INVESTIGATION OF EFFICACY OF A DCBRN-01VN SKIN DECONTAMINANT AGAINST ORGANOPHOSPHATE AGENT

Ngoc Doan Vu[1,*], Minh Dong Le[1], Thi Hien Nguyen[2], Viet Thai Nguyen[1], Phong Le[3]

[1]*Faculty of Physics and Chemical Engineering, Le Quy Don Technical University*

[2]*Faculty of Environment, Vietnam National University of Agriculture*

[3]*Department of Chemistry, Institute of Forensic Sciences*

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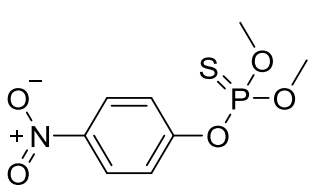
Abstract

This research has been initiated to examine the effectiveness of DCBRN-01VN lotion in degrading some readily available organic phosphorous (OP). UPLC-MS/MS technique was used to measures the amount of residual pesticide via selected ion monitoring. The results showed that lotion effectively degrades OP expediently. The removal efficiency is nearly 100% with MP at 6th min and MT at 2nd min when the molar ratio of KBDO and OP is 10:1. The results *in vivo* test with both solutions (MP and MT) on pig skin showed that the toxic removal efficiency was over 90%, for MP was over 98%.

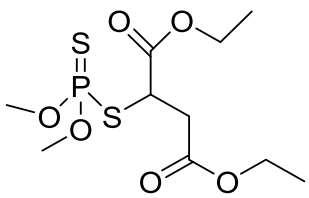
Keywords: *Decontamination; DCBRN-01VN; malathion; methyl parathion.*

1. Introduction

The use of chemical weapons has caused a sizable number of injures and deaths throughout the world in the past century alone, since the time they were first deployed in modern warfare during World War I. Chemical warfare has been long recognized by the defense community as a very real threat to the soldier in the field [1], and a great deal of effort has been devoted to the development of prophylactic and therapeutic measures including skin decontamination. Many potential chemical threats exist, the most likely chemical compounds are still thought to be VX and G-agent [2] (shown in Fig. 1).



Methyl parathion (MP)



Malathion (MT)

Fig. 1. Organophosphorus nerve agents methyl parathion and malathion.

* Email: doanvn@lqdtu.edu.vn

Organophosphorus pesticides are thiols, amides, or esters of phosphonic, phosphonic, phosphoric, or triphosphoric acids with two additional organic side chains of the phenoxy, cyanide, or thiocyanate group [3]. These nerve agents can be classified into four types: G-series agents which were released by Germans include tabun, sarin, soman and V-series agents, where V stands for venomous include VX, VG [4, 5]. Normally, the G-series agents are less toxic compared to the V-series [6]. It is now one of a broader V-series of agents which are classified as nerve agents and have been used as a chemical weapon in various recorded deadly attacks, commonly known as chemical warfare agents [7]. G-type and VX fatalities occur with exposure to tens of milligram quantities via inhalation or absorption through the skin; VX is thus more potent than sarin, another nerve agent with a similar mechanism of action. On such exposure, these agents severely disrupt the body's signaling between the nervous and muscular systems, leading to a prolonged neuromuscular blockade, flaccid paralysis of all the muscles in the body including the diaphragm, and death by asphyxiation [8, 9]. Most of these organophosphate compounds (OPCs) are inactive in their native form which converts to their active forms by a biotransformation process. This biotransformation process happens through oxidation of different groups such as sulfur group (e.g., parathion and malathion. Organophosphate crosses the respiratory epithelial and dermal membrane easily and gets distributed, especially in fat tissues. Then, the biotransformation, these OPCs can interact and inhibit the enzyme acetylcholinesterase (AChE) which leads to the cholinergic crisis. In addition, it also interacts with certain biomolecules other than the AChE [2]. Hence, more effective treatment strategies have also been critically analyzed to soldier in the field.

The efficacy of RSDL Kit against traditional chemical warfare agents (CWAs) (tabun, sarin, soman, cyclohexyl sarin, V-type organophosphorus nerve agents VR and VX,) has been well characterized in both *in vitro* and *in vivo* studies [10-14]. The RSDL Kit is in use in many countries by both military and first responder personnel. Decontamination of riot control agents in order to alleviate their adverse health effects is of particular interest to these government agencies [15].

Though the Geneva Protocol banning the use of chemical weapons was signed in 1925, many nations and groups continued to develop, produce, and stockpile chemical weapons. This activity escalated further during the Cold War and became a major area of concern for world leaders and the United Nations. The Organisation for the Prohibition of Chemical Weapons (OPCW) is the implementing body for the Chemical Weapons Convention, which entered into force on 29 April 1997. The OPCW, with its

193 Member States, oversees the global endeavors to permanently and verifiably eliminate chemical weapons [15]. Although substantial progress has been made in addressing chemical weapon threats, recent activity underscores the need for vigilance and preparedness against chemical attacks.

The current study was undertaken to determine the DCBRN-01VN (new Kit lotion was synthesized in Lequidon laboratory) lotion reactivity against two selected OPCs: methyl parathion and malathion. This study uses LC-MS to further examine the effectiveness of DCBRN-01VN lotion in inactivating the chemicals at several molar ratios of KDBO to test articles.

2. Experiment

2.1. Chemicals and Equipment

Reagents were purchased from commercial suppliers and used as received. DCBRN-01VN (A lotion containing potassium 2,3-butanedione monoxime (KBDO) in monomethoxypolyethylene glycol 550 (MPEG 550) with 0.1722g KBDO per mL) was prepared in the laboratory of Le Quy Don technical university. Acetonitrile, methanol, formic acid, ammonium acetate were purchased from Merck; methyl parathion 98%, malathion 56% and these solution standard 1000 ppm obtained from Macklin in China. A working solution of methyl parathion (as a G-type) 50.0 mg/mL was prepared from methyl parathion 98% and methanol; the working solution of malathion (as a VX type) 0.05 M was prepared from malathion 56% and methanol. Water used in these experiments was purified using a Barnstead International Nanopure Diamond UV Series 1191 ultrapure water system (Barnstead International Inc., Dubuque, IA, USA) to a resistance of 18 MΩ, adsorbents; octadecyl chemically bonded phase silica gel (C18, 43-60 um). Single column, up to 2.1 mm internal diameter (I.D.), up to 100 mm in length with filter or guard column.

2.2. Equipment

MP and MT were detected and quantified by an UPLC-MS/MS system in Academy for Green Growth in Vietnam National University of Agriculture. An Acquity UPLC H-class plus/Xevo TQ-S micro/ultra performance liquid chromatography - mass selective detector (LCMSD) instrument - Tandem Quadrupole Mass Spectrometer (Waters cops in America), controlled by a PC running MASSLYNX Version 4.2 SCN1001, was used for generating LC-MS data, and data processing was carried out using MASSLYNX PROJECT. All mass spectrometer data were acquired using

atmospheric pressure chemical ionization sources in positive ion modes. The gradient program is shown in Table 1.

Table 1. The gradient program

Entry	Time (min)	Flow	Rate (mL/min)			
			%A	%B	%C	%D
1	Initial	0.250	98.0	0.0	0.0	2.0
2	9.00	0.250	2.0	0.0	0.0	98.0
3	10.00	0.250	2.0	0.0	0.0	98.0
4	10.00	0.250	98.0	0.0	0.0	2.0
5	11.00	0.250	98.0	0.0	0.0	2.0

The mobile phase was composed of a mixture Solvent A: Water + 0.1% HCOOH; Solvent B: ACN + 0.1% HCOOH; Solvent C: Acetonitrile; Solvent D: Methanol; Low Pressure Limit: 0.000 psi; High Pressure Limit: 15000.000 psi; Seal Wash Period: 5.00 min; Flow Ramp Rate: 0.45 min.

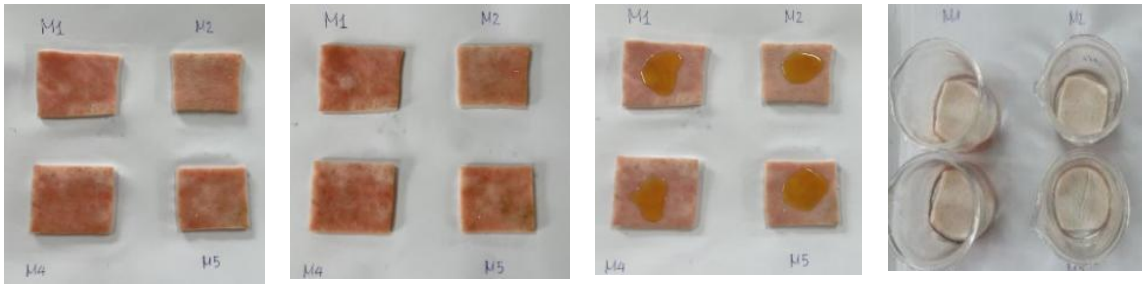
2.3. Liquid phase reaction (in vitro test)

Reactions of 0.5 mL MP 0.19 M or MT 0.05 M with V mL of DCBRN-01VN 1.25 M correspond to molar ratio 1:3; 1:5 and 1:10 were carried out in a glass reaction vial at room temperature and mixed using a magnetic stir bar. Following the desired elapsed time of reaction, acetic acid was added to the vial to quench the reaction. The concentration of acetic acid was added much more than 10 times the molar concentration of KBDO. Dilution of the samples with methanol minimized the ion suppression effect of MPEG (DCBRN-01VN) on pesticide detection by LC-MS. For the liquid organophosphate compounds (methyl parathion, malathion), preparation of calibration and reaction time: 1 min, 2 min, 3 min, 4 min, 6 min.

2.4. In vivo test

Reactions were carried out on a pig skin with a rectangle shape (4 cm × 5 cm) as shown in Fig. 2. The test rate of malathion and methyl parathion solution treatment with DCBRN-01VN material at the ratio of 1:10; the reaction time is 5 minutes. Add 500 μL of 0.19 M MP solution to the center of the pig skin, then continue to drip 760 μL of the test solution (DCBRN-01VN) onto the spot where methyl parathion was applied (samples M1_TN and M2_TN). Add 500 μL of 0.05 M MT solution to the center of the pig skin, then continue to drip 200 μL of the test solution (DCBRN-01VN) onto the spot where

malathion was applied (samples M4_TN and M5_TN). Use a small glass rod to evenly disperse the treatment material solution and the MT solution, then let it stand for 5 minutes. After the reaction time, wipe the surface of the pig skin with a cotton swab and place the entire sample in a beaker containing 20 mL of MeOH so that the sample is submerged in the solvent. After carrying out ultrasonic extraction for 30 minutes at normal temperature, the sample solution was obtained. The sample solution was filtered through a 0.25 mm filter and diluted several times in methanol to become the measuring solution.



Initial sample *Toxify* *Detoxify* *Extract*

Fig. 2. Pig skin was a reaction with OP nerve agents.

2.5. Calibration line

The malathion and methyl parathion calibration curve consists of standard solutions with a concentration of 20 ppb; 40 ppb; 80 ppb; 160 ppb and 240 ppb (MP) or 20 ppb; 40 ppb; 80 ppb; 160 ppb and 320 ppb (MT); prepared from 1000 ppb standard solution and methanol. Calibration lines were shown in Fig. 3.

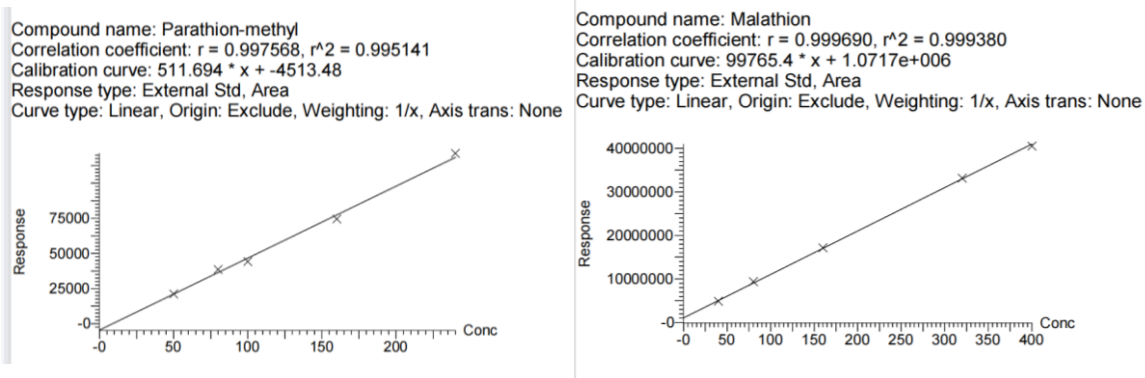


Fig. 3. Calibration curve of organophosphorus.

3. Results and discussion

The 0.05 M (MT) and 0.19 M (MP) working solution were diluted to several times with methanol to the measuring solution, the UPLC-MS/MS results were confirmed concentration was 16,186 ppm and 50,250 ppm, and the dilution factor is 40,000 times and 125,000 times, respectively.

3.1. Efficacy of A DCBRN-01VN against nerve agents

3.1.1. Malathion and methyl parathion were treated with DCBRN-01VN

Figure 4 shows the reaction results of MT and MP with DCBRN-01VN at different rates. As seen, processing performance is very good with the reaction ratio 1:5 and 1:10, the highest processing efficiency reaching 100% with the ratio 1:10 at 6th min, especially MT complete reaction at 2nd min. After 1 minute, the treatment efficiency reached 97.5% with the ratio 1:10 and over 80% with the ratio 1:5. Thus, MT and MP are decomposed quite quickly by DCBRN-01VN material with ratio 1:10. The LC-MS/MS chromatograms of malathion was shown in Fig. 5.

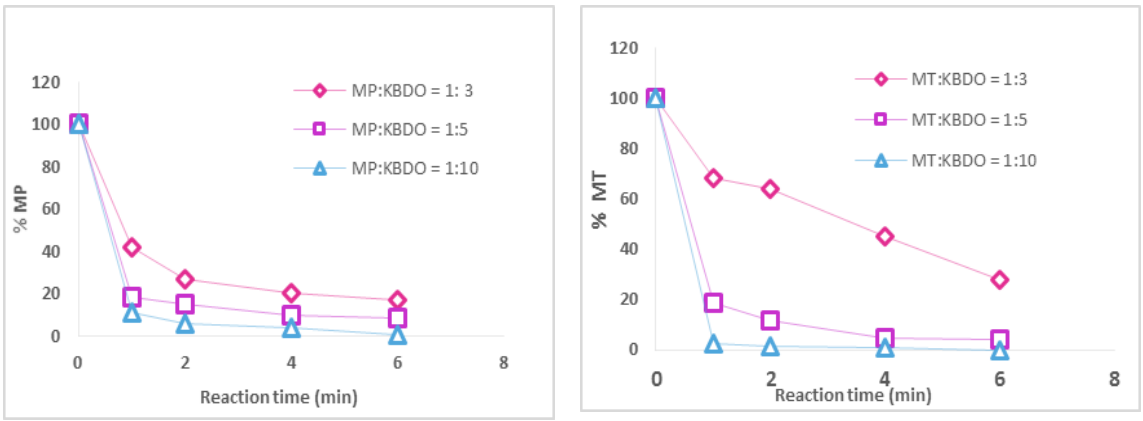


Fig. 4. Reaction of DCBRN-01VN lotion with MP, MT.

3.1.2. In vivo test with DCBRN-01VN

In vivo pig skin samples were extracted with methanol, filtered through a 0.25 mm filter. And then the extracted liquid was diluted several times before measurement. The results are presented in Table 2, the LC-chromatograms were shown in Fig. 6.

Test results on pig skin samples showed that the toxic removal efficiency was nearly completed (> 98%) with MP and that of MT reached nearly 90%. Therefore, the

synthetic solution of DCBRN-01VN is promising to be a very good solution to be used in decontamination.

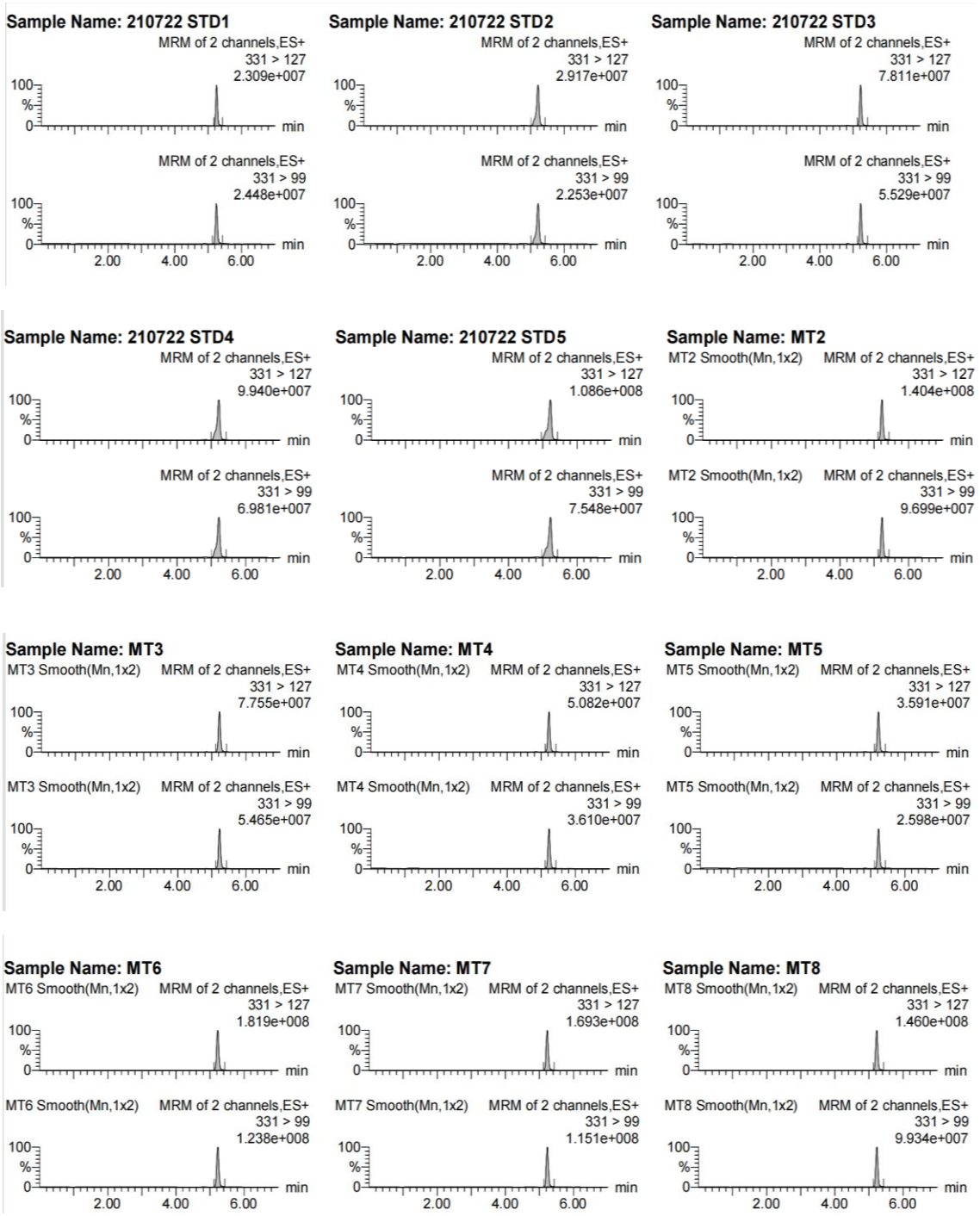


Fig. 5. The LC-MS/MS chromatograms of malathion.

Table 2. Results of DCBRN-01VN in vivo against MP, MT

Entry	Name	Concentrate (ppb)	Coefficient diluted	Concentration of treatment (ppm)	Yield (%)
1	M1_TN	4.5	125,000	562.5	98.88
2	M2_TN	4.8	125,000	600	98.80
3	M4_TN	44.8	40,000	1792	88.93
4	M5_TN	42.6	40,000	1704	89.47

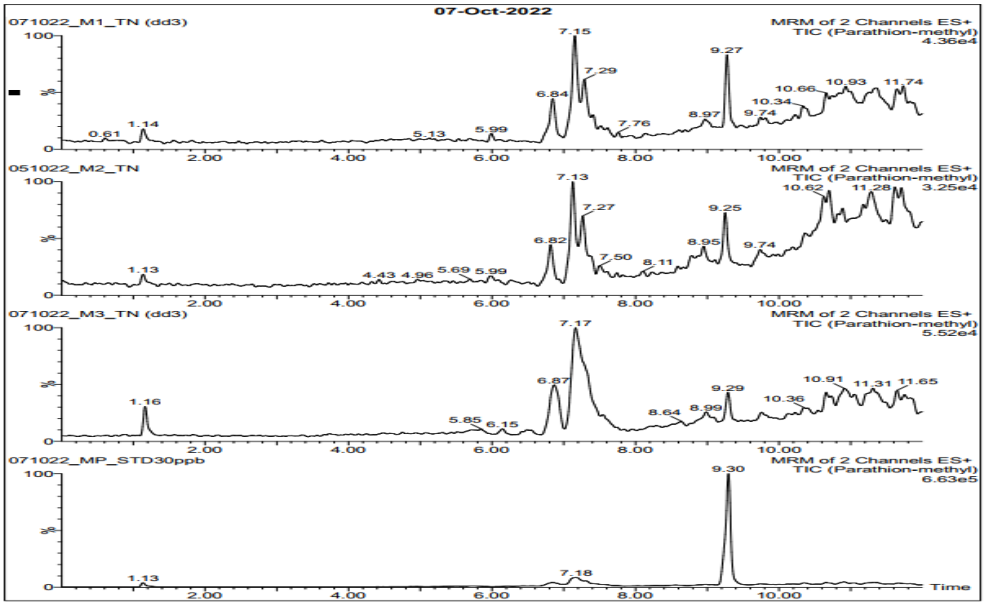


Fig. 6. The LC-chromatograms of samples tested for MP decomposition by DCBRN-01VN in vivo.

3.2. Decomposition mechanism MT and MP of DCBRN-01VN

The active ingredient KBDO in DCBRN-01VN lotion acts as a strong nucleophile and participates in substitution reactions with electrophilic compounds. In the neutralization of OP compounds with DCBRN-01VN, two molar equivalents of KBDO are required for complete reaction. 2,3-butanedione-monoximate reacts first through nucleophilic substitution at the electrophilic phosphorus center, followed by Beckmann fragmentation, which is facilitated by a second equivalent of oximate. The requirement of two molar equivalents of KBDO is consistent with an analogous reaction mechanism for reaction with methyl parathion, as illustrated in Fig. 7.

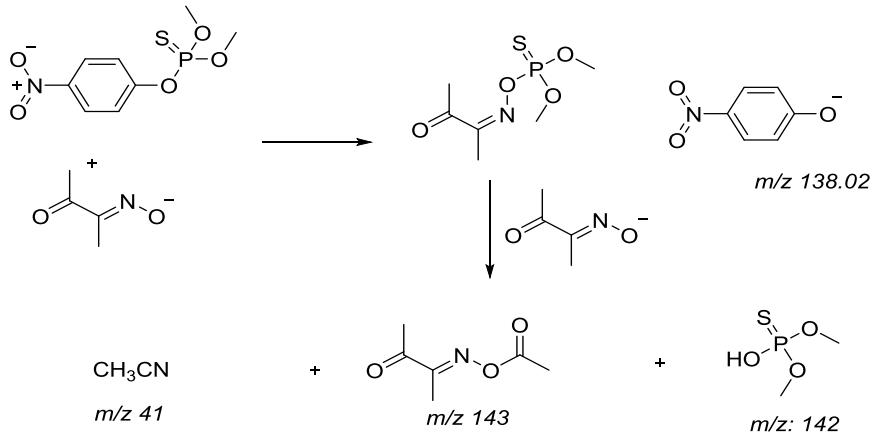


Fig. 7. Mechanism of KDBO and methyl parathion.

The expected reaction mechanism of OP pesticides would be somewhat similar to that depicted in Fig. 8. A for parathion-methyl. In the present study, a few reaction products were detected, e.g., in the case of parathion-methyl, para-nitrophenol (138.2) and O,O-dimethyl thiophosphonic acid (m/z 141.2), to support the proposed mechanism. However, expected reaction products were not detected from other test compounds. The mechanism of reaction between KDBO and OP by analogy to the accepted mechanism of DCBRN-01 lotion degradation of G-type nerve agents BDO- refers to the 2,3-butane-dione-monoximate anion of KBDO.

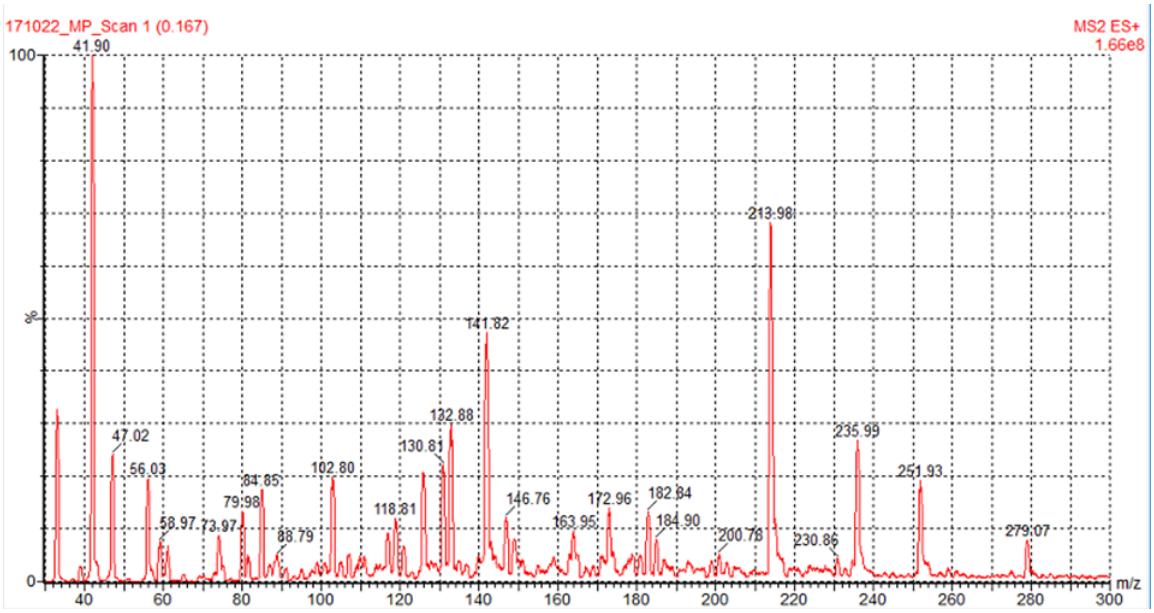


Fig. 8. The MS - scan spectrum of M1_TN.

4. Conclusions

In conclusion, the DCBRN-01VN lotion effectively degrades OP expediently. It was successfully eliminated the chemicals if sufficient molar ratios of the active ingredient, KBDO, to test article are employed. The 5:1 molar ratios were sufficient to achieve complete degradation of the compounds; methyl parathion at 6^{th} min, whereas malathion at 2^{nd} min. The decomposition OP was better with 10:1 molar ratio. The results *in vivo* test with both solutions showed that the toxic removal efficiency was over 90%, for MP was over 98%.

The MS-scan spectrum of the testing sample with methyl parathion suggested that the decomposition process by DCBRN-01VN has cut the link of the aromatic ring and the phosphorus group, creating less toxic products.

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ĐÁNH GIÁ KHẢ NĂNG PHÂN HỦY CHẤT ĐỘC CƠ PHOTPHO (METHYL PARATHION, MALATHION) BỞI SẢN PHẨM DCBRN-01VN

Vũ Ngọc Doãn[a], Lê Minh Đông[a], Nguyễn Thị Hiển[b], Nguyễn Việt Thái[a], Lê Phong[c]

[a]*Khoa Hóa - Lý kỹ thuật, Đại học Kỹ thuật Lê Quý Đôn*

[b]*Khoa Môi trường, Học viện Nông nghiệp Việt Nam*

[c]*Phòng Hóa học, Viện Khoa học hình sự*

Tóm tắt: Nghiên cứu này đã kiểm tra hiệu quả phân hủy một số hợp chất cơ phốt pho sẵn có (OP) của dung dịch DCBRN-01VN. Kỹ thuật UPLC-MS/MS được sử dụng để định lượng các OP còn lại sau phản ứng thông qua các mảnh ion đặc trưng. Kết quả cho thấy dung dịch DCBRN-01VN đã làm giảm OP một cách hiệu quả. Hiệu suất loại bỏ đạt gần 100% với MP ở phút thứ 6 và MT ở phút thứ 2 khi tỉ lệ mol của KBDO và OP là 10:1. Kết quả thử nghiệm *in vivo* với cả 2 dung dịch (MP và MT) trên da heo cho thấy hiệu quả loại bỏ độc tố đều hơn 90%, đối với MP là hơn 98%.

Từ khóa: *Khử nhiễm; DCBRN-01VN; malathion; methyl parathion.*

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