardite II (methyldiphenylurea), akardite III (ethyldiphenylurea), ethylphenylurethane, methylphenylurethane and diphenylurethane [Meyer *et al.*, 2002].

C3. Other additives

Burning rate is of extreme importance: the propellant detonation can destroy the weapon causing injury to the shooter. It can be controlled by the size and geometrical design of the individual grains and by the use of surface coatings. Burning rate depends on the size and shape of the granules as well as the composition [House and Zack, 1977].

When gunpowder is burning in the chamber of a weapon, the internal ballistic energy of the powder charge will be exploited to the best advantage if the gas pressure is kept constant almost up to the emergence of the projectile from the barrel, despite the fact that the gas volume keeps growing during that period, owing to the movement of the projectile. It follows that gas evolution from the powder charge should be slow at first, while accelerating towards the end of the combustion process ("progressive burning").

The ballistic properties of a powder are affected not only by its chemical composition, but also by its shape. Thus, in conventional weapons, it ought to bring about progressive burning, or at least ensure that the surface area of the grain remains constant during combustion.

This is achieved mainly by imparting a suitable shape to the powder granule (in seven-hole powder, the surface area increases during combustion and the combustion is therefore progressive).

The following geometric forms of powder grains are manufactured:

- perforated long tubes,
- perforated tubes, cut short,
- multiperforated tubes,
- flakes,
- strips,
- ball powder,
- cubes,
- rods, cut short (macaroni),
- ring powder.

In the following pictures several powders from cartridges examined during the present research are shown.

Fig. 1. FEDERAL 9 mm Luger cartridge with BallistiClean® primer.

Fig. 2. FIOCCHI 9 mm Luger cartridge with “Leadless” primer.

Fig. 3. HIRTENBERG 9 mm Luger cartridge with “Schadstoff freie Anzundung”.

Fig. 4. RWS/GECO .38 Special cartridge with “No Lead - No Barium - Non erosive” primer.

Fig. 5. SINTOX 9 mm Luger cartridge with SINTOX® primer.

Fig. 6. SPEER LAWMAN 9 mm Luger cartridge with CLEAN FIRE® primer.

Fig. 7. WINCHESTER 9 mm Luger cartridge with SUPER-X® SUPER UNLEADED primer.

Compounds having a softening effect are incorporated into the powders to ease their manufacturing. Pure gelatinizers (plasticizers) without a stabilizing effect include: dibutylphthalate, diamylphthalate and camphor.

Finer-grained powders are used for small arms; tubular powder is mostly employed for guns, powders in the form of flakes and short tubes are employed for mortars, howitzers and other high-angle firearms.

Finer-grained powders can be improved in their ballistic behaviour by "surface treatment", i.e. by allowing phlegmatizing, that are combustion-retarding substances (such as centralite, dibutylphthalate, camphor, dinitrotoluene, etc.), to soak into the powder. When phlegmatizers are infiltrated in the outer layer of the powder grains, the burning rate in the weapon chamber begins slowly and turns progressive by keeping the maximum pressure peak of the combustion curve low.

A number of liquid nitric acid esters other than nitrocellulose have been recently used, including metriol trinitrate, butanetriol trinitrate, triglycol dinitrate and diglycol dinitrate, the latter being the most extensively employed. Such powders have the advantage of producing less heat. This fact is relevant to the service life of the gunbarrels in which these powders, known as "cold propellants", are utilized. The use of DNT to reduce the heat of explosion is common in small arms powders.

Further research for gunbarrel saving propellants led to the development of nitroguanidine powders, in which nitroguanidine is the third energy containing component, beside nitroglycerine (or diglycol dinitrate or triglycol dinitrate) and nitrocellulose. Nitroguanidine acts as muzzle flash suppressor too, producing nitrogen gas which dilutes the combustible muzzle gases. Triple base propellants are unlikely to be encountered in small arms ammunition [Meyer et al., 2002].

Propellants are also used in blank cartridges and other propellant-activated devices which have numerous uses, e. g. to drive turbines, to move pistons, to eject pilots from jet planes, to operate vanes in rockets, to start aircraft engines.

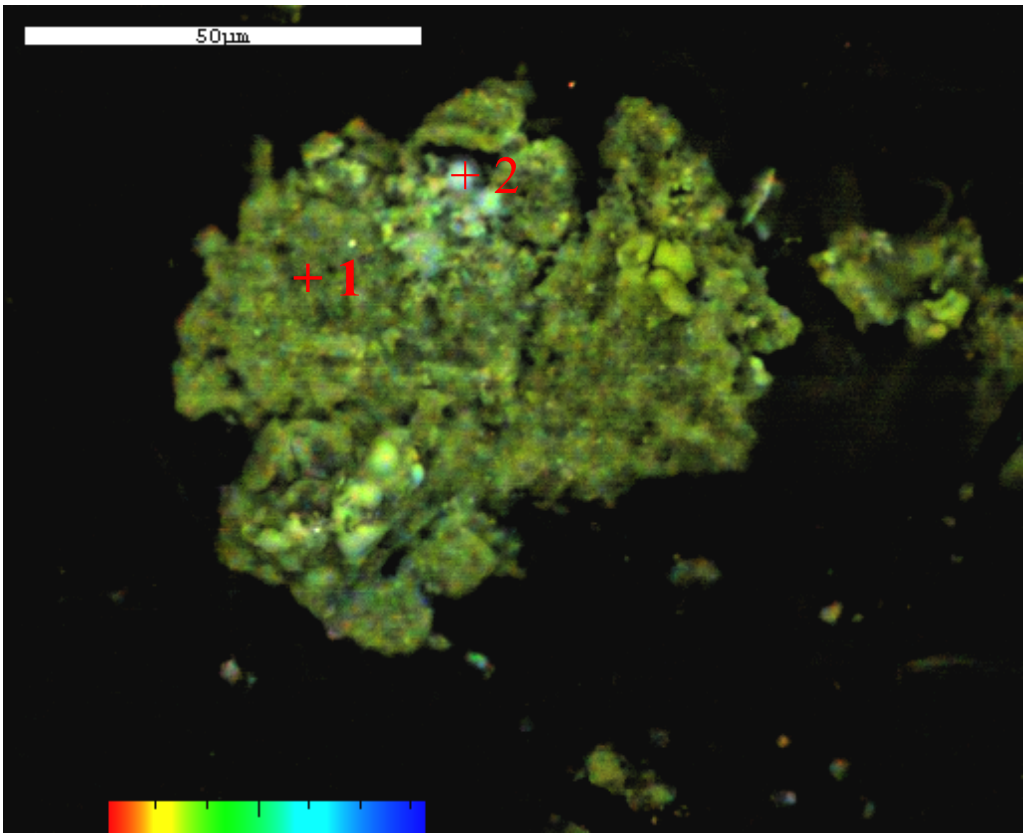
Annexe 3 SEM-EDX analysis of GSR

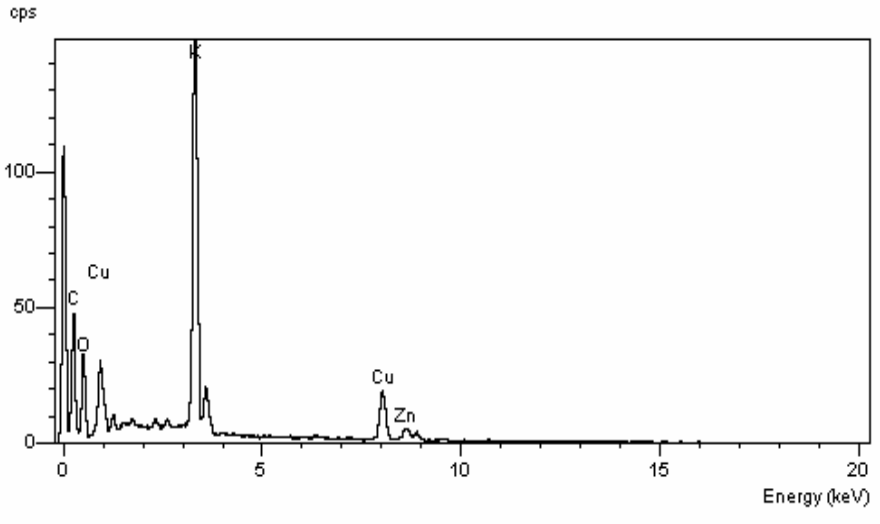
Experimental

There are a few papers about the analysis of gunshot residue (GSR) produced by non-toxic primed ammunition using Scanning Electron Microscopy (SEM). During the present research samples were taken from the inside of spent cartridge cases using a wooden stick and were fixed on an aluminium stub from BAL-TEC AG (Balzers, Austria) with conductive double-sided adhesive tape from Plano GmbH (Wetzlar, Germany). Analyses were conducted using a variable pressure (VP) SEM. Particles were located using the Backscattered Electron (BSE) images and then elemental composition were determined recording EDX spectra. One round of WINCHESTER Super X 9 mm Luger, 115 gr. Silvertip Hollow Point (cartridge A) was shot using a semiautomatic pistol SIG Sauer P226 9 mm Para. The same weapon was then used to shoot one round of WINCHESTER Super X, 9 mm Luger, 115 FMJ Encapsulated, primer Super Unleaded (cartridge B) and one round of GECO SX, 9 mm Luger 124 gr. (cartridge C). Samples were analyzed using a LEO 1430 VP SEM with a Link ISIS 300 detector equipped with GUNSHOT and CAMEO software (Oxford Instruments, High Wycombe, UK).

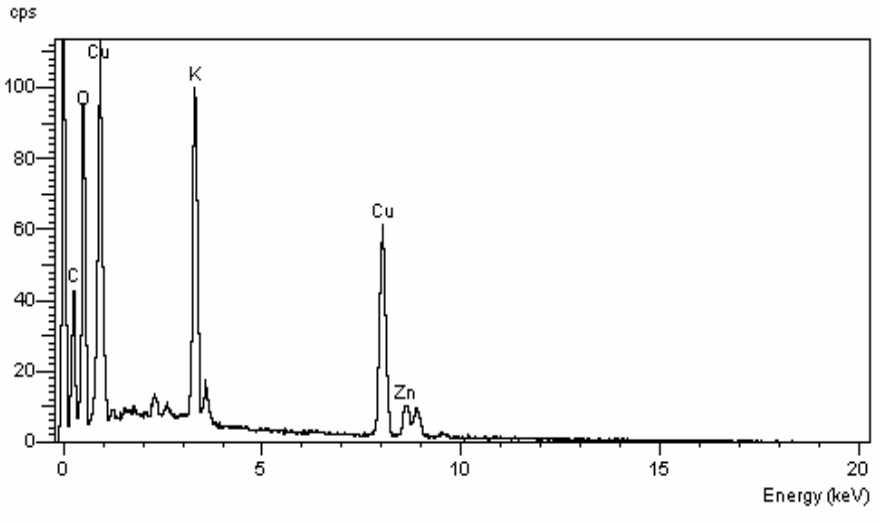
Results

The residue from cartridge A contained Pb, Ba and Sb. In the following picture is the residue from the spent case of cartridge B (WINCHESTER Super Unleaded). Spots 1 and 2 indicated by "+" are where EDX analysis were done. The EDX spectra in the following page show the presence of potassium (base peak in spectrum from spot 1). Copper and zinc are present too.



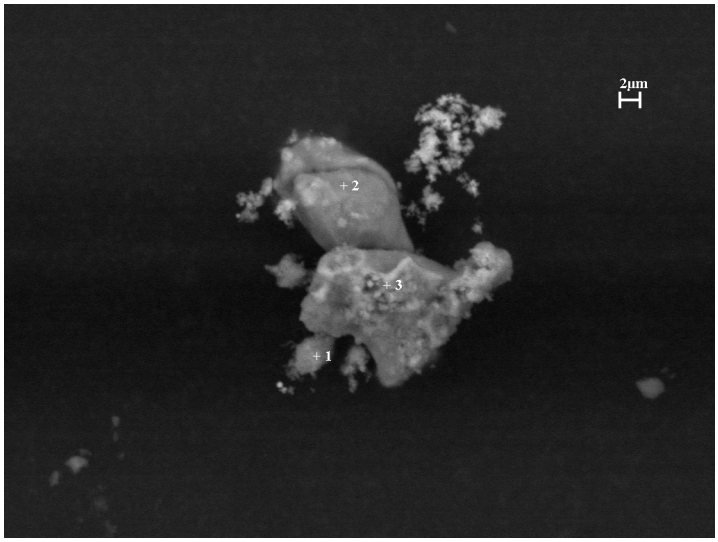


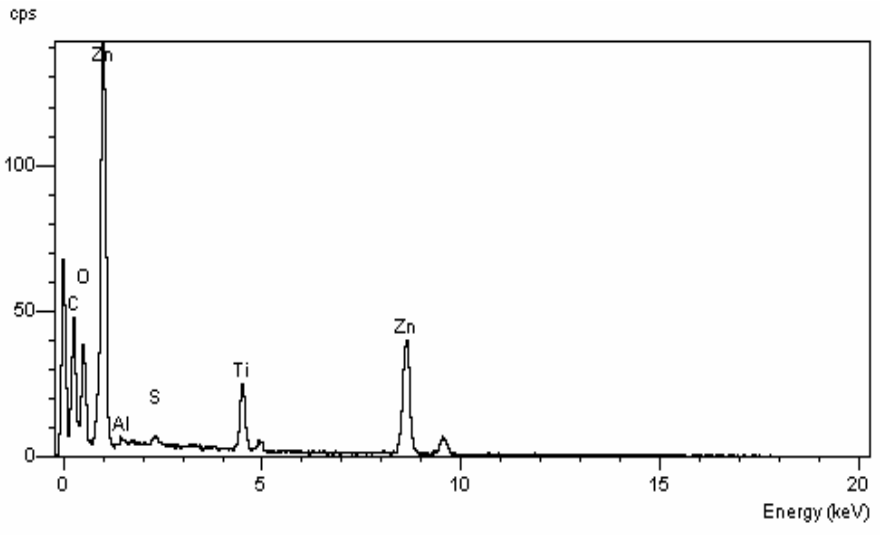
EDX spectrum from spot 1 of WINCHESTER Super Unleaded residue



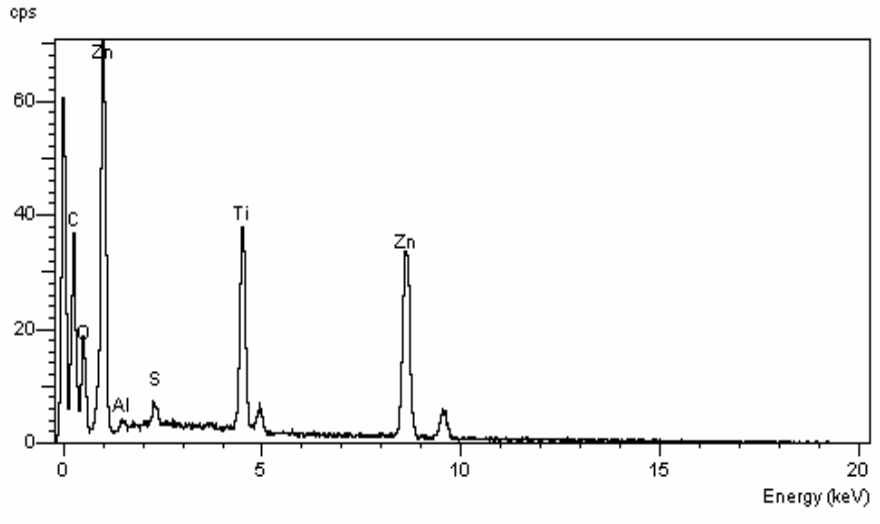
EDX spectrum from spot 2 of WINCHESTER Super Unleaded residue

The residue from the spent case of cartridge C is in the following picture (GECO SX). Spots 1 and 2 indicated by "+" are where EDX analysis were done. The EDX spectra in this and in the following page show the presence of zinc and titanium.

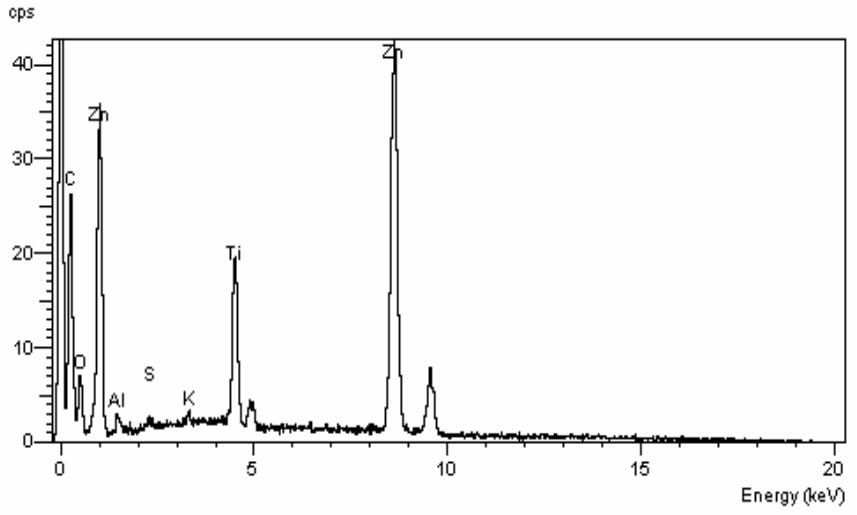




EDX spectrum from spot 1 of GECO SX residue



EDX spectrum from spot 2 of GECO SX residue



EDX spectrum from spot 3 of GECO SX residue

Annexe 4 X-rays diffraction (XRD) analyses of primers

Experimental

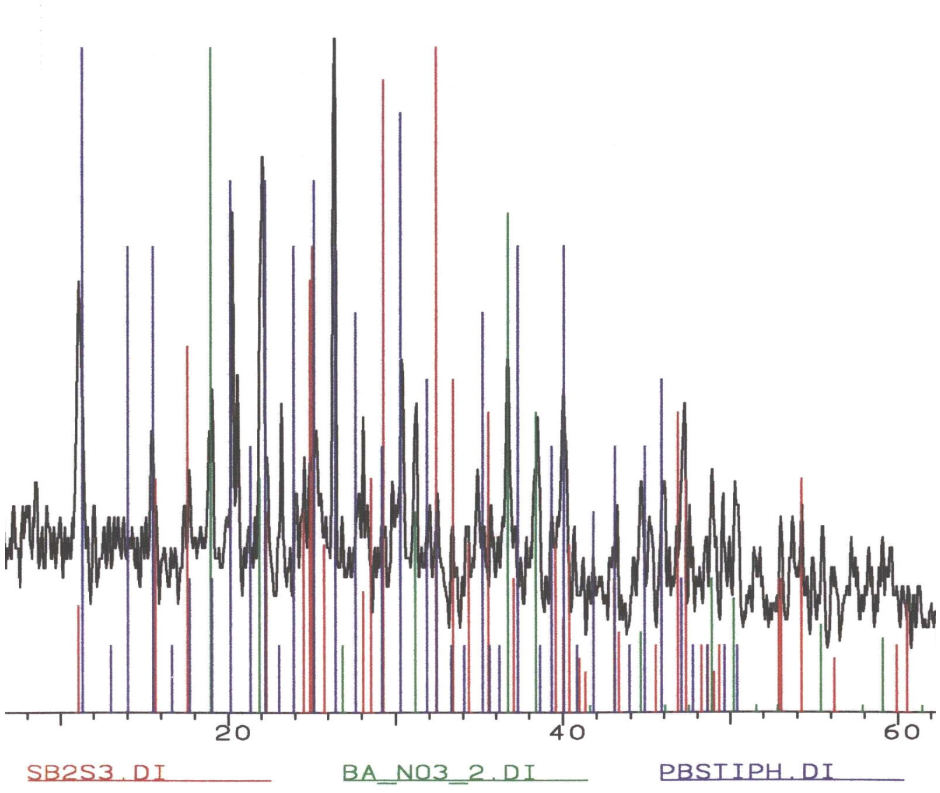
It was not possible to find articles about the analysis of bulk primers by X-ray diffraction. During the present research primer cups were taken from two cartridges, they were opened and primer mixtures were analyzed. X-ray diffraction patterns were obtained using an X-ray diffractometer PHILIPS PW 1800 with CuK_α radiation. The first cartridge was a WINCHESTER Super X 9 mm Luger, 115 gr. Silvertip Hollow Point (cartridge A) and contained a traditional primer; the second one was a SPEER LAWMAN 9 mm Luger, 124 gr. TMJ (cartridge D) and contained a CLEAN FIRE® primer. Analytical conditions were:

focus	LFF (Long Fine Focus)
generator tension [kV]	40
generator current [mA]	30
divergence slit	FINE
irradiated length [mm]	2
starting angle	5,01 [°2θ]
ending angle	79,99 [°2θ]

The diffraction patterns were compared with the ones in the Total Access Diffraction Database Based Upon 1989 PDF-2 PHILIPS using the software PLUS 39.

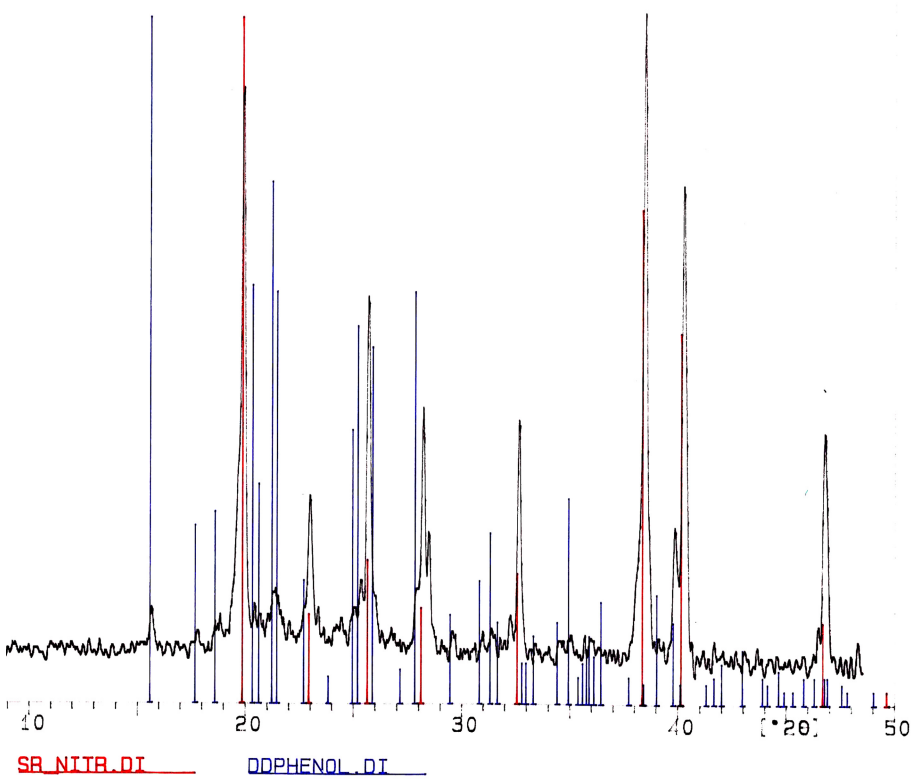
Results

The pattern obtained with the primer from cartridge A (WINCHESTER Super X 9 mm Luger, 115 gr. Silvertip Hollow Point) showed the presence of antimony sulphide (red lines), barium nitrate (green lines) and lead styphnate (blue lines). These compounds are contained in traditional primer mixes.



Diffraction pattern of the primer from the WINCHESTER Super X 9

The pattern obtained with the primer from cartridge D (SPEER LAWMAN 9 mm Luger, 124 gr. TMJ with CLEAN FIRE® primer) showed the presence of strontium nitrate (red lines) and diazodinitrophenol (blue lines). Strontium nitrate is an oxidiser and diazodinitrophenol is an initiating explosive. They are both used in lead-free primer mixes manufacturing.



Diffraction pattern of the CLEAN FIRE® primer from the SPEER LAWMAN TMJ

Annexe 5 Analysis of explosives

A. Introduction

The analysis of explosives is a very important area of forensic science, where analytical chemistry plays a central role. Forensic identification of explosives captured by police forces can play a central role in the investigations against terrorists and organised crime. The analysis of explosive traces is a relatively new area in forensic sciences: the first studies were published in the 1960s [Yinon et Zitrin, 1981]. It can be sub-divided into two topics: post-explosion residues and traces on suspects or on their belongings. General comprehensive schemes for the analysis of post-explosion residues where first described in 1970s. They can include the team approach for processing bomb-scene, visual examination of debris, sample preparation and analysis [Beveridge, 1992]. With the increasing use of explosives by terrorists, law enforcement agencies throughout the world are faced with the problem of detecting explosives and bombs in airline luggage, in mail, etc. and detection of hidden explosives is fundamental for security strategies.

The analysis of explosives is not only a field of interest for forensic science, but for different areas of analytical chemistry. Chemical analysis of explosives is necessary for manufacturing procedures and during storage, to check efficiency and stability of products. Also biological samples from personnel working in manufacturing plants are analyzed to detect and quantitate traces of explosives and their metabolic products. Most explosives are toxic and their production and disposal pose a serious contamination problem. Moreover some organic nitrates are used both as explosives and as drugs. Extraction procedures and analytical methods for the measurement of explosives and their degradation products in soil and water are becoming subjects of increasing interest in analytical chemistry too. The closure of former ammunition plants and military facilities requires accurate characterization of the extent of soil and groundwater contamination.

Yinon and Zitrin published the most important reference works in the field [1999, 1993 and 1981]. In this annexe the most important papers about trace analysis of explosives which can be used in organic GSR analysis are listed and briefly described. Sampling, extraction and concentration procedures are described in Chapter V.

B. Clean-up techniques

Clean-up techniques are fundamental to increase selectivity in procedures for explosive traces analysis with real samples. Post-explosion and organic gunshot residue samples often contain large amounts of contaminants. A sensitive analytical technique often fails in detecting small quantities of explosives in highly contaminated matrix at levels well above detection limit, especially when the detector is not specific. Also when using a specific technique, clean-up can be useful to minimize instrumental deterioration.

The easiest way of cleaning the extract of handswabs, containing traces of nitroglycerine, is probably using a silica TLC plate [Twibell *et al.*, 1982]. The swab extract was applied to the baseline of a silica gel plate over its full width prior to development by cyclohexane-toluene (70:30). A band of silica gel was scraped from the region on the plate centred on the established Rf value of nitroglycerine. The scraped silica was then extracted with acetone and the extract analyzed by TLC and GC. This procedure gave an order of magnitude improvement in detecting nitroglycerine.

Meng and Caddy [1994] used TLC to clean-up ethylcentralite extracts from hand swabs. They used toluene/ethyl acetate to develop the plate before scraping off and extracting ethylcentralite with ethyl acetate for its fluorometric determination.

Solid Phase Extraction (SPE) allows a complete clean-up procedure, removing both polar and non-polar contaminants with two different columns filled with bonded phase sorbents [Strobel and Tontarsky, 1983]. Solutions containing HMX, RDX, TNT, Tetryl and PETN in acetone/water (1:10) were placed on column, washed with acetonitrile/water (20:80) to remove polar contaminants. Explosives were then eluted with methylene chloride/hexane (1:1) directly on a second column, where non-polar contaminants were washed before eluting. Cyclohexyl bonded phase showed better recoveries and superior selectivity compared to C_{18} column to remove polar substances, ethyl and octyl phases are not efficient in retaining explosives from acetone/water

(1:10). The second column was a cyanopropyl; hexane was used for washing it and acetonitrile/water (70:30) to elute the explosives prior HPLC analysis. A diol column resulted not efficient in retaining TNT and PETN from methylene chloride/hexane (1:1). Recoveries were better than 95%, except for HMX.

Amberlite XAD-7 (poly(methylmethacrylate)) was introduced by Douse [1982] to remove interfering lipophilic material from extracts of cotton swabs with ether before GC analysis. After evaporation of ether, ml 3 of n-pentane were used to redissolve the extract and mg 10 of Amberlite XAD-7 porous polymer beads were added to the solution, stirring gently. The beads were subsequently rinsed with n-pentane and extracted with ethyl acetate. All the procedure was repeated a second time using fresh beads. In a following report [Douse, 1985] swab extracts in methyl tert-butyl ether were evaporated to μl 10, dissolved with ml 0.5 n-pentane and passed in an Amberlite XAD-7 column. Extracts were washed with n-pentane and eluted with two portion of ethyl acetate, resulting in a faster clean-up procedure with considerably less sample manipulation. Using methyl tert-butyl ether and n-pentane mixtures to elute from Amberlite XAD-7 columns, instead of ethyl acetate, higher recoveries could be obtained [Douse, 1987]. Amberlite porous polymer beads were also used to trap vapours of volatile explosives. A dynamic headspace sampling apparatus was used by Douse [1989] to clean-up organic gunshot residue prior to the detection of nitroglycerine by capillary column gas chromatography with thermal energy analysis detection. A Luer-lock glass syringe containing the swab from hands or a filter from clothing was connected to a glass column containing mg 18 of Amberlite XAD-7 resin. Nitrogen was passed through the system for 7 minutes at a rate of 200 ml/min, while the syringe was thermostated at 100°C. The elution of the resin started with n-pentane to elute nitrobenzene and 4-nitrotoluene, then with n-pentane/MTBE (1:1) to elute unwanted co-extractives and finally with ethyl acetate to elute nitroglycerine and other explosives.

The analytical technique is very important in determining the clean-up procedure. Lloyd conducted a series of studies in clean-up methods for high performance liquid chromatography with a pendent mercury drop electrode as detector. He first developed a microfilter extraction assembly containing a mixture of alumina, for polar contaminants, and octadecylsilica, for lipophilic contaminants [1983b]. Elution was performed in a centrifuge with methanol/water (100:35). The percentage of water in the elution mixture was important to avoid distortion of the chromatographic peak. Apart from the removal of lipids and lipophilic materials likely to degrade chromatographic performance, the adsorbent removed highly polar electroactive compounds, and trapped out volatile explosives components when the swabbing solvent was removed. The sensitivity limits were on the order of 1-10 ng per used swab, which were subject to variations due to the origin of the swab and the identity of the compounds of interest. In 1985 a test has been published [Lloyd, 1985a] on a wide range of adsorbents to recover traces of explosives from extracts of debris or swabs. Distribution coefficients for the adsorption on ten representative adsorbents of sixteen explosives-related compounds in solution were summarized. Polar selectivity was required for explosives and suitable adsorbents were Amberlite XAD-7 (poly(methylmethacrylate)), Porapak T (poly(ethylene glycol dimethylacrylate)) and Chromosorb 104 (acrylonitrile-divinylbenzene copolymer), in which polar selectivity persisted even in relatively polar solvents. Two kinds of microcolumns were then developed [Lloyd, 1985b]. The first one was a stainless-steel tubing packed with Porapak T. The sample in aqueous solvent was washed with aqueous methanol and the elution started with isopentane to elute nitrobenzene and 4-nitrotoluene and lipophilic materials, followed by diethyl ether, to elute the most of explosives except HMX and tetryl, and acetonitrile to complete desorption. For heavy contaminated sample was used a disposable column with Porapak T, Chromosorb 104 and charcoal with a similar sequence of solvents. When the only explosives of interest were nitrate esters the preferred adsorbent was charcoal. Lloyd [1986a] used charcoal microcolumns (30 mm x 0.6 mm) to clean-up nitroglycerine, 1,2-glyceril dinitrate and 1, 3-glyceril dinitrate in methanol/aqueous phosphate buffer using 50 ml methanol to elute them before HPLC

analysis. The adsorption and exclusion characteristics of nitrocellulose on 12 representative supports were studied under conditions relevant to the trace analysis of nitrocellulose by size-exclusion chromatography with amperometric detection at a mercury cathode [Lloyd, 1984]. It was found, in particular, that in acetonitrile nitrocellulose was largely excluded from Porapak T, whereas it was adsorbed onto this support from mixtures of acetonitrile with diethyl ether. This provided the basis of a microcolumn clean-up technique that enabled nanogram amounts of nitrocellulose to be characterized. The acetonitrile handswab extract was diluted with a 1.5-volume ratio of diethyl ether and eluted from the Porapak T column with acetonitrile. Chromosorb 104 was used for solid phase extraction of swabs, using the same container in which the swabs had been collected to minimize losses of materials, contamination or mislabelling [Lloyd et King, 1990]. Chromosorb 104 was than transferred in a column filled with Amberlite XAD-4 (styrene-divinylbenzene copolymer), which were found better than Porapak T, and elution performed with acetonitrile/water (25:12).

Kolla [1991] used commercial SPE columns 500-mg RP-18 to clean-up acetone extracts. The original samples, whether debris from a bombing scene or swabs, were extracted in a soxhlet extractor with acetone for at least 2 hours. The samples were concentrated on a water bath at 50°C and diluted 1:10 with water. Methanol/water (1:4) was used for washing and elution was performed with ethanol. The methanol was evaporated and the sample were dissolved in acetone for GC analysis or acetonitrile for HPLC. Recoveries were 72% for RDX, 82% for TNT and 94% for PETN.

The Northern Ireland Forensic Science Laboratory (now Forensic Science Agency of Northern Ireland) initially used the technique developed by Lloyd and King [1990] but it was found to be time consuming and the extraction system was adapted and optimized to be suitable in laboratory with a heavy caseload. An SPE tube was used, packed with mg 10 of Amberlite XAD-4 and mg 30 of Chromosorb 104 [Speers *et al.*, 1994]. The organic extracts from swabs or filters were diluted 1:9

with water, washed with acetonitrile/water (1:10) and eluted with acetonitrile. It was demonstrated that the recovery of residues from the Chromosorb 104 - Amberlite XAD-4 SPE column prepared in the laboratory was more efficient (>95%) compared to the commercial ODS (32-47%) and aminopropyl SPE columns (2-9%).

Amberlite-15, a macroreticular, strongly acidic, cation-exchange resin was used for the selective recovery of diphenylamine from handswabs or clothing [Lloyd, 1987].

Later Waters introduced Porapak RDX Sep-Pak®, the first solid phase extraction column specifically designed for the analysis of explosives in water. This column contained a divinylbenzene/vinylpyrrolidone copolymer and meet the QA/QC requirements of EPA method 8330. Recoveries for the 14 compounds of EPA method 8330 were greater than 89% [Bouvier and Oehrle, 1995].

C. Chromatographic analysis

Thin layer chromatography (TLC) is one of the simplest and most widely used chromatographic techniques and it is extensively used in explosive analysis [Yinon and Zitrin, 1981; Yinon and Zitrin, 1993]. TLC is rapid, inexpensive, it can be performed outside a laboratory in open air with a simple equipment and permits the analysis of different samples in the same time using the same plate. It has been used by different workers to separate and identify minor components present in smokeless powder but only in a few recent papers thin layer chromatography was used for gunshot residue detection because it is only moderately sensitive and offers poor quantification [Meng and Caddy, 1997]. Peak [1980] reported a limit of detection of 10 ng for nitroglycerine and 500 ng for nitrocellulose using the nitrite-specific Saltzman-Greiss reagent as visualizing agent [Saltzman *et al.*, 1969]. Diphenylamine and diphenylbenzidine reagents are slightly more sensitive but their reaction with any oxidizing agent and the brown char produced by the acid on co-extracted material render them of little use for hand swab extracts [Twibell *et al.*, 1982]. The same reactions used for developing thin layer chromatography plates are widely used as **chemical tests** in bulk explosive analysis for preliminary results [Yinon and Zitrin, 1993; Yinon and Zitrin, 1981] and in shooting range determination [Bonfanti, 1995; Sellier, 1991; Dillon, 1990; Lichtenberg, 1990]. The earliest techniques used in gunshot residue detection was **"**dermal nitrate" or "paraffin test". Detection was based on a colour reaction produced by a 0.25% solution of diphenylbenzidine in concentrated sulphuric acid.

Gas chromatography (GC) is a well established technique in analytical organic chemistry. Capillary columns allow the separation of highly complex mixtures due to their high efficiency and different detectors have been developed to be used with GC. Their high selectivity and sensitivity contributed to the extensive use of GC, often as the method of choice for thermostable substances with a suitable boiling point.

The compatibility of GC with the analysis of explosives is not self-evident. The relatively high temperatures employed during the GC analysis could lead to decomposition of thermally labile compounds, especially nitrate esters and nitramines. Nitroglycerine has a deflagration temperature of 200 °C [Urbanski, 1964] but the initiation temperatures of single smokeless powder flakes range from 165° C and 193° C and the combustion temperatures range from 184°C and 198°C [DeHaan, 1975]. Kolla showed that the response of a GC for nitroglycerine decreased raising the injector temperature from 80° C. The optimum temperature for explosive analysis including RDX and PETN was 170° C [1994].

The **Flame Ionization Detector** (FID) has been the standard detector in GC for many years. It has been widely used in many areas of organic analysis, especially the analysis of hydrocarbons and other molecules with a high proportion of carbon atoms. The sensitivity of the FID to the different molecules increases with increase in the C/O or C/N elemental ratio in the molecule. The use of FID in GC analysis of explosives has been limited, mainly owing to the relatively low C/O and C/N elemental ratio in nitro-containing explosives. Even in the early use of GC in the analysis of explosives FID gave good results only with explosives having a relatively large number of carbon atoms, such as nitroaromatic compounds. In the analysis of propellants FID has been more useful with additives than with nitrate esters.

The study of the mechanism of aging and expected useful life of smokeless powders involves the measurement of the various propellant stabilizers. The methods normally used in 1970 for analyzing aged propellant samples for residual stabilizers and other components were a combination of infrared and thin-layer chromatography. A new gas chromatography method for the analysis of propellant components was investigated by Trowell [1970]. Solutions of the propellant in ether or methylene chloride were analyzed by GC-FID with a packed column (T_{inj}=70°C, column programmed from 70 to 230°C at 10°C/min). In these conditions mononitroglycerine,

dinitroglycerine and some additives, included nitrated diphenylamines, were detected, but nitroglycerine eluted too late and sensitivity was poor.

A novel nitric oxide selective pyrolysis-chemiluminescence detector called **Thermal Energy Analyzer** (TEA) was introduced in 1975 [Fine *et al.*] for the trace analysis of volatile and non-volatile N-nitroso compounds and could be used both with GC and HPLC. Compounds eluted from the column, passed in a pyrolizer where nitric oxide and other pyrolisis products were formed. They passed into a cold trap where pyrolisis products were trapped while nitric oxide went to a reaction chamber maintained at a reduced pressure, where it was oxidized by ozone to nitrogen dioxide in excited state. This species decayed to its ground state emitting a distinctive and broad near-infrared chemiluminescent radiation centred around 1200 nm [Yan, 1999]. The light intensity was proportional to nitric oxide concentration, and hence to the nitro compound concentration. Thermal Energy Analyzer was used for the trace level identification and determination of explosives in 1981 [Lafleur and Mills]. They analyzed nitrotoluenes, five dinitrotoluenes and 2,4,6-trinitrotoluene. Pyrolyzer temperatures above 800 °C were required to produce optimum yields of nitric oxide from the compounds studied (500°C was the optimal pyrolizer temperature for nitrate esters and nitramines). Analysis were performed with a packed column (T_{inj}=240°C, column programmed from 100 to 240°C at 8°C/min). The GC-TEA method gave linear response over 4 orders of magnitude and a precision of 11% or better at the picomole level.

To improve the limit of detection (LOD) of explosives a capillary column was used [Douse, 1982]. The column passed through a heated transfer line into the hot region of the pyrolizer. This was important to avoid losses due to adsorption of polar explosives. The injector was in splitless mode. The capillary column was a 25 m x 0.3 mm Internal Diameter (ID) OV-101 (T_{inj}=200°C, column programmed from 40°C, where stayed 1 min, to 240°C at 39.9°C/min). Obtained limits of detection were compared with Electron Capture Detector (ECD) performances. For NG was pg 15 with GC-TEA and pg 5 with GC-ECD. For TNT was pg 10 both with GC-TEA and GC-ECD. For RDX

about pg 200 with GC-TEA, 10 with GC-ECD. TEA detector was much more selective than ECD detector, resulting in higher sensitivity in real samples detection. Detection limit for nitroglycerine in hand swab extracts analyzed by GC-ECD corresponded to 5 ng because of the greatly increased background [Twibell *et al.*, 1982].

Thermal energy analysis (TEA) was compared with **Electron-Capture Detection** (ECD) for the analysis of explosives both as pure compounds and in spiked hand-swabs by silica capillary column gas chromatography by Douse [1985]. GC-ECD had been used by Jane *et al.* to detect organic gunshot residue [1983]. Douse analyzed NG, TNT, RDX and 2.4-DNT with substances found in smokeless powder (2-NO_2-DPA, 4-NO_2-DPA, 2.4-diNO_2-DPA) and nitro-compounds suspected to cause false positive results (musk xylol, musk moskene) using a GC with the injector in splitless mode. The capillary column was a 12 m x 0.25 mm ID BP-1 (T_{inj}=175°C, column programmed from 60°C, where stayed 1 min, to 260°C at 39.9°C/min, where stayed 2 minutes). TEA conditions were He flow=8.8 ml/min, T_{pyr}=700°C. A cleaned liner was used every day. TEA was shown to approach the sensitivity of ECD but was more selective, enabling low nanogram levels of explosives in handswabs to be detected.

Douse presented two years later a preliminary paper about an improved method for the trace analysis of explosives by capillary column GC-TEA [1987]. Modifications to the TEA detector which eliminated peak broadening and improved selectivity were described. The ceramic nitroso-specific pyrolisis tube was replaced by a silica capillary column tubing. This modification did not improve the peak shape or the sensitivity but capillary silica tubing had low price, long lifetime and were easy to replace. Electronic modifications were described to improve peak shape and sensitivity. Under optimum conditions 5 pg of NG, TNT and RDX were readily detectable.

Kolla [1991] reported similar modifications to improve GC-TEA performance in trace analysis of explosives from complex mixtures (debris or handswabs). The GC separation was carried on a 15 m x 0.53 mm DB-5 and later on a 8 m x 0.25 mm DB-5 capillary column. The phenyl groups in

polymethyl-phenyl(5%)siloxane (DB-5) enhanced the selectivity of the column for the nitro compounds, with pure polymethylsiloxane (OV-1) the separation of PETN and RDX was not possible. The temperature of the injector was 170°C. The pyrolizer was held at 900°C. The same author suggested later that column DB-5 shouldn't be longer than 10 m, operating from 50° to 250° C, 10°C/min, and columns of higher polarity should be shorter [Kolla, 1994].

High Performance Liquid Chromatography (HPLC) is the method of choice for thermally labile and high-boiling-point compounds. The United States Environmental Protection Agency (EPA) specified method 8330 for the trace analysis of explosive residues in water, soil, or sediment matrixes. Following sonication, extraction with acetonitrile, and preconcentration, analysis for 14 species is performed using high-performance liquid chromatography (HPLC) and **UV detection**.

Isocratic HPLC separations using commercially available C_{18} columns typically take over 30 min and are unable to separate two of the three dinitrotoluene isomers. To fully identify each of the 14 compounds, an additional HPLC run must be performed using a cyano column, leading to an increase in analysis time and sample handling complexity [EPA, 1992].

Diphenylamine and its nitrated derivatives can be used to characterize smokeless gunpowder. The various nitrated congeners of diphenylamine reflect not only the production of the gunpowder, but also its storage career and thermal history following manufacture. Espinoza and Thornton used HPLC with UV detection at 254 nm [1994]. The column was a Phenomenex Ultramex 5C18 (25 cm x 4.6 mm i.d.) reversed-phase C_{18} column. Separation were obtained isocratically with methanol/water/triethylamine (74:25:1).

Bouvier and Oehrle [1995] were able to identify all of the method 8330 components in less than 30 min but were unable to achieve baseline resolution. They used a C-8 stationary phase with a water-isopropanol (82:18 v/v) mobile phase. The composition of mobile phase and the temperature were optimized. Photodiode-array detection provided peak identification and confirmation. Porapak RDX Sep-Pak® SPE cartridges permitted to detect less than 0.125 µg/l of all analytes with a recovery

better than 89%. Unfortunately UV detection was too little sensitive for nitrate esters and nitramine and too little selective for dirty samples. Analyzing extracts of debris from bombing scenes or from handswabs, UV traces (210 nm) showed the elution of many matrix components.

Harvey and Clauss [1996] described an on-line trace enrichment system that combined a divinylbenzene-vinylpyrrolidone co-polymer precolumn with a reversed-phase C_{18} HPLC analytical column. This arrangement allowed quantitative preconcentration of TNT and RDX from water on the resin sorbent, followed by complete transfer of analyte to the analytical column for separation and UV absorbance detection at 254 nm. A detection limit down to 20 pg/ml was demonstrated for TNT by preconcentrating 50 ml of sample. To the knowledge of the authors it was the lowest limit of detection that has been demonstrated for the analysis of TNT by HPLC. Further reductions in detection limits could proceed by more selective detection technique, due to the increasing prominence of matrix interferences with larger sample volume.

Fine *et al.* [1984] showed that **TEA**, interfaced to both a gas and a high performance liquid chromatograph, could successfully perform analyses of "real world" explosives, post-explosion debris, handswabs, and human plasma. Because of the selectivity of the technique, there was no need for sample clean-up before analysis. The method used was specific to nitro-based explosives at a sensitivity of 4 to 5 pg injected on-column.

In 1991 Kolla used HPLC to confirm GC-TEA results if the concentration was high enough. The column was a Lichrospher RP-18 (125 x 4.6 mm). The mobile phase was methanol/water (47:53), flow 1 ml/min, UV detection at 210 nm. He considered that the best choice was HPLC with more selective and sensitive detection such as photolysis-electrochemical detection [Selavka and Krull, 1986]. Engelhardt, Meister and Kolla [1993] photolytically decomposed the compounds coming from the column to nitrite, which could be detected selectively by the Griess reaction and monitored at a wavelength where interference of other organic solutes was less likely. The detection limits for esters and nitramines after post-column reaction were around 100 pg, instead of 100 μg without

photolysis. For separation they used RP-8 and RP-18 columns with methanol/water (50:50 w/w) or acetonitrile/water (50:50 w/w) mixtures. One year later Kolla [1994] investigated the potential of gas chromatography, high-performance liquid chromatography and ion chromatography for application to the determination of traces of explosives. In gas chromatography a chemiluminescence detector (TEA) showed the best performance but for high-performance liquid chromatography postcolumn derivatization was preferred. HPLC-TEA was used for analysis of smokeless powder [Bender, 1983] but its performance was poorer than GC-TEA. The limit of detection for nitroglycerine using HPLC-TEA was 500 pg, compared with 15 pg using GC-TEA [Douse, 1983].

Bratin *et al.* [1981] used reductive and oxidative **electrochemical detection** with liquid chromatography to determine nitro aromatics, nitrate esters, nitramines and diphenylamine in military explosives and single and double base smokeless powders. They developed a sensitive and highly selective method for the detection of organic gunshot residue on the hands of individuals who had discharged a weapon. The mechanism of reduction of explosives was studied and limits of detection (s/n=3) in reductive mode were 170 pg for RDX, 140 pg for TNT, 380 pg for NG, 160 pg for DNT, 400 pg for PETN. Diphenylamine, 2-nitrodiphenylamine and 4-nitrodiphenylamine were analyzed in oxidative mode, resulting in more complex chromatograms due to interference from phenolic compounds present in the lipid layer of the skin. DPA limit of detection in oxidative mode was 39 pg. The HPLC column for DPA was a 125 x 4.9 mm i.d. packed with Spherisorb and mobile phase was methanol/water (65:35) [Jane *et al.*, 1983].

In 1983 Lloyd introduced the **Pendant Mercury Drop Electrode** (PMDE), found to be the best solution for explosive analysis, in conjunction with a 3-µm particle size HPLC column packing ODS-Hypersil [Lloyd, 1983a]. Glassy carbon electrodes could not withstand the high voltage necessary for the analysis of explosives (-1,0 V vs. Ag/AgCl). Detection limits reported for a mercury film electrode (MFE) technique were approximately ten-fold higher than with PMDE and

MFE was found to be subject to contamination problems. PMDE could be renewed during or at the start of a chromatogram, results were highly reproducible and detection limits for fourteen nitrate and nitro compounds were lower than 50 pg after injecting 20 µl of sample, the linear range extending in excess of four orders of magnitude.

The HPLC-PMDE technique was used in a procedure to analyze extracts from hand-swabs [Lloyd, 1983b], permitting detection limits after clean-up on the order of 1-10 ng per swab used. The column used was a 150 x 4.5 mm ODS-Hypersil (3 micron particle size) with 10 µl loop, the mobile phase was deoxygenated aqueous phosphate (0.035M, pH=3)/methanol (86:100 v/v) with a flow rate of 1.0 ml/min. Usually the injection volume was 20 µl, but slightly higher resolution was obtained with 10 µl volume. In the same condition Lloyd studied the presence of previously-undetected compounds in samples collected from skin that had been in contact with materials containing nitroglycerine. The compounds identified were 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate. These are impurities of glyceryl trinitrate, but a significant amount of them was found to be produced on the skin surface. Chromatograms obtained from the samples are highly characteristic and, depending on the time since contact, could exhibit dinitrate responses comparable to the response due to any remaining glyceryl trinitrate [1986b].

In 1984 PMDE detector was used to detect nitrocellulose traces after **size-exclusion chromatography** [Lloyd]. Acetonitrile was found better than THF or ethyl acetate, common eluent in size-exclusion chromatography, because of its ion-solvating characteristics. The effect of variation of response to NC and NG with working electrode potential and eluent flow rate was studied: NC could be detected in amounts as small as 100 pg, with a linear range extending to approximately 200 ng. Samples of 21 common plastic materials were processed: the only substance likely to be confused with nitrocellulose is nitrostarch, which occur as a rare explosive component. A single and a double-based propellant, a semigelatinous explosive, ammon-gelignite, two NC-based spray paints and three celluloid-type materials (an adhesive, a fingernail lacquer and a wood

filler) were analyzed. In 1986 a complete procedure to detect nitrocellulose from gunshot in hand-swabs using HPLC-PDME was presented [Lloyd, 1986c].

To confirm HPLC-PMDE results the use of GC-TEA after trapping of HPLC peaks was suggested [Lloyd, 1991]. A four-port sampling valve between the HPLC column and the detector was inserted. Selected peaks could be trapped on microcolumns packed with either Chromosorb-104 or Porapak-T. The eluate of the microcolumn was later analyzed using GC-TEA for confirmation. Dahl and Lott used HPLC with oxidative electrochemical detection to analyze diphenylamine, ethylcentralite and 2-nitro diphenylamine [1987]. The same authors compared organic and inorganic analysis in test firings using 20 handguns and 20 different types of ammunition of various calibre. Stabilizers were detected in 19 out of 20 tests, AAS analysis using graphite furnace gave positive identification in 14 out of 20 residues [Dahl *et al.*, 1987]. The Metropolitan Police Forensic Science Laboratory of London compared GC-ECD, GC-TEA and HPLC with hanging mercury dropping electrode. The HPLC column was 15 cm x 4.6 mm ID Hypersil ODS 3 μm, used with a 1 cm column guard. The mobile phase was phosphate buffer (0.025M pH=3)/methanol (65 :100). Limits of detection (S/N=3) for nitroglycerine were: GC-ECD=9pg, GC-TEA=9pg, HPLC(10 μl injected)=19 pg [Corless, 1993]. The Forensic Science Service Laboratory of Birmingham designed a combined swabbing kit to recover explosives and gunshot residue from skin surfaces and hair, while clothing were examined by vacuum filtration. Samples were screened-up for explosives by HPLC-PMDE and the peaks were trapped and confirmed by GC-TEA. The LOD of the combined techniques corresponded to around 1 nanogram of explosive on a hand swab. If necessary the inorganic components of GSR were detected by SEM-EDX [King, 1995]. A problem with analysis of organic explosive residues by high-performance liquid chromatography with a pendant mercury drop electrode detector was the need of manually deoxygenate samples before injection. McKeown and Speers [1996] introduced an automated system based on a programmable autoinjector and a universal switching valve with on-line nitrogen. In 1996 HPLC with electrochemical detection in the oxidative and reductive mode was applied to the analysis of nitroaromatics, nitramines,

aminoaromatics and nitrophenols in groundwater samples from the surroundings of a former ammunition plant [Lewin *et al.*, 1996]. Electrochemical detection allowed selective and sensitive detection of 22 substances with either amino or phenol groups in oxidative mode with a glassy carbon electrode (detection limits between 60 and 500 pg). For sixteen nitroaromatics and nitramines the analysis in reductive mode suffered of high background current, in spite of good degassing of sample and mobile phase and the use of stainless-steel mobile-phase transfer lines. The employment of a gold and a mercury film (Au/Hg) electrode did not improve the sensitivity of the detection.

Another sensitive and selective mode of detection used in HPLC is **fluorimetry**. Ethylcentralite is similar to urea herbicides and several papers described detection of this class of compounds. The phenylurea herbicides can give rapid catalytic hydrolysis on silica gel at elevated temperatures. The anilines produced can be analyzed after derivatization with heptafluorobutyrric acid anhydride. Analysis done on a gas chromatograph equipped with an electron-capture detector permitted detection limits in the 1-5 picogram range [de Kok *et al*., 1984; de Kok *et al.*, 1981]. Methoxuron and its breakdown product (3-chloro-4-methoxyaniline) were analyzed by thin-layer and high-performance liquid chromatography with fluorescence detection after derivatization with dansyl chloride to yield fluorescent derivatives [Lantos *et al.,* 1984]. The various procedures involved extraction, clean-up, catalytic hydrolysis of herbicides to anilines, liquid or gas chromatographic fractionation and/or separation, chemical derivatization and detection. Meng and Caddy developed a fluorescence method to detect EC in gunshot residue [1994]. Shooters hands were sampled with cotton wool swabs. The swabs were extracted by a syringe elution procedure and the extracts cleaned by TLC. The cleaned samples were hydrolyzed to yield N-ethylaniline, which was dansylated directly on a TLC plate. After two-dimensional developing, the fluorescent dansyl-N-ethylaniline spot was scraped off and extracted for fluorimetric determination. Recording the fluorescence spectra from 420 to 620 nm permitted a LOD of 1 ng of standard solution and 5 ng of spiked samples [Meng and Caddy, 1995]. High-performance liquid chromatography analysis with

fluorescence detection of ethyl centralite and 2,4-dinitrotoluene in gunshot residue after derivatization with 9-fluorenylmethylchloroformate was used later [Meng and Caddy, 1996]. 2,4-dinitrotoluene was reduced with $Fe(NH_4)_2$ $(SO_4)_2$ at 155°C for 20 minutes before derivatization. The analytical column was a home-made ODS column, 125 x 4.9 mm i.d., packed with 5 μm particles. Samples were separated at a flow rate of 0.9 ml/min using an isocratic eluent composed of citrate buffer (pH was adjusted with ammonia solution to 6.6)/acetonitrile (22:78 v/v). Excitation and emission wavelength were 266 nm and 302 nm. The limits of detection were 200 pg for ethyl centralite and 1 ng for 2,4-DNT.

A relatively new analytical method is HPLC-NMR (LC probe heads have been commercially available since the early 1990s). Its first application to environmental problem was published in 1997 [Godejohann *et al.*]. Water samples from an ammunition hazardous waste site were analyzed using the continuous flow mode at very low flow rates. At a flow rate of 0.006 ml/min, it was possible to detect less than 29 nmol (5 μg) of 1,3-dinitrobenzene injected on a 75 mm x 4 mm reversed phase C_{18} column (particle size, 5 μm).

Another technique suitable for analysis of thermally labile or non-volatile explosive is **supercritical fluid chromatography** (SFC). Supercritical fluids are gases under pressure and heated above the critical temperature, resulting in liquid-like densities and gas-like diffusivities and viscosities. The use of supercritical fluids (CO_2, N_2O, NH_3 or SF_6) as mobile phases in supercritical fluid chromatography resulted in higher separation efficiency than in HPLC. Douse analyzed traces of explosives using a 6.8 m x 0.05 mm i.d. SB Octyl Superbond cross-linked flexible silica capillary column [1988]. Thermal energy analyzer permitted minimum detectable levels of 23 pg for nitroglycerine, 40 pg for pentrite and 60 pg for tetryl [Douse, 1988]. Munder [1990] used a bonded methylpolysiloxane capillary column (SB methyl-100, 5 m x 100 μm i.d.) and a bonded 50% cyanopropyl phenylpolysiloxane capillary column (DB 225, 10 m x 50 μm) while Griest *et al.* used a 250 mm x 1 mm i.d. Deltabond Cyano column packed with 5-μm particles [1989]. Virtually all

HPLC and GC detectors can be used with SFC. Fourier transform infrared spectrometry, UV absorption, flame ionization detector (FID) and electron capture detector (ECD) [Ashrof-Khorassni and Taylor, 1989] were used. The last three detectors were all connected on-line to achieve greater selectivity and sensitivity. Solvating gas chromatography was used by Bowerbank *et al.* to analyze nitroglycerine and other explosives with chemiluminescence detection [2000].

D. Mass spectrometric analysis

In 1977 high performance liquid chromatography (HPLC) and chemical ionization (CI) **mass spectrometry** (MS) was applied to the isolation and identification of explosives [Vouros *et al.*]. Fractions of the eluent were collected at the outlet of the HPLC column, evaporated and transferred to the probe tip of the mass spectrometer. The use of ammonia as reagent gas for chemical ionization of NG, TNT, RDX and 1,3,5,7,-tetranitro-1,3,4,7,-tetraazacyclooctane (HMX) was evaluated and its advantages over methane, water, hydrogen, and isobutane discussed. **For the first time NG molecular ion was detected**, together with the characteristic peaks of mass spectrum of nitroglycerine (M+H=228, 228-HNO_3=165, CH_2ONO_2=76, NO_2=46). The column was a 25 cm x 3.1 mm i.d. packed with silica (Partisil). NG and TNT were separated with a mobile phase of 2% (by volume) isopropanol in heptane, but NG eluted unretained.

GC with a Mass Spectrometry (MS) detector was used to detect and identify organic GSR [Mach *et al.*, 1978]. A revolver was loaded with cartridges containing nitroglycerine, dinitrotoluene, diphenylamine, ethylcentralite and dibutylphthalate, was encased in, and fired through a plastic bag: several powder flakes of partially burned smokeless powder were found after each firing. Of 15 separate flakes analyzed, NG and DPA were found in all 15 cases, DNT and EC in 13 and DBP in 3. Hand samples taken using acetone-soaked cotton swabs or tape lifts were analyzed directly, without clean-up procedures: no trace of smokeless powder, aside from partially burned flakes, were detected on a person's hand immediately after having fired a gun. Limits of detection for NG, DNT, DPA, EC and DBP were considered around 50 ng.

Under electron impact (EI) conditions explosive compounds such as nitroglycerine undergo considerable fragmentation and, in most cases, the NO_2^+ ion (46) constitutes the principal fragment in the spectrum, while the molecular ion peak is usually undetectable. In view of the relatively lower energy processes associated with chemical ionization mass spectrometry (CI-MS)

several investigators explored this approach. In 1982 Parker *et al.* used HPLC with negative ion chemical ionization mass spectrometry, found to be more sensitive than positive ion CI, to analyze TNT, RDX, tetryl and PETN. The MS analysis was carried out online, using a direct liquid insertion probe LC-MS interface. The columns were RP-18 and RP-8 reversed - phase (10 cm x 4.6 mm, particle size 10 μm), mobile phases were acetonitrile/water (1:1) and methanol/water (1:1) at flow rate of 1 ml/min [Parker *et al.,* 1982].

Yinon and Hwang [1983] described the use of a high performance liquid chromatography-mass spectrometry system for the separation and identification of EGDN, TNT, tetryl, 2,4-DNT, RDX, PETN using positive-ion chemical ionization. Separation was done on a C_8 reversed - phase column (10 cm x 4.6 mm i.d. LiChrosorb), using acetonitrile/water (1:1) and methanol/water (1:1) as mobile phases, followed by UV and mass spectrometry (MS). The MS analysis was carried out online, using a direct liquid insertion probe LC-MS interface. The chemical ionization mass spectrum of NG was characterized by a small peak at m/z 228 (M+H=228) and typical fragment ions at m/z 183 (228-CH_3NO) and at m/z 165 (228-HNO_3). The 2,4-DNT spectrum included the M+H=183 peak and a major peak at m/z 153 (183-NO).

In 1986 the use of thermospray interface (TSP) was reported [Voyksner and Yinon]. A series of explosives were analyzed and the method gave better sensitivity in the negative ion mode under chemical ionization conditions. The spectra of the explosives analyzed provided molecular weight information with few fragment ions. Detection limits from 200 pg for TNT to 5 ng for ammonium picrate were obtained under full scan mass spectrometer conditions. TSP-HPLC-MS was useful in separating and identifying components in commercial explosives from hand swabs with excellent sensitivity and selectivity. The plasticizers primarily were detected in the positive ion mode while the explosives were more sensitive with negative ion detection.

Berberich *et al.* [1988] investigated the application of liquid chromatography-thermospray-mass spectrometry (LC-TSP-MS) to the separation and identification of commercial and military

explosives. The TSP was operated in the filament-on negative ionization mode, which yielded unique spectra for the following explosive compounds: RDX, HMX, TNT, DNT and monomethylamine nitrate (MMAN). The detection limit for PETN was less than 2.5 pg. Components of double-based smokeless powder also yielded positive ion spectra and spectra which were obtained from the residues of a double-based smokeless powder allowed identification of the pure explosive compounds.

Speers *et al.* [1994] developed a sensitive GC-MS method to detect trace of additives from smokeless powders. The column was 15 m x 0.32 mm Rtx-1. Temperature was held at 85°C for 1 min, then raised 30°C/min to 250°C and held for 5 min. After a first injection in Selected Ion Monitoring (SIM) of the MS detector (169 for DPA, 120 for EC and 134 for MC) confirmation was obtained with a second injection in SIM with three or four masses of the specific compound found in the previous injection. LOD was 10 pg for each compound and positive results after one shot of .357 magnum was obtained (DPA and EC>1 ng).

Yinon [1996] used Gas Chromatography Mass Spectrometry (GC-MS) with a Temperature-Programmed Injector for trace analysis of explosives in water. The analyzed explosives included TNT, 2,4,6-N-tetranitro-N-methylaniline (tetryl), RDX, HMX, PETN and a series of dinitrotoluene isomers. Using a septum programmable injector (SPI), thermal decomposition, even for the thermolabile explosives, was minimal. For all the explosives except for DNT isomers SPI was at a temperature of -5°C for 0.3 min, then programmed from -5°C to 250°C at a rate of 200°C/min. The column used was 15 m x 0.255 mm DB-1, temperature was held at 80°C for 2 min, then raised 25°C/min to 250°C and held for 2 min. For five DNT isomers SPI was at a temperature of 45°C for 0.5 min, then programmed to 260°C at a rate of 150°C/min. The column used was 15 m x 0.255 mm DB-5MS, temperature was held at 50°C for 2 min, then raised 25°C/min to 260°C and held for 2 min. The mass spectrometer used was an ion trap and the ionization mode was electron ionization (EI). Traces of explosives in water in the range of 5-100 ppb could be detected and identified.

PETN produced a mass spectrum containing one single ion at m/z 46 and could be identified at a minimal amount of 100 ppb by starting the GC run at 50°C.

The introduction of tandem mass spectrometry permitted more selective procedures for forensic identification of explosives [Yinon, 1991]. Casetta and Garofolo presented in 1994 a work featuring HPLC-MS-MS parent-ion scan experiments. Separation was performed with a C_{18} column end-capped for acidic compounds, 2x100 mm. Flow rate was 200 µl/min and a gradient of acetonitrile in water varying from 0% to100% in 10 minutes was used. HMX, ethileneglycol dinitrate (EGDN), RDX, NG, TNT, tetryl, DNT, PETN, hexanitrostilbene (HNS), triazidotrinitrobenzene (TNTAB), tetranitroacridone (TENAC), hexanitrodiphenylamine (HEXIL), nitroguanidine (NQ) were analyzed by a Perkin Elmer Sciex API III triple-quadrupole mass spectrometer equipped with an atmospheric-pressure ion source as detector for liquid chromatography. Using the parent-scan technique it was possible to detect explosives within a mixture applying the selectivity of tandem mass analysis.

Garofolo *et al.* presented later a method for quantitative determination of thermostable explosive compounds using API III [1996a]. The explosives were dissolved in dimethylformamide and the quantitative analysis was performed by combined liquid chromatography-tandem mass spectrometry using a phenyl column. The mobile phase consisted of a gradient of 5mM aqueous ammonium acetate and acetonitrile saturated with ammonium acetate. Selected reaction monitoring experiments were performed by setting the first quadrupole to pass the $[M-H]^-$ or $[M+acetate]^-$ ions of a selected explosive, while simultaneously setting the third quadrupole to pass its most characteristic fragment ion. An extensive statistical analysis of results was carried out, which showed a linear response between 0.8 ng and 12.8 ng for hexanitrostilbene, between 0.4 ng and 6.4 ng for hexanitrodiphenylamine, and between 0.1 ng and 1.6 ng for tetranitroacridone.

In 1996 the use of a new microflow particle beam interface in HPLC analysis of explosive was reported [Cappiello *et al*., 1996]. The new interface permitted greater vaporization efficiency and

negligible thermal decomposition in the ion source of the mass spectrometer. A reversed-phase 250-mm-i.d. C_{18} packed capillary column was laboratory-made and used. Acetonitrile was preferred to methanol because of its lower viscosity. NG showed the best sensitivity with acetonitrile/water (50:50). Electron capture ionization was carried out with methane used as a reagent gas and allowed selective and sensitive determination. Detection limit for NG was 60 pg, based on a signal-to-noise ratio of 5:1 in selected ion monitoring (m/z 62) mode after column elution.

Electrospray ionization (ESI) was used to conduct the mass spectral investigation of a series of explosives, using an ESI-ion trap mass spectrometer [Yinon *et al.*, 1997]. The mass spectral results, obtained by tandem mass spectrometry-collision-induced dissociation (daughter-ion, parent-ion and neutral loss) using the ion trap were compared to mass spectra previously obtained.

Doolan *et al.* [1998] studied a number of explosives and constituents of smokeless powders by liquid chromatography coupled with electrospray mass spectrometry. The spectra generated included protonated molecular ions, adduct ions and in some instances fragmentation patterns resulting from collision induced dissociation. Chromatography was performed on a 5 μm particle size 100 x 2.1 mm ODS column. Mobile phase was 50:50 acetonitrile:25mM ammonium acetate at a flow rate of 0.5 ml/min. The column eluent was split to allow 35 ml/min into the electropray source.

In contrast to GC–MS, libraries for the identification of unknown substances separated using HPLC and detected by means of API-MS are not available. Schreiber *et al*. constructed mass spectral libraries for the analysis of samples containing explosives and pesticides, successfully applied on real samples. They found that the composition of eluents normally used for HPLC–API–MS had no significant effect on the mass spectra and thus no effect on the results of the library search [2000].

Wu *et al.* [2001, 1999] published two papers about the utilization of MS-MS in detection of GSR. They described a method where only methylcentralite (MC), a stabilizer having a structure similar to ethylcentralite, was detected following a single transitions 241 $\rightarrow$ 134. Tong *et al.* [2001]

described a novel method for determination of diphenylamine (DPA) and its nitrated derivatives by tandem mass spectrometry.

Electrospray ionization Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectrometry in the analysis of some explosive compounds was studied by Wu *et al.*. They demonstrated the potential of ESI-FTICR, due to its very high mass resolving power and mass accuracy. Elemetal composition of active and nonactive ingredients of explosives was determined [2002].

Evans *et al.* [2002] analyzed explosive compounds by HPLC-MS using an ion trap detector. They studied trinitrotoluene, nitroglycerine, pentaerythritol tetranitrate and hexahydro-1,3,5-trinitro-1,3,5-triazine under negative ion atmospheric pressure chemical ionization (APCI) conditions and found that the limit of detection was improved, in some cases by several orders of magnitude, by complexation with chlorine.

Takada *et al.* developed a new detection system to detect vapours from explosives, based on a novel ion source for atmospheric pressure chemical ionization (APCI). They called the technique "counter-flow introduction" (CFI). The ion source was installed in an ion-trap mass spectrometer and was used to conduct on-line detection of explosives within a few seconds without sample pretreatment [2002].

A gradient reversed-phase HPLC-ESI-MS method was published by Mathis and McCord. The method was optimized for the simultaneous separation and detection in the positive ion mode of diphenylamine, along with isomers of its nitroso and nitro derivatives, centralite I and II, in addition to dialkylphthalate acid esters. The procedure was used to differentiate several unburned powders by their additive profile [2003].

E. Capillary elecrophoresis and electrochromatography

Micellar Electrokinetic Capillary Electrophoresis (MEKC) was introduced in 1984 by Terabe *et al.* and is based on both chromatographic and electrophoretic phenomena [Terabe *et al.*, 1984]. When an electrophoretic analysis is performed in a fused silica capillary, in proximity of the inside wall of the capillary an electrical double layer is formed, due to deprotonation of silica. If the analytical buffer pH is higher than 4, the charged buffer moves towards the positive electrode, causing the so called electro-osmotic flow (EOF). Sodium dodecyl sulphate in water at concentrations higher than 10 mM forms charged micelles, which would migrate in an electric field towards the negative electrode, unless the EOF in the capillary were faster, as it generally is. Neutral substances can be separated in a capillary under the influence of an electric field because of the distribution equilibrium between the bulk solvent, moving with the EOF speed, and the inside of the mycelles moving slower. Micellar electrokinetic capillary electrophoresis provides very rapid and efficient separations (more than 100.000 theoretical plates / meter) of high boiling point and thermolabile compounds. In 1991 MEKC was employed to examine spent ammunition casings for the presence of organic gunshot residue and to separate a test mixture of nitrotoluenes [Northrop *et al.*]. Twenty-six substances were separated in a capillary of effective length of cm 62 and internal diameter of μm 100 in less than 10 min with efficiencies in excess of 200 000 theoretical plates. The effects of different experimental parameters were studied: SDS concentration caused an increasing of k' as addition of a tetraalkylammonium salt did, pH caused only slight modification due to influence to ionic strength and current. Capillary diameter (50 μm and 150 μm worked worse) and injection time effect (the efficiency decreased with longer injection time) were also studied. Multiple-wavelength analysis provided UV spectral profiles of the constituents for use with selective wavelength monitoring. Northrop and Mac Crehan studied later procedures to collect, prepare and analyze gunshot residue with MEKC with 25 mM SDS in 2.5 mM sodium tetraborate pH=8.5 and β-naphthol was used as internal standard [1992]. Several swabs moistened with ethanol

or acetone were tested but tape lifting was preferred. Kennedy *et al.* analyzed EGDN, HMX, RDX, NG, TNT, PETN and tetryl by MEKC [1995]. Separation was completed in about 11 minutes with a good peak shape for all the explosives except for PETN. UV detection at 214 nm permitted calculated limit of detection LOD in the picograms region. A complete protocol for the recovery of the organic GSR under a variety of sampling conditions were evaluated and improved for MEKC analysis [Mac Crehan *et al.*, 1998]. The collection of residue samples where external contaminants such as grease or blood were present on the residue substrate were investigated using both tape lifts and solvent swab protocols. In addition, residue component recovery using supercritical fluid extraction techniques was preliminary evaluated for samples contaminated with blood. MEKC analysis were performed in 15 minutes with a fused silica capillary uncoated 75 μm ID, 55 cm length with 25 kV, with fixed wavelength UV at 200 nm. It was impossible to distinguish all the 38 smokeless powders analyzed. Shooting tests were performed on polyester/cotton cloths, sampled by tape lifting. NG e DPA were detected in all the tests, even in presence of hand lotion, engine oil, weapon oil or sweat. When blood was present a supercritical fluid extraction prior to analysis was successfully tested.

The applicability of capillary electrochromatography to analyze 14 nitroaromatic and nitramine explosive compounds was presented by Bailey and Yao [1998]. Capillary electrochromatography is performed in capillary filled with non porous particles of stationary phases used in HPLC. A separation with a baseline resolution was achieved with ODS for all of the compounds of EPA method 8330 in less than 7 min, featuring efficiencies of over 500.000 theoretical plates / meter. Using more aggressive running conditions, 13 of the 14 compounds were separated in less than 2 min.

Reardon *et al.* used MEKC to determine the additive composition of seven reloading powders [2000]. They found a good agreement between results after analyzing powders and samples from firing tests. In 2001 Northrop described a procedure to collect GSR samples for both MEKC and

SEM analysis and described the results of an extensive study about a group of 100 subjects who had not recently fired a firearm. NG, DPA and EC were never found [Northrop, 2001a]. Samples from firing range were analyzed following both the MEKC and SEM-EDX. Results obtained were compared. The value of MEKC was demonstrated but organic GSR could not persist on skin for more than one hour [Northrop, 2001b]. In the same year MacCrehan *et al.* published a paper about the effect of changing ammunition on the composition of additives in organic GSR [2001]. Analyses after firing two to ten rounds of a new ammunition did not show any traces of the organic components of the initial powder. Moreover was demonstrated that organic GSR composition does not depend on the primer type (conventional leaded and new lead-free primers). Reardon and MacCrehan [2001] developed a quantitative extraction technique for determining the organic additives in smokeless powders. This procedure was used later to study how to associate gunpowder and residues from commercial ammunition after "compositional analysis" [MacCrehan *et al.*, 2002]. A numerical propellant to stabiliser (P/S) ratio was calculated with seven boxes of commercial .38 calibre ammunition. Samples from four of the seven boxes were correctly identified using the P/S ratio alone and all the seven samples could be differentiated after both visual comparison and P/S calculation. Recently MacCrehan *et al.* [2003] demonstrated the possibility to analyze organic GSR after hair combing by followed by MEKC. NG was detected in most of the shooting tests.

Annexe 6 API 3000

The PE-SCIEX API 3000 is a tandem triple-quadrupole mass spectrometer. During the present research the effluent from the chromatographic column was diverted to the TurboIonSpray source of the mass spectrometer. Several mass spectrometric parameters have to be optimised in order to obtain the highest possible abundance of the analytes. The first parameters to be optimised are those having an effect on the spray formation: the voltage of the capillary, which affects the intensity of the ions and the gases (see Fig. 1). One of the most important parameter, however, is the Declustering Potential (DP). An excessive DP value determines in-source fragmentation. The Focusing Potential (FP) and the Entrance Potential (EP) control the passage of the ions from the source to the first quadrupole of the mass spectrometer.

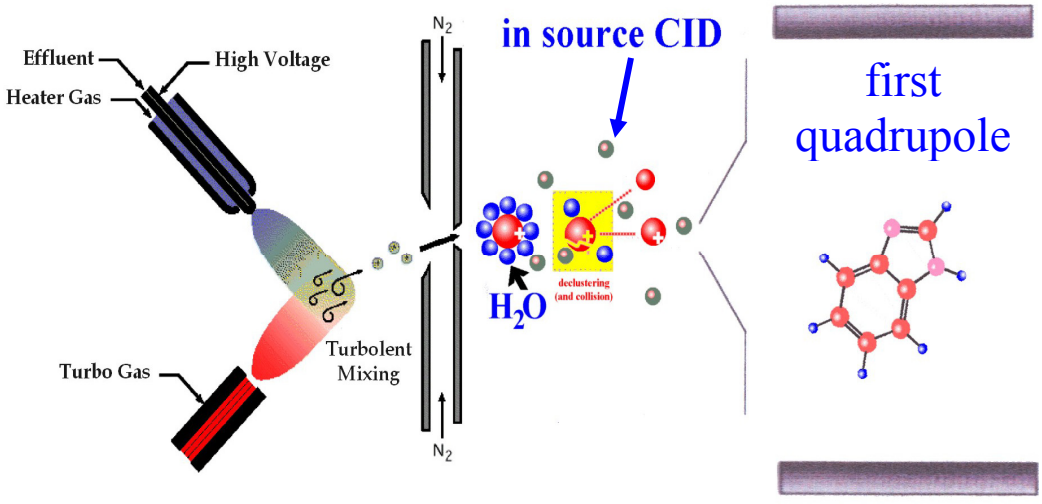


Fig. 1. The TurboIonSpray source.

In Fig. 2 is shown a clustered ion entering the ion spray source and the ion path through the different zones of the spectrometer (declustering, focusing, extraction, filtration).

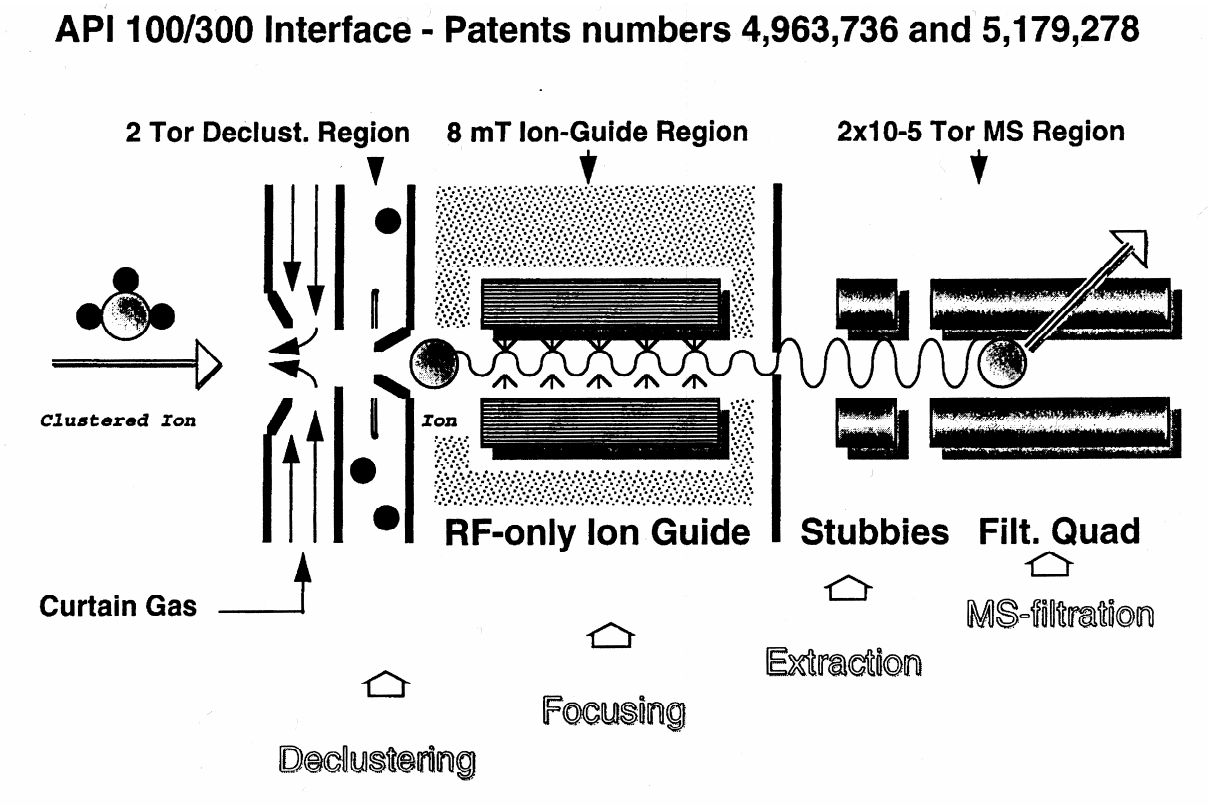


Fig. 2. The TurboIonSpray source.

In the first quadrupole it is possible to select the ions going into the second quadrupole, where can be fragmented by collision induced dissociation. The Collision Energy (CE) control the velocity of ions entering the second quadrupole, an higher velocity determines increased dissociation. The Cell Exit Potential (CXP) control the ions exiting the collision cell and entering the third quadrupole (see Fig. 3).

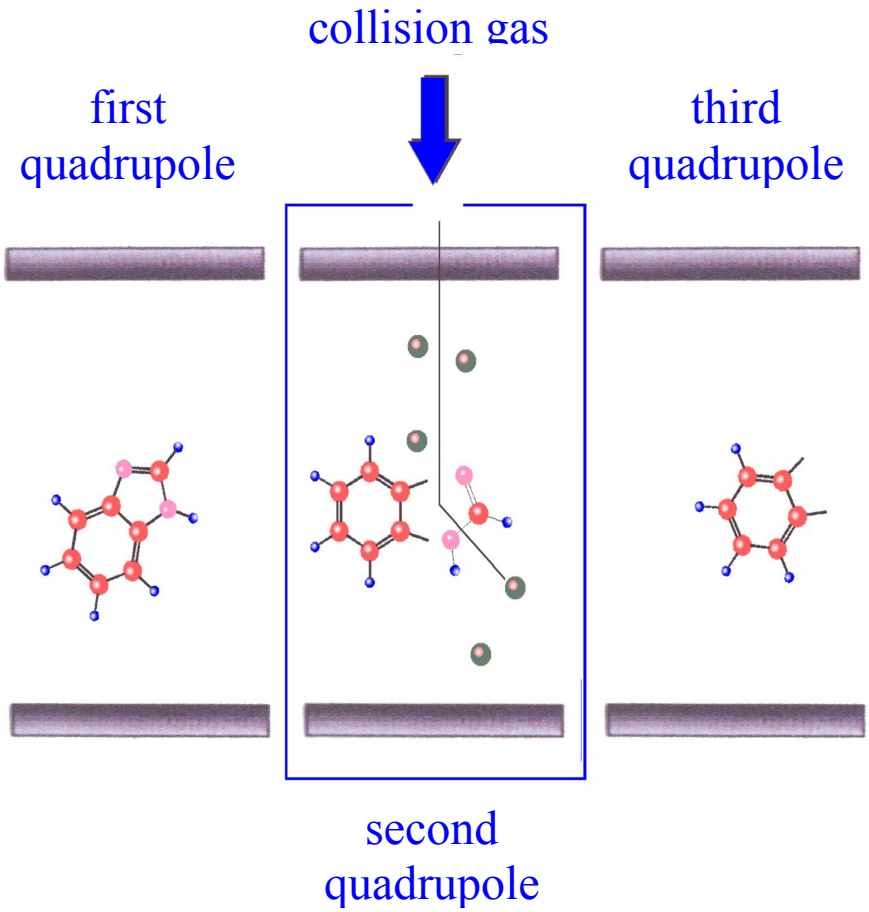


Fig. 3. The tandem triple-quadrupole mass spectrometer.

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