HPLC Method Development for the Fast Separation of a Complex Explosive Mixture

Benmalek Boulesnam, Fahima Hami, Djalal Trache, and Toudert Ahmed Zaid

Abstract−The growing threat of terrorism in many parts of the world has called for the urgent need to find rapid and reliable means of analyzing explosives. This is in view to help forensic scientists to identify different swabs from post-blast debris. The present study aims to achieve an efficient separation and identification of a mixture of sixteen explosive compounds (including nitroaromatics, nitramines, and nitrate esters) by high performance liquid chromatography using a diode array detection (HPLC/DAD) and an Agilent Poroshell 120 EC-120 C_{18} column at two wavelengths (235 and 214 nm). Relevant chromatographic parameters such as capacity factors, resolution, selectivity and number of theoretical plates have been optimized in order to achieve the best separation of the different components. In this respect, the effects of various parameters such as gradient time, column temperature, flow rate of mobile phase and initial percentage of organic mobile phase on the separation of these compounds were investigated. It was revealed that the method allowed a fairly acceptable separation of all the compounds in less than 15 minutes except for two isomers, namely 4-A-2,6-DNT, 2-A-4,6-DNT and 2,6- DNT which could not be resolved by the used C_{18} column. These shortcoming notwithstanding, the authors believe the developed method produced satisfactory results and demonstrated sensitive and robust separation, furthermore indicating that the HPLC developed method can be both fast and efficient for the analysis of complex mixtures of explosive compounds.

***Keywords*−HPLC method development, UV detection, explosives, optimization.**

NOMENCLATURE

HPLC	High Performance Liquid Chromatography
DAD	Diode Array Detection
FPD	Flame Photometric Detector
UV	Ultraviolet
US EPA	United States Environmental Protection Agency
F	flow rate (mL/min)
HRMS	High resolution mass spectrometry
MeOH	methanol
k	capacity factor
α	selectivity factor
N	number of theoretical plates
R	resolution
t_R	retention time (min)
t_G	gradient time (min)
XRD	X-ray diffractometry
XRF	X-ray fluorescence spectroscopy
Δt_R	difference in retention times for two peaks (min)
$\Delta Ø$	gradient range, equal to the final value of $Ø$ in the gradient ($Ø_f$) minus the initial value ($Ø_0$)
$Ø$	volume fraction of B solvent in the mobile phase
$Ø_f$	value of $Ø$ for mobile phase at end of gradient
$Ø_0$	value of $Ø$ for mobile phase at start of gradient

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Benmalek Boulesnam is with the Explosives and Explosions Department, "Institut National de Criminalistique et de Criminologie-INCC/GN", Bouchaoui – Algeria (e-mail: boulesnam.benmalek@gmail.com).

Fahima Hami is with the Microtrace Department, "Institut National de Criminalistique et de Criminologie-INCC/GN", Bouchaoui – Algeria (e-mail: hami.fahima@gmail.com).

Djalal Trache is with Laboratoire des Matériaux Energétiques (UER-PE), Ecole Militaire Polytechnique, Bordj El Bahri 16046 – Algeria (e-mail: djalaltrache@gmail.com).

Toudert Ahmed Zaid is with Laboratoire de Valorisation des Energies Fossiles, Ecole Nationale Polytechnique, El Harrach 16200 Algiers –Algeria (e-mail: toudert.ahmed-zaid@g.enp.edu.dz).

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I. INTRODUCTION

Conventional munitions constituents such as nitroaromatics, aminoaromatics, nitramines, and nitrate esters are the most common used organic high explosives by either armed forces or terrorist groups around the world. There is a current need to improve security/screening methods for explosive detection or recognition. Such methods can be applied for either environmental considerations or forensics. The stat-of-the-art of the analysis of such explosives during the last few decades has shown that the application of high performance liquid chromatography (HPLC) allowed obtaining a high degree of accuracy and precision. Such analytical tool presents an obvious advantage over gas chromatography, because it is carried out at room temperature and the above-mentioned explosives are known to present a low vapor pressure [1]. Being nondestructive, HPLC can be utilized for the combined analysis of both volatile and nonvolatile materials.

The analysis of explosives mixtures by liquid chromatography equipped by UV [2-6] or FPD [7] detectors has already been carried out. However, such combinations often required extending analysis time. Recent chromatography methods based on mass spectrometric detection have been revealed to be efficient [8-10, 11-13], due to high level of confirmation and accuracy. Nevertheless, UV absorbance detection remains one of the universal methods used in micro separations due to its simplicity, ease-of-use and low cost [5]. Furthermore, most of organic compounds can be analyzed by HPLC equipped by UV detectors. This latter displays further advantages such as rapidity, accessibility, durability, low toxicity and cost efficiency. However, it is demonstrated that the detection of

explosive mixtures is challenging because of poor mass transfer efficiencies and long analysis times.

At present, there is no simple method, which efficiently separates and quantifies munitions constituents or mixtures of explosives [6,14].

On the other hand, the identification of trace explosives can be extremely difficult because of the complexity of the different matrices that can be investigated due to their low content in explosive compounds. Thus, sophisticated analytical techniques that are sensitive, robust, fast and cost-effective are often required. A comprehensive review dealing with the high performance liquid chromatography methods for the analysis of explosives was reported by Gaurav et al. [5]. Mohamad Afiq Mohamed Huri et al. published an exhaustive review concerning the analysis of explosive residue from the forensic point of view [15]. This latter reported the approaches to track traces of explosives and the respective extraction methods. These authors provided a deep insight on the methods used to analyze the explosive residue as well. However, this research area remains an ongoing subject that needs more investigations to find new efficient approaches. Other advanced techniques such as nuclear magnetic resonance (NMR) have made it possible to identify the structural composition of explosives from post-blast debris [16] while combined techniques including HPLC-HRMS, XRD and XRF were used to gain fingerprints of various brands of explosives when including the analysis of additives and by-products [17]. These authors claim that these combined methods of analysis can be useful for the creation of a database on explosives that enables to assign specific formulations to certain manufacturers and countries of origin.

The objective of this work is to implement an analytical technique using HPLC equipped with photodiode array detector (HPLC/DAD) for the rapid separation of a mixture of 16 explosive substances by optimizing the separation through the variation of relevant chromatographic parameters such as capacity factors, resolution, selectivity and number of theoretical plates.

II. Materials and Methods

II.1. Chemicals and Materials

Explosive standards solutions used in this study were purchased from AccuStandard™ and supplied in a solvent in 1 mL size glass ampoules dissolved in acetonitrile (AcN) or methanol (MeOH) (or a mixture of both (AcN: MeOH (1:1)) at a concentration of 1000 µg/mL concentration. The list of the sixteen studied compounds is given in Table I. All solutions were stored in amber glass vials at 4°C to avert degradation.

Methanol, of HPLC-grade, purchased from VWR (Fontenay Sous Bois, France), was degassed prior to use. Organic-free reagent water was used as mobile phase in linear gradient elution mode.

Table I
Explosive compounds under study

Compounds	Abbreviations
Nitroaromatics and nitramines	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX (C1)
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX (C3)
1,3,5-Trinitrobenzene	1,3,5-TNB (C4)
1,3-Dinitrobenzene	1,3-DNB (C5)
Nitrobenzene	NB (C6)
3,5-Dinitroaniline	3,5-DNA (C7)
2,4,6-Trinitrophenylméthylnitramine	Tetryl (C8)
2,4,6-Trinitrotoluene	2,4,6-TNT (C9)
4-Amino-2,6-Dinitrotoluene	4-A-2,6-DNT (C10)
2-Amino-4,6-Dinitrotoluene	2-A-4,6-DNT (C11)
2,6-Dinitrotoluene	2,6-DNT (C12)
2-Nitrotoluene	2-NT (C13)
4-Nitrotoluene	4-NT (C14)
3-Nitotoluene	3-NT (C15)
Nitrate esters	
Ethylene glycol dinitrate	EGDN (C2)
Pentaerythritoltetranitrate	PETN (C16)

II.2. Analytical Instrumentation

An Agilent Model 1200 HPLC, coupled with a diode array detector (DAD), was used to separate the mixture of explosives. Experimental monitoring and data acquisition are performed by using HPLC ChemStation for LC 3D systems, Rev. B.04.03 Software. The employed analytical column was an Agilent Poroshell 120 EC-120 C_{18} (4.6 x 150 mm, 4 µm). The mobile phase was a MeOH – H_2O mixture. The elution mode is a linear gradient from 5% to 100% of MeOH during 15 min, with a mobile phase flow rate of 1.2 mL/min. The column temperature was 25°C and two wavelengths were used for the detection (214 nm and 235 nm).

II.3. Sample Preparation

Using explosive standard solutions (AccuStandard, purity > 99%), a mixture solution containing 10 ppm of each of the following compounds: NB, 2-NT, 3-NT, 4-NT, EGDN, 1,3-DNB, 2,6-DNT, 3,5-DNA, 2-A-4,6-DNT, 4-A-2,6-DNT, 1,3,5-TNB, RDX, 2,4,6-TNT, Tetryl, HMX, PETN was prepared by dissolving explosive standards solutions in methanol HPLC grade.

III. Separation Optimization

The goal of the present study is to separate a mixture of sixteen (16) explosive substances with an adequate peak resolution higher than 1.5, and through a fast and complete separation. These requirements can be achievable by optimizing chromatographic parameters such as the capacity factor (k), factor of selectivity α, and the number of theoretical plate N. Once ‘‘best’’ values of k and α have been established (optimization of selectivity), the resolution and the run time will depend only on N. The experimental conditions that favor a fast separation include small particles and short columns of the stationary phase, in addition to high flow rate of the mobile phase**.** [18]

Based on literature and availability reasons, a C_{18} packed column described in the above section 2 was selected. Poroshell 120 columns are based on superficially porous particle technology, which features a solid silica core and a porous outer layer providing higher chromatographic efficiencies, fast and high-resolution separations.

III.1. Initial separation test

Starting with the initial separation conditions mentioned in section 2, the chromatogram given in Fig. 1 was obtained.

The elution mode (gradient) was justified by a $\Delta t_R / t_G$ Ratio > 4 (calculated value = 4.6). Based on their polarity, the studied compounds will leave the column, where the most polar one will be the first to be eluted. As can be seen, the majority of the compounds are well separated, except the compounds (C10, C11 and C12). The UV spectra of these compounds match those of 4-A-2,6-DNT, 2-A-4,6-DNT, and 2,6-DNT

respectively. These co-eluted compounds are indeed difficult to separate with most of commercial C_{18} columns and are baseline resolved by specific columns such as Acclaim E1 Explosives Analytical Columns, designed for US EPA Method 8330 [19]. So, a separation optimization needs to be performed.

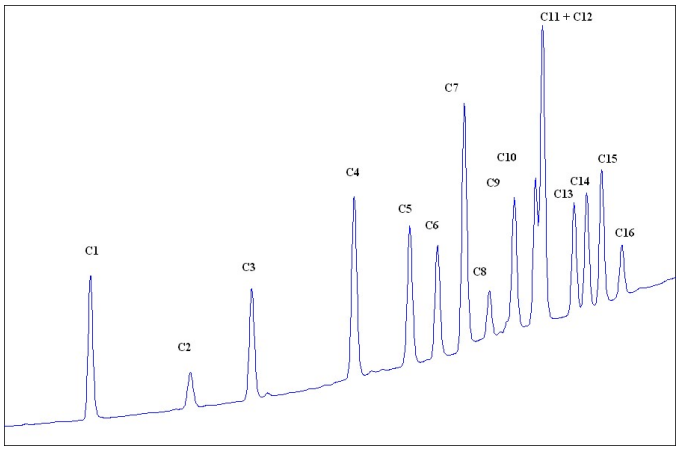

Fig. 1: Chromatogram of the mixture of explosives at 214 nm with concentration of 10 ppm. HPLC conditions: Poroshell 120 EC-120 C_{18} (4.6 x 150 mm, 4 µm), MeOH-H_2O mixture at flow rate of 1.2 mL/min, injection volume = 5 µL, elution mode: linear gradient from 5% to 100% of MeOH in H_2O during 15 min, column temperature: 25°C.

III.2. Effect of capacity factor (k)

The capacity factor k in isocratic elution is usually controlled by varying the mobile-phase composition. In elution gradient mode, the variation of the applied gradient duration (t_G), affects the capacity factor (k). The usual separation goal is to reach $k \leq 10$ for all peaks because this corresponds to narrower and taller peaks, which improves the detection at short run times. Fig. 2 shows the effect of the increase of t_G on the peak separation whereas Table II, Table III and Table IV display the effect of t_G on the capacity factors (k), the number of theoretical plates (N), the resolutions (R) and selectivity (α) of each detected peak.

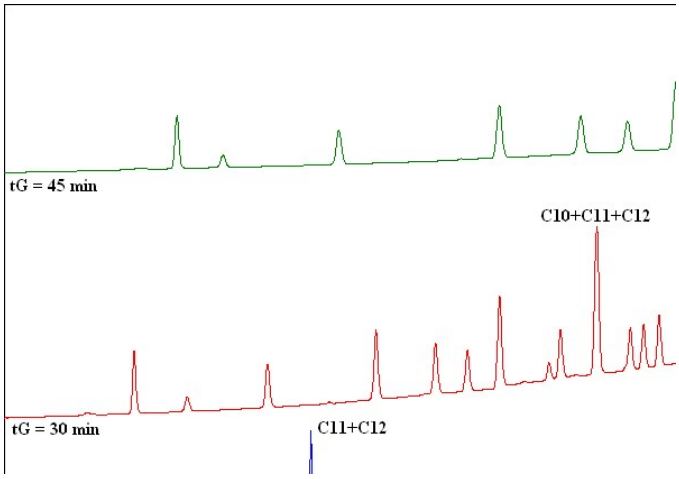

Fig. 2: Effect of t_G increase on the separation profile. HPLC conditions: Poroshell 120 EC-120 C_{18} (4.6 x 150 mm, 4 µm), MeOH-H_2O mixture at flow rate of 1.2 mL/min, injection volume = 5 µL, elution mode: linear gradient from 5% to 100% of MeOH in H_2O for 15, 30 and 45 min, column temperature: 25°C.

As can be seen from Fig. 2, the application of an elution gradient of 15 min allowed a good separation of the sixteen explosive compounds. However, the application of higher elution gradient durations of 30 min and 45 min resulted in an overlap "coelution" of the peaks (2,6-DNT, 2-A-4,6-DNT) and (2,6-DNT, 2-A-4,6-DNT, 4-A-2,6-DNT) respectively. This indicates that t_G = 15 min is the most appropriate gradient duration with k values ranging between 4.0 and 9.2.

With the employment of Poroshell 120 EC-120 C_{18} column, the retention of compounds increases in the following order: PETN > 3-NT > 4-NT > 2-NT > 2,6-DNT > 2-A-4,6-DNT > 4-A-2,6-DNT > 2,4,6-TNT > Tetryl > 3,5-DNA > NB > 1,3-DNB > 1,3,5-TNB > RDX > EGDN > HMX. It should be noted that no reversal elution occurred in the separation runs what can cause changes of the relevant parameters during the optimization steps, except when changing the initial percentage of the organic phase (MeOH). However, as was already mentioned, 4-A-2,6-DNT and 2-A-4,6-DNT isomers could not be separated and are co-eluted with 2,6- DNT.

Table II
Influence of t_G on k

t_G (min)		15	30	45
Compound	**Peak order**		**k**	
HMX	1	4.0	5.2	6.0
EGDN	2	5.0	6.2	6.8
RDX	3	5.6	7.6	9.0
1,3,5-TNB	4	6.6	9.6	11.9
1,3-DNB	5	7.1	10.7	13.4
NB	6	7.4	11.3	14.2
3,5-DNA	7	7.7	11.9	15.1
Tetryl	8	7.9	9.5	16.9
2,4,6-TNT	9	8.2	13.0	-
4-A-2,6-DNT	10	8.4	13.7	18.1
2-A4,6-DNT	11	8.4	-	-
2,6-DNT	12	-	-	-
2-NT	13	8.7	14.3	19.0
4-NT	14	8.9	14.5	19.3
3-NT	15	9.0	14.8	19.7
PETN	16	9.2	15.5	20.9

Table III
Influence of t_G on N

t_G (min)		15	30	45
Compound	**Peak order**		**N**	
HMX	1	50316	47341	42390
EGDN	2	49494	38099	33400
RDX	3	74861	58838	49587
1,3,5-TNB	4	96123	92537	80411
1,3-DNB	5	114878	106316	90758
NB	6	129999	120123	100272
3,5-DNA	7	135268	128584	111341
Tetryl	8	158320	180379	113503
2,4,6-TNT	9	157171	163642	-
4-A-2,6-DNT	10	177625	151348	160783
2-A4,6-DNT	11	164951	-	-
2,6-DNT	12	-	-	-
2-NT	13	206239	230409	217680

4-NT	14	214348	242032	203886
3-NT	15	219374	248145	226031
PETN	16	228438	276265	256827

TABLE IV
INFLUENCE OF t_G ON R AND α

t_G (min)		15		30		45	
Compound	**Peak order**	**R**	**α**	**R**	**α**	**R**	**α**
HMX	1	-	-	-	-	-	-
EGDN	2	10.0	1.25	7.4	1.19	5.5	1.14
RDX	3	5.9	1.12	10.1	1.24	12.1	1.31
1,3,5-TNB	4	10.4	1.18	14.0	1.26	16.3	1.33
1,3-DNB	5	5.6	1.08	7.6	1.11	8.0	1.13
NB	6	2.9	1.04	4.1	1.05	4.4	1.06
3,5-DNA	7	2.8	1.04	4.1	1.05	4.6	1.06
Tetryl	8	2.7	1.03	6.6	1.08	9.0	1.12
2,4,6-TNT	9	2.7	1.03	1.6	1.02	-	-
4-A-2,6-DNT	10	2.3	1.03	4.6	1.05	5.5	1.07
2-A4,6-DNT	11	0.8	1.01	-	-	-	-
2,6-DNT	12	-	-	-	-	-	-
2-NT	13	3.5	1.04	4.4	1.04	5.0	1.05
4-NT	14	1.4	1.01	1.9	1.02	2.0	1.02
3-NT	15	1.7	1.02	2.2	1.02	2.3	1.02
PETN	16	2.3	1.02	5.2	1.04	6.5	1.06

III.3. SELECTIVITY (α) OPTIMIZATION

For a further improvement of the separation, relative retention (peak spacing, selectivity, or separation factor α) is then adjusted by varying the organic solvent, temperature or type of column [17]. In order to improve the resolution of co-eluting isomers, the column temperature was carried over the range of 25 – 38°C.The separation runs were performed under the following conditions: a MeOH-H_2O mixture with a flow rate of 1.2 mL/min is used as a mobile phase and the linear gradient as elution mode is varied from 5% to 100% of MeOH in H_2O. The duration of the analysis is around 15 min, whereas the column temperatures used are, respectively, 25, 28, 30, 32, 35 and 38°C. The corresponding chromatograms are shown in Fig. 3.

The effect of temperature on the capacity factors (k), the number of theoretical plates (N), the resolutions (R) and selectivity (α) of recorded peaks is shown in Table V, Table VI, Table VII and Table VIII respectively.

As can be seen from the chromatograms (Fig. 3), the two compounds (2,6-DNT and 2-A-4,6-DNT) overlap at temperatures of 25°C and 28°C while the peaks (4-A-2,6-DNT and 2-A-4,6-DNT) overlap at temperatures of 35°C and 38°C, respectively, also the peaks (NB and 3,5-DNA) overlap at temperatures of 38°C.

Regarding the capacity factors (k), they are all within the optimal domain, whatever the temperature is (Table V).

During the optimisation of column temperature, the co-elution of peaks of 4-A-2,6-DNT and 2-A-4,6-DNT, especially at 30 and 32°C, was the major issue as it had limited the resolution so far (R < 1.5). From Fig. 3 obtained, when running the samples at different temperatures, it was clearly seen that the increase of the column temperature substantially affects the resolution of the NB and 3,5-DNA compounds, where the highest resolution of different compounds is obtained at T = 32°C.

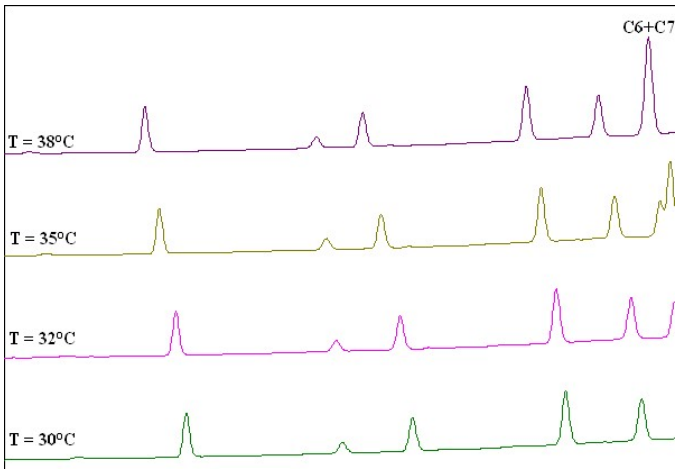

Fig.3: Effect of column temperature on separation profile. HPLC conditions: Poroshell 120 EC-120 C18 (4.6 x 150 mm, 5 µm), MeOH-H_2O mixture at flow rate of 1.2 mL/min, injection volume = 5 µL, elution mode: linear gradient from 5% to 100% of MeOH in H_2O for 15 min.

TABLE V
INFLUENCE OF COLUMN TEMPERATURE ON k.

T (°C)		25	28	30	32	35	38
Compound	**Peak order**	**k**					
C1	1	4.0	3.9	3.8	3.7	3.6	3.5
C2	2	5.0	4.9	4.9	4.8	4.8	4.7
C3	3	5.6	5.5	5.4	5.3	5.1	5.0
C4	4	6.6	6.5	6.4	6.4	6.3	6.1
C5	5	7.1	7.0	7	6.9	6.8	6.6
C6	6	7.4	7.3	7.2	7.2	7.1	7.0
C7	7	7.7	7.5	7.4	7.3	7.2	-
C8	8	7.9	7.8	7.7	7.6	7.5	7.4
C9	9	8.2	8.1	8	7.9	7.8	7.7
C10	10	8.4	8.2	8.1	8.1	8.0	7.8
C11	11	8.4	8.3	8.2	8.1	-	-
C12	12	-	-	8.3	8.2	8.1	8.0
C13	13	8.7	8.7	8.6	8.5	8.4	8.3
C14	14	8.9	8.8	8.7	8.6	8.6	8.5
C15	15	9.0	8.9	8.9	8.8	8.7	8.6
C16	16	9.2	9.2	9.1	9.0	9.0	8.9

TABLE VI
INFLUENCE OF COLUMN TEMPERATURE ON N

T (°C)		25	28
Compound	**Peak order**	**N**	
HMX	1	50316	49762
EGDN	2	49494	50174
RDX	3	74861	72544
1,3,5-TNB	4	96123	97881
1,3-DNB	5	114878	113353
NB	6	129999	131215
3,5-DNA	7	135268	134113
Tetryl	8	158320	164861
2,4,6-TNT	9	157171	160916
4-A-2,6-DNT	10	177625	167242
2-A-4,6-DNT	11	172478	51819
2,6-DNT	12	-	-
2-NT	13	206239	200383

4-NT	14	214348	208222
3-NT	15	219374	213310
PETN	16	228438	225669

TABLE VI
INFLUENCE OF COLUMN TEMPERATURE ON N (CONTINUED)

T (°C)		**30**	**32**	**35**	**38**
Compound	**Peak order**	**N**			
C1	1	49786	49370	47722	45752
C2	2	51625	52159	51381	49621
C3	3	71402	70862	68289	65721
C4	4	99265	98391	95914	94143
C5	5	112891	111894	109241	104377
C6	6	130194	131786	126031	99043
C7	7	133100	132649	115456	-
C8	8	164761	161625	154060	147515
C9	9	172095	168921	170255	129397
C10	10	330984	377306	57513	63760
C11	11	223214	315890	-	-
C12	12	274220	217809	183513	168383
C13	13	202108	200554	193880	185785
C14	14	207674	208074	198628	193769
C15	15	210452	210788	205318	199153
C16	16	226431	216383	219000	219600

TABLE VII
INFLUENCE OF COLUMN TEMPERATURE ON R.

T (°C)		**25**	**28**	**30**	**32**	**35**	**38**
Compound	**Peak order**	**R**					
HMX	1	-	-	-	-	-	-
EGDN	2	10.0	10.8	11.4	11.9	12.4	12.8
RDX	3	5.9	5.3	4.9	4.5	3.9	3.3
1,3,5-TNB	4	10.4	10.9	11.2	11.5	11.8	12.1
1,3-DNB	5	5.6	5.6	5.6	5.5	5.4	5.3
NB	6	2.9	3.0	3.2	3.3	3.4	3.5
3,5-DNA	7	2.8	2.2	1.8	1.4	0.8	-
Tetryl	8	2.7	3.2	3.5	3.8	4.2	4.5
2,4,6-TNT	9	2.7	2.9	3.1	3.2	3.4	3.3
4-A-2,6-DNT	10	2.3	1.9	2.0	1.8	1.1	0.8
2-A-4,6-DNT	11	2.3	0.7	0.7	0.8	-	-
2,6-DNT	12	-	-	0.9	1.2	1.1	1.5
2-NT	13	3.5	2.6	4.2	4.1	4.0	4.1
4-NT	14	1.4	1.4	1.4	1.4	1.4	1.4
3-NT	15	1.7	1.8	1.8	1.8	1.8	1.8
PETN	16	2.3	2.6	2.8	2.9	3.1	3.3

TABLE VIII
INFLUENCE OF COLUMN TEMPERATURE ON α.

T (°C)		**25**	**28**	**30**	**32**	**35**	**38**
Compound	**Peak order**	**α**					
HMX	1	-	-	-	-	-	-
EGDN	2	1.25	1.27	1.28	1.3	1.32	1.34
RDX	3	1.12	1.11	1.1	1.09	1.08	1.07
1,3,5-TNB	4	1.18	1.19	1.2	1.2	1.22	1.23
1,3-DNB	5	1.08	1.08	1.08	1.08	1.08	1.08
NB	6	1.04	1.04	1.04	1.04	1.05	1.05
3,5-DNA	7	1.04	1.03	1.02	1.02	1.01	-
Tetryl	8	1.03	1.04	1.04	1.05	1.05	1.06
2,4,6-TNT	9	1.03	1.03	1.03	1.04	1.04	1.04
4-A-2,6-DNT	10	1.03	1.02	1.02	1.02	1.02	1.01
2-A-4,6-DNT	11	1.03	1.01	1.01	1.01	-	-
2,6-DNT	12	-	-	1.01	1.01	1.02	1.02
2-NT	13	1.04	1.04	1.04	1.04	1.04	1.04
4-NT	14	1.01	1.01	1.01	1.01	1.01	1.01
3-NT	15	1.02	1.02	1.02	1.02	1.02	1.02
PETN	16	1.02	1.03	1.03	1.03	1.03	1.03

III.4. COLUMN EFFECTIVENESS OPTIMIZATION (N): EFFECT OF MOBILE PHASE FLOW RATE

A further separation improvement may be possible by varying some column conditions (such as the column length, the flow rate, and the particle size), in order to improve the column plate number N. The mobile phase flow rate effect is indeed investigated. The previously optimized conditions such as the capacity factor and the column temperature (32°C) are maintained. The flow rate variation is performed in an inverse trend to the duration of the elution gradient. Thus, a decrease in flow rate by one-half will correspond to double of the elution gradient duration. The chromatographic parameters obtained are presented in Table IX, Table X, Table XI, Table XII and Table XIII.

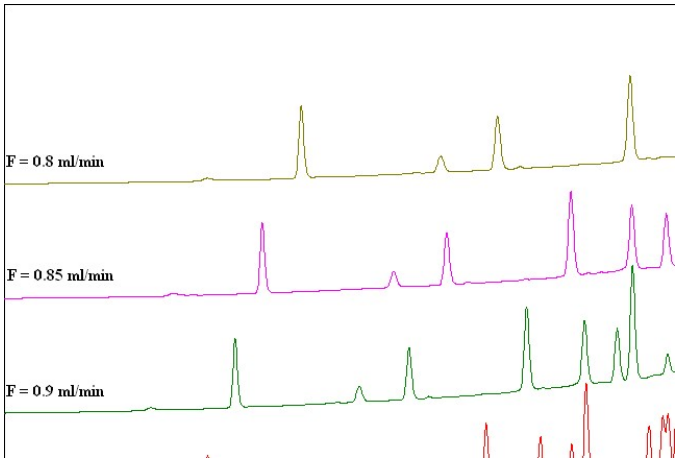

Fig. 4: Effect of flow rate on the separation performance. Separation conditions: elution mode: linear gradient from 5% to 100% of MeOH in H_2O in 15 min, injection volume = 5 µL, column temperature: 32°C.

As can be seen from Fig. 4, the 16 compounds are all separated whatever the applied flow rate. It should be noted that at a flow rate of 0.7 mL/min, the time required for the separation of the sixteen substances is much longer, with an increase of roughly 50%. However, the capacity factors of the different separated substances remain within the optimum range. As was expected, the number of theoretical plates increases with a decrease of the flow rate.

Concerning the resolutions, it seems that their values are fairly close within the investigated domain of flow rates. Based on these observations, a compromise between t_R, R, and N was considered satisfactory for a flow rate of 0.9 mL/min.

TABLE IX
EFFECT OF LIQUID PHASE FLOW RATE ON t_R

Flow(mL/min)	**0.8**	**0.85**	**0.9**	**0.95**	**1.0**
Compound			**t_R**		
HMX	8.85	8.28	7.86	7.45	7.06
EGDN	10.95	10.25	9.72	9.22	8.73
RDX	11.81	11.05	10.47	9.95	9.41
1,3,5-TNB	13.79	12.91	12.24	11.63	11.01
1,3-DNB	14.76	13.82	13.10	12.45	11.79
NB	15.32	14.34	13.59	12.91	12.24
3,5-DNA	15.58	14.58	13.82	13.13	12.45
Tetryl	16.17	15.14	14.36	13.64	12.95

2,4,6-TNT	16.69	15.63	14.82	14.08	13.36
4-A-2,6-DNT	16.94	15.86	15.04	14.28	13.56
2-A-4,6-DNT	17.03	15.95	15.12	14.36	13.63
2,6-DNT	17.18	16.09	15.25	14.49	13.76
2-NT	17.79	16.67	15.80	15.01	14.25
4-NT	18.02	16.88	16.00	15.20	14.44
3-NT	18.30	17.14	16.25	15.44	14.66
PETN	18.77	17.57	16.67	15.83	15.04

TABLE X
EFFECT OF LIQUID PHASE FLOW RATE ON k.

Flow (mL/min)		**0.8**	**0.85**	**0.9**	**0.95**	**1.0**
Compound	Peak order			**k**		
HMX	1	3.7	3.7	3.7	3.7	3.7
EGDN	2	4.9	4.8	4.9	4.9	4.8
RDX	3	5.3	5.3	5.3	5.3	5.3
1,3,5-TNB	4	6.4	6.3	6.4	6.4	6.4
1,3-DNB	5	6.9	6.9	6.9	6.9	6.9
NB	6	7.2	7.2	7.2	7.2	7.2
3,5-DNA	7	7.3	7.3	7.3	7.3	7.3
Tetryl	8	7.7	7.6	7.6	7.7	7.7
2,4,6-TNT	9	7.9	7.9	7.9	7.9	7.9
4-A-2,6-DNT	10	8.1	8.0	8.0	8.1	8.1
2-A-4,6-DNT	11	8.1	8.1	8.1	8.1	8.1
2,6-DNT	12	8.2	8.1	8.2	8.2	8.2
2-NT	13	8.5	8.5	8.5	8.5	8.5
4-NT	14	8.6	8.6	8.6	8.7	8.7
3-NT	15	8.8	8.7	8.8	8.8	8.8
PETN	16	9.0	9.0	9.0	9.1	9.1

TABLE XI
EFFECT OF LIQUID PHASE FLOW RATE ON N

Flow (mL/min)		**0.8**	**0.85**	**0.9**	**0.95**	**1.0**
Peak order				**N**		
C1	1	64723	63347	60882	57707	55814
C2	2	58826	58660	57767	57622	55733
C3	3	90062	86259	85604	82398	80347
C4	4	124956	119581	117616	111927	109806
C5	5	136523	133911	132714	128401	123456
C6	6	159956	155109	155118	151431	146315
C7	7	171166	164257	161346	155204	149189
C8	8	200065	186948	198195	189478	182309
C9	9	211432	198988	199255	193421	186085
C10	10	207915	207383	219216	221841	211009
C11	11	176601	179830	198470	197487	129095
C12	12	219183	228468	270426	265321	214104
C13	13	256388	248446	240285	233506	222446
C14	14	263325	255489	248564	240460	230839
C15	15	266250	264247	252705	245534	238434
C16	16	319770	305640	288809	277017	264889

TABLE XII
EFFECT OF LIQUID PHASE FLOW RATE ON R

Flow (mL/min)		**0.8**	**0.85**	**0.9**	**0.95**	**1.0**
Compound	Peak order			**R**		
HMX	1	-	-	-	-	-
EGDN	2	13.1	13.1	12.8	12.7	12.5
RDX	3	5.1	5.0	4.9	5.0	4.8
1,3,5-TNB	4	12.7	12.4	12.4	12.1	12.1
1,3-DNB	5	6.1	6.1	6.0	5.9	5.8
NB	6	3.5	3.5	3.5	3.4	3.4
3,5-DNA	7	1.7	1.7	1.7	1.6	1.6
Tetryl	8	4.0	3.9	4.0	4.0	4.0
2,4,6-TNT	9	3.5	3.5	3.5	3.4	3.4
4-A-2,6-DNT	10	1.7	1.6	1.7	1.7	1.7
2-A-4,6-DNT	11	0.6	0.6	0.6	0.6	0.5
2,6-DNT	12	1.0	1.0	1.1	1.1	0.9
2-NT	13	4.3	4.3	4.4	4.4	4.1
4-NT	14	1.6	1.6	1.6	1.6	1.5
3-NT	15	2.0	2.0	1.9	1.9	1.9
PETN	16	3.4	3.3	3.3	3.2	3.2

TABLE XIII
EFFECT OF LIQUID PHASE FLOW RATE ON **α**

Flow (mL/min)		**0.8**	**0.85**	**0.9**	**0.95**	**1.0**
Compound	Peak order			**α**		
HMX	1	-	-	-	-	-
EGDN	2	1.30	1.30	1.30	1.30	1.30
RDX	3	1.10	1.10	1.10	1.09	1.09
1,3,5-TNB	4	1.20	1.20	1.20	1.20	1.20
1,3-DNB	5	1.10	1.10	1.10	1.08	1.08
NB	6	1.00	1.00	1.00	1.04	1.04
3,5-DNA	7	1.00	1.00	1.00	1.02	1.02
Tetryl	8	1.00	1.00	1.00	1.04	1.05
2,4,6-TNT	9	1.00	1.00	1.00	1.04	1.04
4-A-2,6-DNT	10	1.00	1.00	1.00	1.02	1.02
2-A-4,6-DNT	11	1.00	1.00	1.00	1.01	1.01
2,6-DNT	12	1.00	1.00	1.00	1.01	1.01
2-NT	13	1.00	1.00	1.00	1.04	1.04
4-NT	14	1.00	1.00	1.00	1.01	1.01
3-NT	15	1.00	1.00	1.00	1.02	1.02
PETN	16	1.00	1.00	1.00	1.03	1.03

III.5. EFFECT OF CHANGES IN THE GRADIENT

The usual goal of a change of the initial-%B is to shorten the run time, by removing the empty space in the early part of the gradient chromatogram. In the following section, the effects of a change of initial-%B are investigated by varying gradient time t_G in proportion to $\Delta\emptyset$, thus holding ($\Delta\emptyset/t_G$) constant.

Fig. 5 illustrates the effects of a change in initial-% methanol during the separation of the explosives mixture.

The effect of the initial %MeOH on the capacity factors (k), the resolutions (R), the number of theoretical plates (N) and selectivity (α) of recorded peaks is shown in Table XIV, Table XV, Table XVI and Table XVII respectively.

An increase in initial %MeOH results in a rather rapid elution of the first compounds, resulting in a decrease of the capacity factor values. In this case the acceptable values of (k) correspond to initial% in MeOH ≤ 30%, when values of (k) are varied between 1.4 and 7.1.

Regarding the peak resolutions, the obtained results show that an increase of the initial % in methanol causes a slight decrease of the resolutions. Once again, resolutions of peaks (C10, C11, and C12), are rather low. These peaks are those of compounds 2-A-2,4-DNT, 4-A-2,6-DNT and 2,6-DNT respectively, which are hardly separated on a C_{18} column.

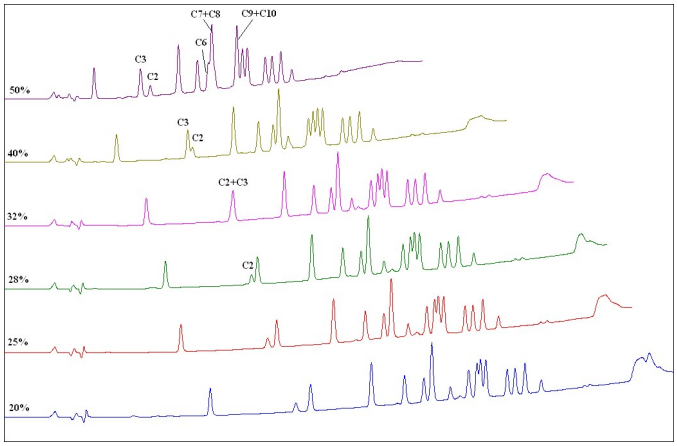

Fig. 5: Effect of a change in initial %Methanol for the gradient separation of the explosives mixture sample. Conditions: (150 × 4.6 mm, 4µm) C_{18} Poroshell 120 column; temperature: 32°C; injection volume = 5 µL, Flow rate: 0.9 mL/min; initial % MeOH: 20, 25, 28, 32, 40 and 50.

It can be inferred that, by increasing the initial percentage % of the organic phase (MeOH), especially starting from 40%, the dilution order varied between EGDN (C2) and RDX (C3). As it is shown in Fig. 5, at 50 %, the peaks of 3,5-DNA and Tetryl overlap which induced the decreasing of the resolution.

TABLE XIV
EFFECT OF INITIAL % IN MEOH ON (k) OF RECORDED PEAKS

Initial %MeOH		**20**	**25**	**28**	**32**		**40**	**50**
	Peak order	**k**				Peak order	**k**	
C1	1	2.1	1.7	1.4	1.1	1	0.7	0.3
C2	2	3.4	3.0	2.7	2.4	3	1.8	1.2
C3	3	3.6	3.1	2.8	-	2	1.8	1.0
C4	4	4.5	4.0	3.6	3.2	4	2.4	1.6
C5	5	5.0	4.4	4.1	3.7	5	2.8	1.9
C6	6	5.3	4.7	4.4	3.9	6	3.0	2.1
C7	7	5.4	4.8	4.5	4.0	7	3.1	2.1
C8	8	5.7	5.1	4.7	4.2	-	3.3	-
C9	9	6.0	5.4	5.0	4.5	8	3.6	2.5
C10	10	6.1	5.5	5.1	4.6	-	3.6	-
C11	11	6.2	5.5	5.2	4.7	9	3.7	2.6
C12	12	6.3	5.6	5.3	4.8	10	3.8	2.7
C13	13	6.6	6.0	5.6	5.1	11	4.1	2.9
C14	14	6.7	6.1	5.7	5.2	12	4.2	3.0
C15	15	6.8	6.2	5.8	5.3	13	4.3	3.2
C16	16	7.1	6.5	6.1	5.6	14	4.5	3.3

TABLE XV
EFFECT OF INITIAL % IN METHANOL ON N OF RECORDED PEAKS

Initial %MeOH		**20**	**25**	**28**	**32**
Compound	Peak order	**N**			
C1	1	25173	18389	14469	10623
C2	2	38054	31584	31527	20142
C3	3	47298	40452	35124	-
C4	4	68290	55231	50332	41692
C5	5	84048	70237	60910	52399
C6	6	98017	81500	72286	62526
C7	7	99605	83001	74647	63395
C8	8	125853	105483	90365	79287
C9	9	122221	105185	95014	84315
C10	10	127185	101584	86813	70866
C11	11	98589	77992	67183	63831
C12	12	128035	111100	102257	90318
C13	13	154715	132560	120192	103323
C14	14	161318	136848	124075	107459
C15	15	165945	141695	127752	111521
C16	16	189782	161170	147357	127396

TABLE XV
EFFECT OF INITIAL % IN METHANOL ON N OF RECORDED PEAKS (CONTINUED)

Initial %MeOH		**40**	**50**
Compound	Peak order	**N**	
C1	1	7674	5890
C2	3	16425	13551
C3	2	19210	10627
C4	4	30198	18041
C5	5	38452	23351
C6	6	44241	42438
C7	7	44456	18294
C8	8	37916	-
C9	9	37737	30126
C10	10	37272	-
C11	11	54038	35293
C12	12	62762	36953
C13	13	76108	46346
C14	14	79384	48612
C15	15	81875	51230
C16	16	91153	56612

TABLE XVI
EFFECT OF INITIAL % IN METHANOL ON (R) OF RECORDED PEAKS

Initial %MeOH		**20**	**25**	**28**	**32**		**40**	**50**
Compound	Peak order	**R**				Peak order	**R**	
C1	1	-	-	-	-	1	-	-
C2	2	15.4	15.6	15.9	14.7	3	0.8	1.9
C3	3	2.5	1.6	1.1	-	2	13.9	9.5
C4	4	10.8	10.3	10.0	8.7	4	7.4	5.6
C5	5	5.9	5.8	5.7	5.5	5	4.8	3.7
C6	6	3.6	3.5	3.4	3.3	6	2.9	2.5
C7	7	1.5	1.4	1.4	1.3	7	1.1	0.7
C8	8	3.6	3.3	3.1	2.8	-	1.7	-
C9	9	3.6	3.6	3.7	3.8	8	3.4	4.4
C10	10	1.5	1.5	1.4	1.3	-	0.7	-
C11	11	0.6	0.6	0.7	0.7	9	0.8	1.1
C12	12	0.9	0.9	0.9	0.9	10	1.0	1.0
C13	13	4.1	4.1	4.2	4.1	11	4.0	3.7
C14	14	1.6	1.5	1.5	1.5	12	1.5	1.4
C15	15	2.0	1.9	1.9	1.9	13	1.9	1.8
C16	16	3.2	3.1	3.1	3.0	14	2.8	2.2

TABLE XVII
EFFECT OF INITIAL % IN METHANOL ON α OF RECORDED PEAKS

Initial % MeOH		**20**	**25**	**28**	**32**		**40**	**50**
	Peak order	**α**				Peak order	**α**	
C1	1	-	-	-	-	1	-	-
C2	2	1.62	1.79	1.93	2.17	3	1.04	1.14
C3	3	1.07	1.05	1.03	-	2	2.61	3.12
C4	4	1.25	1.28	1.29	1.32	4	1.34	1.36
C5	5	1.11	1.12	1.13	1.14	5	1.16	1.18
C6	6	1.06	1.06	1.07	1.07	6	1.08	1.09
C7	7	1.02	1.02	1.02	1.03	7	1.03	1.02
C8	8	1.05	1.05	1.05	1.05	-	1.05	-
C9	9	1.05	1.05	1.06	1.07	8	1.09	1.18
C10	10	1.02	1.02	1.02	1.02	-	1.02	-
C11	11	1.01	1.01	1.01	1.01	9	1.02	1.03
C12	12	1.01	1.01	1.02	1.02	10	1.02	1.03
C13	13	1.05	1.06	1.06	1.07	11	1.08	1.10
C14	14	1.02	1.02	1.02	1.02	12	1.03	1.04
C15	15	1.02	1.02	1.03	1.03	13	1.03	1.04
C16	16	1.04	1.04	1.04	1.04	14	1.05	1.05

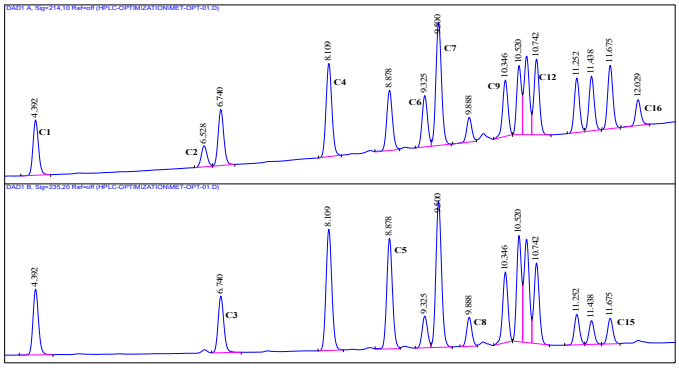

Fig. 6: Chromatogram of separation of 16 explosives after optimization steps.

Based on the above optimization approaches, a compromise given below was obtained for the best separation conditions (Fig. 6):

- Column: Agilent Poroshell 120 EC-120 C_{18} (150 x 4.6 mm, 4µm);
- Mobile phase: MeOH – H_2O;
- Column Temperature: 32°C;
- Liquid phase flow rate: 0.9 mL/min;
- Elution mode: linear gradient 25 à 100% in MeOH during 15 min.

IV. Conclusion

An Agilent Poroshell 120 EC-120 C_{18} (4.6 x 150 mm, 4 µm) was used to provide a means for a rapid screening of a mixture of 16 commercial and military grade explosives. The optimization of relevant chromatographic parameters allowed achieving a fairly acceptable separation of all the compounds in less than 15 minutes except for the amino-dinitrotoluene isomers (2-A-4,6-DNT and 4-A-2,6-DNT and 2,6-DNT) which could not be resolved by the C_{18} column whatever the conditions that were chosen. This method is intended to be applied to the analysis of soils contaminated with explosive residues. The optimized chromatographic technique will be used after solid phase extraction preparation method, in order to purify the samples by eliminating all sorts of contaminations. This activity is currently under way.

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Benmalek BOULESNAM was born on February 21, 1979 in Algeria. In December 2005, he holds a master's degree in Chemical Engineering from the Ecole Nationale Polytechnique and is currently preparing a doctoral thesis on the fast analysis of complex mixtures of explosives. He works within the National Institute of Criminalistics and Criminology (INCC) of the Gendarmerie Nationale as a Judicial Expert in Analysis and Investigation of Fire & Explosives. Since 2018, he has also held the position of Head of the Laboratory for the Analysis of hazardous Chemicals at the INCC.

Fahima HAMI was born on January 07, 1988 in Tizi-Ouzou. In December 2018, she holds a master's degree in Chemical Engineering from the Military Polytechnic School of Bordj El Bahri. She works within the National Institute of

Criminalistics and Criminology (INCC) of the Gendarmerie Nationale as a Judicial Expert in Trace Analysis.

Djalal Trache, born on November 17, 1982 in M'Chedallah (Bouira), has been working as an Associate Professor at Ecole Militaire Polytechnique (EMP), Algeria, since 2016. He is an Associate Editor for Applied Nanoscience and Journal of Nanostructure in Chemistry (Springer) and a member of the editorial board of several reputed journals. He has made several presentations at national and international conferences, published over 100SCI scientific papers in the field of chemical sciences/materials science in mainstream journals such as ChemEng J, Green Chem, CarbohydrPolym, Nanoscale, and Fuel. He has also published eight book chapters and three books. His total citation is more than 3000 times, including 6 ESI highly cited papers with an h index of 27. He is a reviewer for more than 80 international journals in prestigious publishers such as Springer, Nature, Science Direct, Wiley, Taylor and Francis, ACS, and RSC. He has particular expertise in energetic materials, bio-based materials, polymer composites, and their characterizations. He also has interests in nanomaterials and their applications, phase equilibria and kinetics. Besides, he has successfully supervised many engineer, MSc and doctoral students.

Toudert AHMED ZAID, born on August 15, 1954 in Ain El Hammam (Tizi-Ouzou), is Professor at the Chemical Engineering Department (ENP, Algiers). He has an experience of 20 years in the domain of surfactants and dispersed systems. He has authored or co-authored more than thirty papers mainly in this research area.