ISSN 1230-3801 Zeszyt 130 nr 2/2014, 79-87

VALIDATION OF THE ASSAY AND PURITY DETERMINATION OF THE FOX-7 (1,1-DIAMINO-2,2-DINITROETHYLENE) USING HPLC METHOD

Agata KAMIEŃSKA DUDA, Piotr KASPRZAK
Military Institute of Armament Technology

Abstract:1,1-diamino-2,2 dinitroethylen (DADNE, FOX-7) is a novel explosive with low sensivity. The valuable properties is a result of specific chemical construction, in which inter- and intramolecular hydrogen bonds stabilize the structure. From the reason of low sensivity, FOX-7 has a very broad spectrum of use for elaborating many kinds of munnition. Hence a very important aspect is to conduct the research of this substance to determine chemical purity, assay content of water etc. The HPLC method is the one of the analytical technique which has a very high precision of determination of the purity and assay of the substance. The aim of this work was to compile a new method of the assay and purity determination of FOX-7. The C18-Xbridge column and the mobile phase: Acetonitryle: Water: Triethylamine were used. Through application of the C18- XBridge column and mobile phase the substrate MPD and main substance FOX-7 had very good resolution and the analysis time was relatively short. The validation of the new method has been performed: selectivity, specificity, linearity, precision, assay and limits of detection and quantitation. We have also checked the stress tests of the FOX-7 in acidic, basic and oxidative conditions.

Keywords: validation, FOX-7, DADNE, HPLC method, assay, purity

WALIDACJA OZNACZANIA CZYSTOŚCI I ZAWARTOŚCI FOX 7 (1,1-DIAMINO-2,2-DINITROETYLENU) METODĄ HPLC

Agata KAMIEŃSKA DUDA, Piotr KASPRZAK
Wojskowy Instytut Techniczny Uzbrojenia

Streszczenie:1,1-diamino-2,2 dinitroetylen (DADNE, FOX-7) jest nowoczesną małowrażliwą substancją wybuchową. Jej cenne właściwości są rezultatem specyficznej budowy chemicznej, w której wewnątrz i zewnątrz cząsteczkowe wiązania wodorowe stabilizują strukturę, powodując jej małą wrażliwość na bodźce. Dzięki takim właściwościom, FOX-7 może być zastosowany do elaboracji wielu rodzajów małowrażliwej amunicji. Dlatego tak ważnym aspektem jest prowadzenie badań chemicznych uwzględniających czystość i zawartość chemiczną tej substancji. Metoda HPLC jest nowoczesną techniką, szeroko stosowaną do określenia tych parametrów z dużą dokładnością i precyzją. Celem naszej pracy było zwalidowanie nowej metody oznaczania czystości i zawartości FOX-7. Do oznaczania tych parametrów użyto kolumny C-18 X-Bridge natomiast jako fazy ruchomej mieszaniny acetonitrylu trietyloaminy: wody zmieszanej w odpowiednim stosunku. Zastosowanie właśnie takiej kolumny oraz fazy z modyfikatorem pozwoliło na podzielenie zanieczyszczenia MPD od substancji głównej w odpowiednio krótkim czasie z dobrą rozdzielczością oraz symetrią pików. Walidacja metody obejmowała: selektywność, specyficzność, precyzję, dokładność oraz limity detekcji oraz oznaczalności. Podczas walidacji zostały również przeprowadzone testy stresowe w warunkach kwaśnych, zasadowych oraz utleniających.

Słowa kluczowe: walidacja, FOX-7, DADNE, metoda HPLC czystość, zawartość

1. Introduction

Several studies performed in the past decade have indicated increasing interest in the synthesis and characterization of highly energetic materials with low sensitivities. The FOX-7 is one of these energetic materials, which is strong dipolar and very stable material which decomposes only above 220 °C [1-4]. Latypov was the first team which synthesized 1,1-diamino-2,2-dinitroethylene with density 1.885 g/cm^3 and heat formation of 32 kcal/mol. DADNE was prepared by nitration of 2-methyl imidazole with concentrated nitric and sulphuric acid to give a mixture of parabanic acid and 2-(dinitromethylene)-4,5-imidazolidinedione. This latter compound was further treated with aqueous ammonia solution to produce DADNE [4-6]. There are also a few recent reviews on its synthesis [7-8]. The assay and purity of FOX-7 determination plays very important role in controlling the process of synthesis and also during the process of exploitation. The TLC method was commonly used to control of the synthesis process of this substance [9]. In the literature we also found only few publication about controlling purity of FOX-7 and some intermediate products using HPLC method, where PGC Hypercarb column was used [10-12].
The certain aim of this work was to find a fast and less expensive method by which we could determine purity and assay at the same time.

The C18 X-Bridge column was used in the presented method. The C18 column is a very popular column on which we can separate many chemical substances with different chemical properties [13-14]. The main problem was to separate FOX-7 and its intermediates because of extremely polar properties of these substances. Application of a modifier triethylamine to mobile phase gave us the chance to separate the substrate MPD (2-methylpyrimidine-4,6-dione) from the main substance FOX-7 in quite good retention time.

2. Materials and Methods

Reagent and chemicals

The FOX-7-working standard and intermediate MPD were kindly donated by Military University of Technology, Warsaw Poland. Acetonitryle (ACN, HPLC gradient grade), water (gradient grade), Triethylamine (TEA, 99,0% purity) were obtained from Merc (Darmstad, Germany).
Mobile phase were filtred through 0.45μm, 47 mm membrane filters from Millipore (Millipore Corporation, USA)

Solution preparation

The stock solutions of the MPD and FOX-7 were dissolved in the solution of ACN: water: TEA, in the ratio 50:50:0.1 (v/v/v). The concentration for assay and purity test of FOX-7 was 0,4 mg/mL. The concentration for linearity of MPD and FOX-7 in purity test was in the range of 0.0002 mg/mL – 0.0048 mg/mL. The concentration for FOX-7 in assay test was in the range of 0.32 mg/mL - 0.60 mg/mL. For accuracy of MPD of the purity test the concentration was in the range of 0.00032 mg/mL – 0.00048 mg/mL and the concentration of the main substance on each level of MPD was 0,4 mg/mL in this mixture. In accuracy of FOX-7 of assay test the concentration of the FOX-7 was in the range of 0.24 - 0.48 mg/mL. Perpetrated solutions were obtained by dilution with the same dissolvent as stock solution.

Chromatographic system and conditions

HPLC analyses were performed using a Waters Alliance Liquid Chromatogram with PDA detector set at 270 nm. The software Empower 2.0 was used for instrument control and data

acquisition. The Waters X-Bridge C18 column 250mm length, 4,6 mm diameter and with 5µm pore size, resistant to pH range 1-12. The injection volume was 20µL.
Purity and assay test were performed in the same condition.
- Temperature of the column was 25°C.
- The mobile phase was:
  - phase A: ACN:TEA in the ratio (100:0,1), (v/v);
  - phase B: water : TEA in the ratio (100:0,1), (v/v).

The gradient shown in table 1 was used to separate the substances.

Table 1. Gradient for the HPLC analyze of FOX-7

Time [min]	Flow [mL/min]	Phase A [%]	Phase B [%]
	0.8	75.0	25.0
10	0.8	65.0	35.0
12	0.8	75.0	25.0
17	0.8	75.0	25.0

3. Results and discussion

Optimization of the chromatographic condition

Best chromatographic conditions were achieved by optimizing the wavelength of detection, mobile phase composition and a flow rate. Chromatographic conditions were optimized to achieve appropriate plate numbers, a peak symmetry, resolution and a tiling factor.

Method validation procedure

The method for determination of related substance of MPD in FOX-7 was validated in order to demonstrate that the developed technique is suitable for its intended purpose, i.e. content and determination of relative concentration of impurities in the explosive material.
The validation consisted of studying the system suitability test, selectivity, linearity, precision, accuracy, robustness and the limits of detection and quantitation of the method according to PN-EN ISO/IEC 17025 [15]. We also checked the stability of FOX-7 in acidic, oxidative and basic conditions [16].
System suitability test

The average number of theoretical plates per column was >4500, the USP tailing factor <1,4 and the resolution between the MPD and FOX-7 was >2. The chromatogram shown in Fig 1. presents the separated peaks of FOX-7 and MPD.

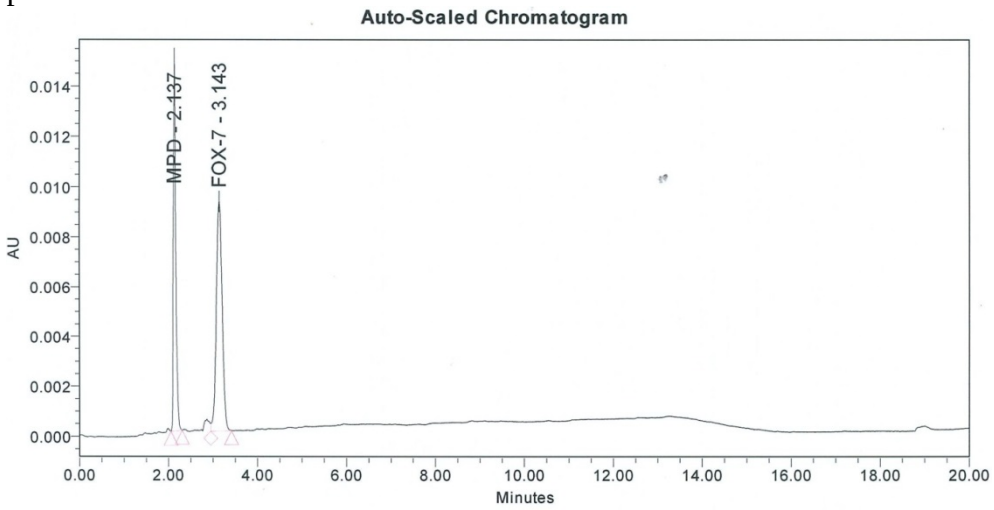


Fig.1. Chromatogram of FOX-7 and MPD of the sst solution

Specificity and selectivity

The specificity of the method was checked by using standard samples of FOX-7, solution of impurity coming from synthesis path and the background control sample. The selectivity of the method was checked by subjecting the samples of FOX-7 in order to stress conditions with involved oxidative, basic and acidic environment [16].

The comparison of the chromatograms obtained with these different solutions confirmed that there was no interference between the FOX-7 and its related substance MPD. The stress test results of the FOX-7 are presented in table 2.

Table 2. Purity of FOX-7 subjected to stress condition

Purity [%]		
0,1M HCl 3h, 25°C	**1M HCl 3h, 25°C**	**0,1 M HCl 3h 60°C**
MPD=0,55	MPD=1,01	MPD=3,84
3% H_2O_2 , 3h, 25°C	**9% H_2O_2 , 3h, 25°C**	
MPD=6,01	MPD=11,8	
0,1M NaOH, 3h, 25°C	**0,1M NaOH, 3h, 60°C**	
MPD=0,57	MPD=7,72	

In all stress conditions the main substance FOX-7 degrade to MPD. In the checked method we didn't notice any others impurities. We have also checked the peak purity of pure FOX-7 see fig 2 and FOX-7 under different conditions. In all cases the purity angle was under purity threshold, which suggests that under the main peak any other peak coming from degradation didn't appear. The acid stress illustrate, that there was not a large decomposition of FOX-7 in the 0,1M and 1M HCl, 25°C, 3h condition. The FOX-7 subjected to 0,1M HCl, 60°C, 3h condition degraded to 3,84% of MPD. In oxidative condition FOX- 7 was not stable. The assay of MPD increased from 6,01 % to 11,8% in 3% H2O2, 3h, 25°C and 9% H2O2, 3h, 25°C relatively. In basic condition only 0,1M NaOH, 3h, 25°C FOX-7 was stable, the assay of MPD was 0,57%. But the increasing of temperature to 60°C caused that FOX-7 decomposed to 7,72 % of MPD.

Linearity

Linearity was observed when mean response area was plotted against concentration, using the last square and regression method (Table 3). Three standard solutions were analyzed at six different concentration levels from 0.00032 mg/mL – 0.00048 mg/mL for MPD and FOX-7 for purity test and 0.24 - 0.48 mg/mL for assay test of FOX-7. Criterion of approval [15] has been as follows: correlation coefficient $R \geq 0.995$ – for low concentration in purity method and $R \geq 0.998$ for high concentrations in assay method. Results of the linearity test in the assay and purity determination of FOX-7 are shown in table 3.

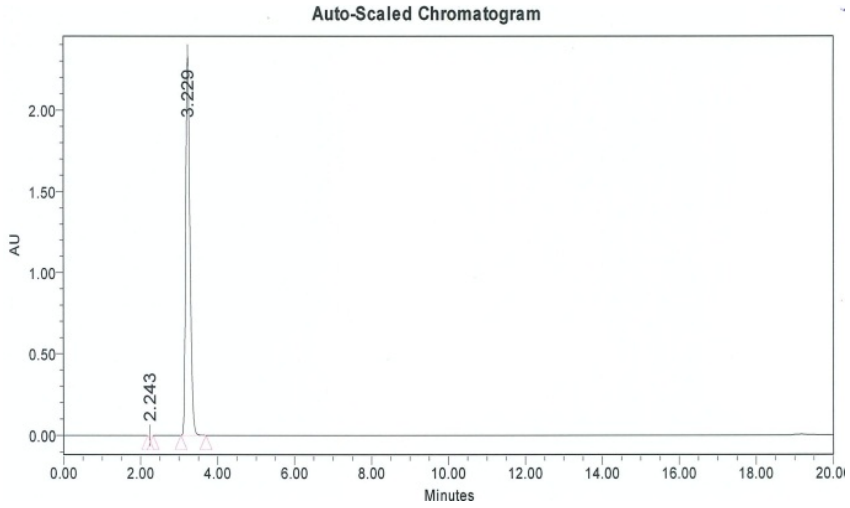
Limits of detection and quantitation.

The limits of detection and quantitation were determined on the basis of standard deviation of the response (y-intercept) (S_{yx}) and slope of the calibration curve at low concentration levels (a) according to equation (1), and (2). The results of DL, and QL for MPD and FOX-7 are presented in table 4. The chromatogram of FOX-7 and MPD on the level of detection limit are shown in Fig.3

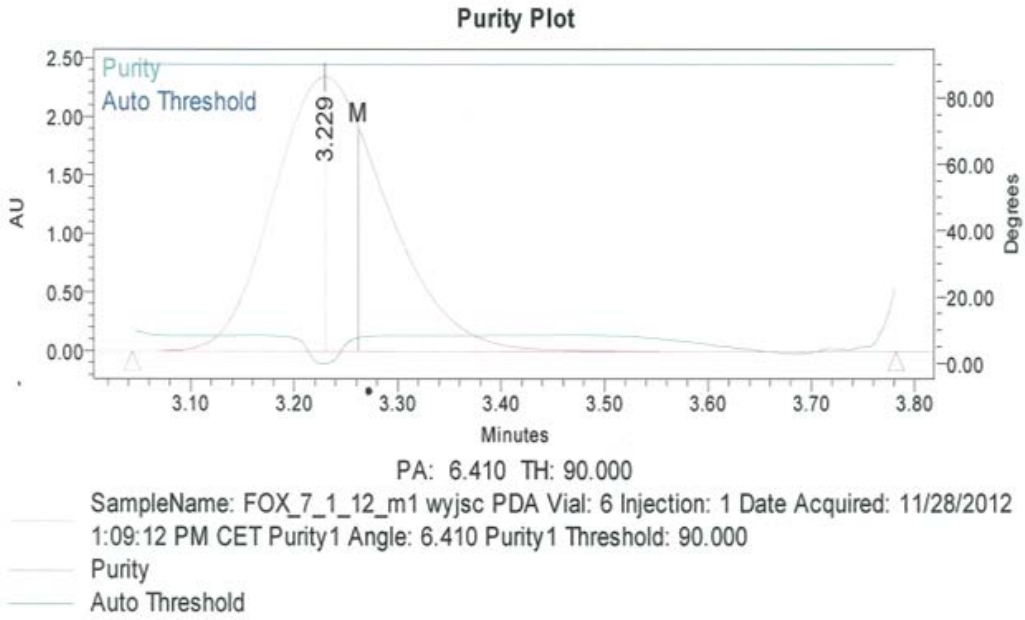
Precision

Precision of the analytical method has been described in a quantitative way by relative standard deviation obtained by establishing the ratio between the standard deviation and the mean chromatographic response. RSD for HPLC method is a function of random errors arising from the column, the injector and the integrator device. Furthermore, precision has also been affected by solutions preparation steps. The comparison of both series results was performed with the test F- Snedecor.The criteria of approval [15] have been as follow:

a)



a)



c)

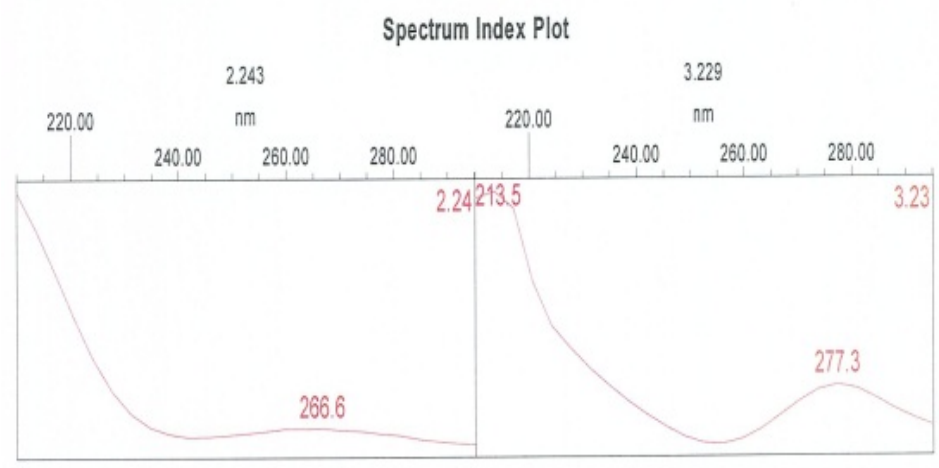


Fig. 2. a) Chromatogram of the examined FOX-7, b) Results of the peak purity of examined FOX-7, c) UV spectrum of MPD (retention time 2.24 min) and FOX-7 (retention time 3.2 min)

Table 3. Results of the linearity test in the assay and purity determination of FOX-7

Test	Substance	Statistical parametrs		
		Coefficient of determination R^2	Regression equations	Residual Standard Deviation (RSD)
Purity	FOX-7	0.995	y = 26634488 x - 148	302.98
	MPD	0.996	y = 32438758x + 433	514.77
Assay	FOX-7	0.999	y = 30089097x +55508	81290.44

$$QL = \frac{10 \cdot S_{yx}}{a}; \qquad (1)$$

$$DL = \frac{3.3 \cdot S_{yx}}{a}; \qquad (2)$$

RSD within one group should not exceed 5 %, RSD between groups should not exceed 10 %, the F-test calculated from Eq.3, F value $\leq F_{a,f1,f2}$ (value read from F-Snedecor distribution boards for 6 measurements $F_{a,f1,f2}$=5,05, µ=0,05).

$F=RSD_1^2/RSD_2^2$ were (S1> S2); (3)

Repeatability

Six consecutive injections of standard solutions of FOX-7 c=0,4 mg/mL were analyzed.

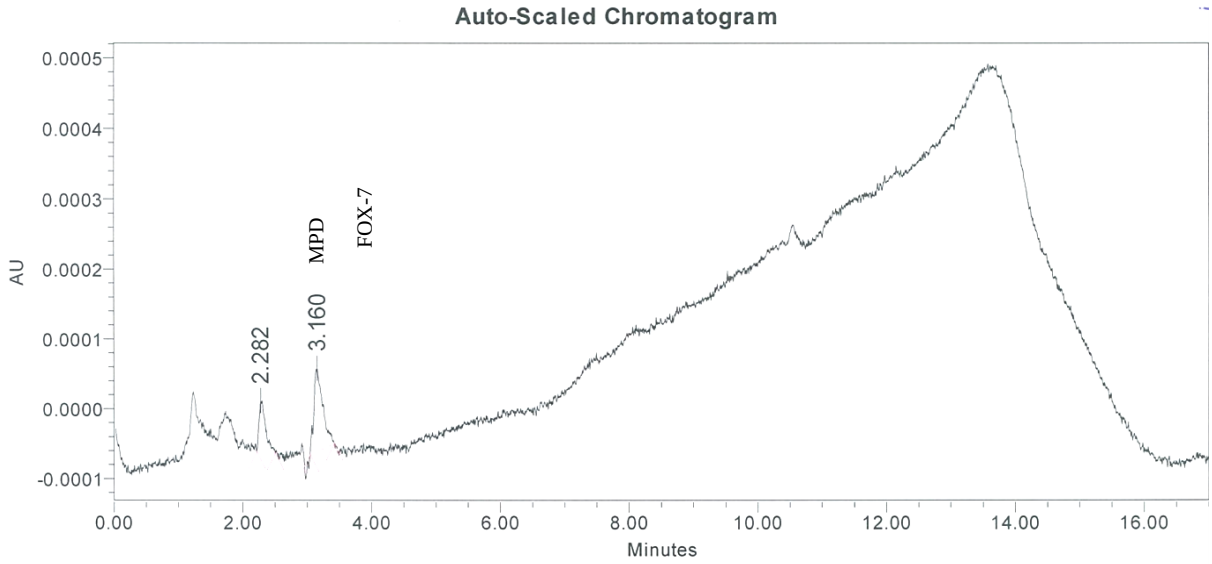


Fig. 3. Chromatogram of FOX-7 and MPD on the level of DL.

Table 4. Results of DL, QL test for MPD and FOX-7

Substance	DL	QL
FOX-7	0.04 [µg/ml]	0.11 [µg/ml]
MPD	0.05 [µg/ml]	0.16 [µg/ml]

Intermediate precision

Six consecutive injections of standard solutions of FOX-7 c=0,4 mg/mL on two different days by different analyst and different column and reagents were performed to evaluate the intermediate precision of the method. Results of the repeatability and the intermediate precision are presented in table 5.

Table 5. Precision of the assay and purity method of the FOX-7.

No	Purity		Assay	
	Assay of MPD Repeatability [%]	**Assay of MPD Intermediate Precision [%]**	**Assay of FOX-7 Repeatability [%]**	**Assay of FOX-7 Intermediate Precision [%]**
1	0.56	0.57	99.7	100.7
2	0.56	0.57	98.9	100.5
3	0.57	0.57	99.6	101.1
4	0.55	0.57	99.6	100.3
5	0.55	0.57	98.8	101.5
6	0.56	0.56	99.8	99.3
Mean	0.56	0.57	99.4	100.6
SD	0.01	0.003	0.43	0.76
RSD	0.94	0.53	0.43	0.75
RSD between groups	0.93		0.81	
F-test	3.15		3.04	

All acceptance criteria in method precision were fulfilled.

Accuracy

Accuracy of the purity method was determined basing on tests of the related substance of FOX-7 in solutions. The solutions were containing appropriate quantities of main substance and its impurity (MPD) . The analysis was carried out as specified in the validated method and then the % recovery was calculated. Accuracy study was determined by comparison of two values: the relative concentrations for samples of FOX-7 and known impurity MPD and relative concentrations obtained in the experiment for these substances. Criteria of approval [15] were as follows: Mean recovery value should be within the range of 95 - 105 % for purity method, and 98-102% for assay method. Each recovery value should be within a range of confidence interval (Mean ± 2SD). The results of accuracy for assay and purity method are collected in table 6 and 7.

Table 6. Results of accuracy for known impurity of the FOX-7

Theoretical relative concentration of MPD [%]	Theoretical relative concentration of MPD [mg/ml]	Concentration of MPD in solution [mg/ml]	Recovery [%]	Mean [%]
0.80	0.000358	0.000364	101.7	101.6
	0.000383	0.000387	101.1	
	0.000334	0.000341	102.1	
0.10	0.000447	0.000422	94.5	96.2
	0.000479	0.000477	99.6	
	0.000418	0.000395	94.5	
0.12	0.000536	0.000509	94.9	96.8
	0.000575	0.000569	98.9	
	0.000502	0.000484	96.5	
Mean				98.2
SD				3.13
RSD				3.19
Mean ± 2SD				91.9 - 104.46

Table 7 Results of accuracy for main substance FOX-7

Theoretical relative concentration of FOX-7 [%]	Theoretical relative concentration of FOX-7 [mg/ml]	Concentration of FOX-7 in solution [mg/ml]	Recovery [%]	Mean [%]
80	0.3208	0.3160	98.5	98.8
	0.3236	0.3205	99.1	
	0.3232	0.3197	98.9	
100	0.4036	0.4032	99.9	99.6
	0.4024	0.3986	99.1	
	0.4084	0.4077	99.8	
120	0.4856	0.4866	100.2	99.5
	0.4832	0.4791	99.2	
	0.4824	0.4789	99.3	
Mean				**99.3**
SD				0.43
RSD				0.44
Mean ± 2SD				98.5 - 100.2

In this method for accuracy of FOX-7 all acceptance criteria were fulfilled.

4. Conclusion

The certain aim of this work was to find a fast and less expensive method by which we could determine purity and assay of FOX-7 at the same time despite a considerable polarity of main substance and intermediate (MPD). It was only possible to separate this compounds on a C18-XBridge column using TEA as a modifier to a mobile phase.

Despite the MPD retention time was quite short, the validation provides that separation method of this two compounds was suitable. We have checked the specificity of the method and there wasn't any unknown impurities coming from main substance or mobile phase which could impose with the MPD peak. After the purity and assay of FOX-7 has been checked, the method proved to be appropriately selected and meet acceptance criteria. Such parameters as symmetry factor, resolution, theoretical plates were satisfactory. During the process of validation the stability of MPD and FOX-7 solutions was also examined. The results show that these solutions were stable for at least 48h in room temperature.

Conducted stress tests prove FOX-7 stability in 0,1M NaOH and 0,1M HCl w 3h, in the temperature of 25°C.

FOX-7 and MPD are linear in the scope of concentration 0.00032 mg/mL – 0.00048 mg/mL for MPD and FOX-7 for purity test and 0.24 - 0.48 mg/mL. Calculated limits of detection and quantitation amount respectively for MPD - 0.04 [µg/mL], 0.11 [µg/mL] and for FOX-7 - 0.05 [µg/mL], 0.16 [µg/mL]. Moreover, the method seems precise, selective and specific.

5. Acknowledgements

We thank Dr. Zbigniew Chyłek from Institute of Chemistry, Millitary Univercity of Technology for distribution of FOX-7 and MPD substance on which we could prepare our validation.

References

[1] N. Latypov, J. Bergman, A. Langlet, U. Wellmar, U. Bemm, Synthesis and reactios of 1,1-diamino-2,2-dinitroethylene, *Tetrahedron* (54) 11525-11536 1998

[2] N. Latypov, A. Langlet, U. Wellmar, Chemical compound suitable for use as an explosive, intermediate and method for preparing the compound, *US Patent* 6 312 538 B1, Nov. 6, 2001

[3] B. Janzon, H. Bergman, C. Eldsater, C. Lamnevik, H. Ostmark, Ostmark, FOX-7 - a novel, high performance, low vulnerability high explosive for warhead applications, in: *Proceedings of the 20th International Symposium on Ballistics*, Orlando, *FL, USA*, 23–27 September, 2002

[4] G. Herve, G. Jacob, Novel illustrations of the specific reactivity of 1,1-diamino-2,2-dinitroethene (DADNE) leading to new unexpected compounds, *Tetrahedron* (63) 953-959 2007

[5] G. Herve, G.Jacob, N. Latypov, The Reactivity of 1,1-diamino-2,2dinitroethene (FOX-7), *Tetrahedron*, (61) 6743-6748 2005

[6] M. Anniyappan, M.B. Talwar, G.M. Gore, S. Venugopalan, B.R. Gende, Synthesis, characterization and thermolysis of 1,1-diamino-2,2-dinitroethylene (FOX-7) and its salts *J. Hazardous Materials* (137) 813-819 2006

[7] Z. Chyłek, S. Cudziłło, J. Błądek, S. Pietrzyk, Application of Thin Layer Chromatography for Monitoring of FOX-7 Synthesis, Propellants Explos. Pyrotech. (34) 321-325 2009

[8] A. Gindulyte, L. Massa, L. Huang, J. Karle, Proposed mechanism of 1,1-diamino-2,2-dinitroethylene decomposition: A functional theory study, *J. Phys. Chem. A* 1999, (103), 11045-11051

[9] J. Błądek, S. Cudziło, S. Pietrzyk, Z. Chyłek, Zastosowanie TLC do monitorowania syntezy 1,1-diamino-2,2dinitroethen, Biul. WAT, (4) 283-290 2006

[10] H. Ostmark, H. Bergman, U. Bemm, P. Goede, E. Holmgren, M. Johansson, A. Langlet, N. Latypov, A. Pettersson, M.L Pettersson, N. Wingborg, C. Vorde, H. Stenmark, L. Karlsson, M. Hihkio, *32nd International Annual Conference of ICT*, Karlsruhe, Germany, 2001

[11] B. Buszewski, M.Michel, S. Cudziłło, Z. Chyłek, High Performance Liquid Chromatography of 1,1-diamino-2,2-dinitroethene and Some Intermediate Products of its Synthesis, *J. Hazardous Materials*, (164) 1051-1058 2009

[12] E. Holmgren, P. Goede, N. Latypov, Porous Graphitc Carbon (PGC) – A. Convenient Column Packing Materials for HPLC Analysis of FOX-7, *32 nd International Annual Conference of ICT*, Karlsruhe, Germany, 2001;

[13] C. Wang, Z. Guo, Z. Lang, X. Zhang, X. Liang, Overloading study of basic compounds with a positively charged C18 column in liquid chromatography, *J. Chrom. A* (128) 60-66 2013

[14] Repeatability and Reproducibility of retention data and band profiles on reversed phase liquid chromatographic columns: results obtained with symmetry C18 columns, *Tenth Symposium of Column Chromatography*, M. Kele, G.Guiochon *J. Chrom. A* (830) 55-79 1999

[15] PN. EN ISO/IEC 17025:2005

[16] Stability of New Drug Substances and Products - ICH - *Guidance for Industry* Q1A(R2)