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

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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 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 C8 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

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\begin{align*}
HPLC & : \text{High Performance Liquid Chromatography} \\
DAD & : \text{Diode Array Detection} \\
FPD & : \text{Flame Photometric Detector} \\
UV & : \text{Ultraviolet} \\
US EPA & : \text{United States Environmental Protection Agency} \\
F & : \text{flow rate (mL/min)} \\
HRMS & : \text{High resolution mass spectrometry} \\
MeOH & : \text{methanol} \\
k & : \text{capacity factor} \\
\alpha & : \text{selectivity factor} \\
N & : \text{number of theoretical plates} \\
R & : \text{resolution} \\
t_R & : \text{retention time (min)} \\
t_G & : \text{gradient time (min)} \\
XRD & : \text{X-ray diffractometry} \\
XRF & : \text{X-ray fluorescence spectroscopy} \\
\Delta t & : \text{difference in retention times for two peaks (min)} \\
\alpha & : \text{selectivity factor} \\
R & : \text{resolution} \\
N & : \text{number of theoretical plates} \\
G & : \text{gradient range, equal to the final value of } G \text{ in the gradient } (G) \text{ minus the initial value } (G_0) \\
\Delta G & : \text{gradient range, equal to the final value of } G \text{ in the gradient } (G) \text{ minus the initial value } (G_0) \\
\theta & : \text{volume fraction of B solvent in the mobile phase} \\
\Omega & : \text{value of } \theta \text{ for mobile phase at end of gradient} \\
\Omega_0 & : \text{value of } \theta \text{ for mobile phase at start of gradient}
\end{align*}
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1. 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 explosive substances by optimizing the separation through detector (HPLC/DAD) for the rapid separation of a mixture of sixteen (16) explosive substances with an adequate peak theoretical plates.

TABLE I. EXPLOSIVE COMPOUNDS UNDER STUDY

| Compounds Description | Abbreviations |
|-----------------------|---------------|
| Nitramine compounds   |               |
| 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)  |
| Nitroamines          |               |
| 2,4,6-Trinitrophenylmethyl nitramine | Tetryl (C8) |
| 2,4,6-Trinitoluene   | 2,4,6-TNT (C9) |
| 4-Amino-2,6-Dinitroguanidine | 4-A-2,6-DNT (C10) |
| 2-Amino-4,6-Dinitroguanidine | 2-A-4,6-DNT (C11) |
| 2,6-Dinitroguanidine | 2,6-DNT (C12) |
| 2-Nitroguanidine     | 2-N (C13)     |
| 4-Nitroguanidine     | 4-N (C14)     |
| 3-Nitroguanidine     | 3-N (C15)     |

NITRO EXTRAS

Ethylene glycol dinitrate | EGDN (C2) |

Pentaerythritol tetranitrate | 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-18 (4.6 x 150 mm, 4 μm). The mobile phase was a MeOH – H₂O 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 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 C₁₈ 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 Δtᵣ/₉₀ 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 C18 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.

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 (tG), affects the capacity factor (k). The usual separation goal is to reach k ≤ 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 tG on the peak separation whereas Table II, Table III and Table IV display the effect of tG on the capacity factors (k), the number of theoretical plates (N), the resolutions (R) and selectivity (α) of each detected peak.

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, 4-A-2,6-DNT) and (2,6-DNT, 2-A-4,6-DNT, 4-A-2,6-DNT) respectively. This indicates that tG = 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 C18 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

| tG (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

| tG (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 |

Fig. 1: Chromatogram of the mixture of explosives at 214 nm with concentration of 10 ppm. HPLC conditions: Poroshell 120 EC-120 C18 (4.6 x 150 mm, 4 μm), MeOH-H2O mixture at flow rate of 1.2 mL/min, injection volume = 5 μL, elution mode: linear gradient from 5% to 100% of MeOH in H2O during 15 min, column temperature: 25°C.

Fig. 2: Effect of tG increase on the separation profile. HPLC conditions: Poroshell 120 EC-120 C18 (4.6 x 150 mm, 4 μm), MeOH-H2O mixture at flow rate of 1.2 mL/min, injection volume = 5 μL, elution mode: linear gradient from 5% to 100% of MeOH in H2O for 15, 30 and 45 min, column temperature: 25°C.
TABLE IV
INFLUENCE OF tG ON R AND α

| tG (min) | 15 | 30 | 45 |
|----------|----|----|----|
| Compound     | R α | R α | R α |
| HMX            | -   | -   | -   |
| EGDN          | 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-H2O 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 H2O. 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-H2O mixture at flow rate of 1.2 mL/min, injection volume = 5 µL, elution mode: linear gradient from 5% to 100% of MeOH in H2O for 15 min.

TABLE V
INFLUENCE OF COLUMN TEMPERATURE ON k.

| T (°C) | 25 | 28 | 30 | 32 | 35 | 38 |
|--------|----|----|----|----|----|----|
| Compound  | Peak order | k |
| HMX      | 4.0 | 3.9 | 3.8 | 3.7 | 3.6 | 3.5 |
| EGDN     | 5.0 | 4.9 | 4.9 | 4.8 | 4.8 | 4.7 |
| RDX      | 7.4 | 7.3 | 7.2 | 7.2 | 7.1 | 7.0 |
| 1,3,5-TNB| 7.7 | 7.5 | 7.4 | 7.3 | 7.2 | - |
| 1,3-DNB  | 7.9 | 7.8 | 7.7 | 7.6 | 7.5 | 7.4 |
| NB       | 8.2 | 8.1 | 8.0 | 7.9 | 7.8 | 7.7 |
| 3,5-DNA  | 8.4 | 8.3 | 8.2 | 8.1 | - | - |
| Tetryl   | 8.7 | 8.6 | 8.5 | 8.4 | 8.3 | - |
| 2,6-DNT  | 8.9 | 8.8 | 8.7 | 8.6 | 8.5 | 8.4 |
| 2-NT     | 9.0 | 8.9 | 8.8 | 8.7 | 8.6 | 8.6 |
| 4-NT     | 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      | 50316 | 49762 |
| EGDN     | 49494 | 50174 |
| RDX      | 74861 | 72544 |
| 1,3,5-TNB| 96123 | 97881 |
| 1,3-DNB  | 114878 | 113353 |
| NB       | 129999 | 131215 |
| 3,5-DNA  | 135268 | 134113 |
| Tetryl   | 158320 | 164861 |
| 2,4,6-TNT| 157171 | 160916 |
| 4-A-2,6-DNT| 177625 | 167242 |
| 2-A4,6-DNT| 172478 | 171589 |
| 2,6-DNT  | - | - |
| 2-NT     | 206239 | 200383 |
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 | 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 | - | - |
| Tetryl | 8 | 2.7 | 3.2 | 3.5 | 3.8 | 4.1 | 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 | 1.1 | 1.1 | 1.09 | 1.08 | 1.07 |
| 1,3,5-TNB | 4 | 1.18 | 1.19 | 1.2 | 1.22 | 1.23 | 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 | 1.05 | 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 | 1.04 | 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 | - | - |

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 | 14.39 | 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 |

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.
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 $\Delta \phi$, thus holding $(\Delta \phi / t_G)$ 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 ($\alpha$) 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 C18 column.
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**

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

**TABLE XV**

\[
\begin{array}{cccccc}
\text{Initial % MeOH} & 20 & 25 & 28 & 32 & 40 & 50 \\
\hline
\text{Compound} & \text{Peak order} & \text{N} & \text{R} \\
C1 & 1 & - & - & - & - & - \\
C2 & 2 & - & - & - & - & - \\
C3 & 3 & - & - & - & - & - \\
C4 & 4 & - & - & - & - & - \\
C5 & 5 & - & - & - & - & - \\
C6 & 6 & - & - & - & - & - \\
C7 & 7 & - & - & - & - & - \\
C8 & 8 & - & - & - & - & - \\
C9 & 9 & - & - & - & - & - \\
C10 & 10 & - & - & - & - & - \\
C11 & 11 & - & - & - & - & - \\
C12 & 12 & - & - & - & - & - \\
C13 & 13 & - & - & - & - & - \\
C14 & 14 & - & - & - & - & - \\
C15 & 15 & - & - & - & - & - \\
C16 & 16 & - & - & - & - & - \\
\end{array}
\]

**TABLE XVI**

\[
\begin{array}{cccccc}
\text{Initial % MeOH} & 20 & 25 & 28 & 32 & 40 & 50 \\
\hline
\text{Compound} & \text{Peak order} & \text{N} & \text{R} \\
C1 & 1 & - & - & - & - & - \\
C2 & 2 & - & - & - & - & - \\
C3 & 3 & - & - & - & - & - \\
C4 & 4 & - & - & - & - & - \\
C5 & 5 & - & - & - & - & - \\
C6 & 6 & - & - & - & - & - \\
C7 & 7 & - & - & - & - & - \\
C8 & 8 & - & - & - & - & - \\
C9 & 9 & - & - & - & - & - \\
C10 & 10 & - & - & - & - & - \\
C11 & 11 & - & - & - & - & - \\
C12 & 12 & - & - & - & - & - \\
C13 & 13 & - & - & - & - & - \\
C14 & 14 & - & - & - & - & - \\
C15 & 15 & - & - & - & - & - \\
C16 & 16 & - & - & - & - & - \\
\end{array}
\]
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