APPLICATION OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY TO THE ANALYSIS OF THE NITROGEN MUSTARD AND THE EVALUATION OF EXTRACTION METHODS USED FOR ITS HANDLING

Turan KARAYILANOĞLU[1]*, Eduard BERENSHTEIN[2], Levent KENAR[1], Nahum KITROSSKY[2], Üçler KISA[3], Mordechai CHEVION[2]

GAZ KROMATOGRAFİSİ-KÜTLE SPEKTROMETRİSİNİN NİTROJEN MUSTARD ÖLÇÜMÜNDE UYGULANMASI VE İŞLEM SIRASINDA KULLANILAN EKSTRAKSİYON YÖNTEMLERİNİN DEĞERLENDİRİLMESİ

***Özet**: Bu çalışmada, nitrojen mustardın (HN2) (2,2-dichloro-N-methyl diethylamine hydrochloride) metanol, hekzan, diklorometan ve izopropil alkol/kloroform ile ekstraksiyonu sonrası trimetilsilil türevlendirmesi ile Gaz Kromatografisi-Kütle Spektrometrisi ile (GC-MS) saptanmasını yönteminden bahsedilecektir. Recovery (geri kazanım) değerleri karşılaştırıldığında, hem diklorometan hem de izopropil alkol/kloroform ile ekstraksiyonun diğer organik çözücülerle olan ekstraksiyon yöntemine göre % 80 daha fazla bir recovery değerinin elde edildiği gözlendi. Bu çalışmada örnekleme ve analiz yöntemi toprak örneklerinde uygulandı ve toprak örneğine ait extraktlarda, HN2'nin GC-MS yöntemi ve kapiler kolon kullanılarak ölçümü yapıldı. GC-MS yönteminde kullanılan ısıya ve HN2'nin fiziksel ve kimyasal yapısına bağlı olarak gün içi CV değeri %3.1-6.5, günler arası CV değeri de %.3.5-6.5 arasında bulundu. Elde edilen geri kazanım ise %87.1 ile %95.4 arasında idi. Çalışmanın bulguları değerlendirildiğinde, HN2'nin mikrogram düzeyinde miktarının ölçülebilmesi için GC-MS yönteminin önerilebileceği sonucuna varıldı.*

***Anahtar Kelimeler:** Nitrogen mustard, GC-MS, derivatizasyon.*

***Summary:** We describe a procedure based on extraction of nitrogen mustard (HN2) (2,2'-dichloro-N-methyl diethylamine hydrochloride) with organic solvents like methanol, hexane, dichloromethane and isopropyl alcohol / chloroform separately followed by trimethylsilyl derivatization and gas chromatographic-mass spectrometric (GC-MS) detection. Compared to recovery efficiencies, it was observed that the recoveries in both dichloromethane and isopropyl alcohol / chloroform extractions were better than other organic solvent extractions as superior of 80%. In this study, the sample handling and analysis procedure were applied to soil samples, and HN2 was identified during GC-MS analyses of soil extracts on capillary column. According to the oven temperature program used in GC-MS method which is determined by taking into consideration the physical and chemical properties of HN2, we evaluated the intra-day and inter-day precision expressed as CV. The intra-day precision was determined between 3.1 and 6.5 % CV range, and the inter-day precision was also found 3.5 to 6.5 % CV. The recovery was also found between 87.1 and 95.4 %. Taking into consideration the results of this study, we concluded that the GC-MS method applied regarding to our running program may be recommended for measuring the quantity of HN2 in micrograms, on conditions, extraction methods with high recovery by using trimethylsilyl derivatization process.*

***Key Words:** Nitrogen mustard, GC-MS, derivatization*

INTRODUCTION

Nitrogen mustard (mechlorethamine, bis (chloroethyl) methylamine; HN2) was one of the first effective anticancer agents employed clinically (1,2).

1Department of NBC, Gulhane Military Medical Academy, Ankara-Turkey
2Department of Cellular Biochemistry, Hebrew University - Hadassah Medical School, Jarusalem, Israel
3Department of Biochemistry, Kırıkkale University, Kırıkkale-Turkey

Derivatives of HN2 are also effective therapeutic agents, even though they are highly carcinogenic (3). Nitrogen mustard alkylates DNA preferentially at N-7 position of guanine and N-3 of adenine, resulting in the formation of monoadducts and crosslinked adducts (4,5). HN2 often mimics ionizing radiation in many respects, so it is genotoxic and produce a wide array of mutations (6,7). A number of chemical compounds have been tried in order to trap HN2 in a stable form that can then be analyzed. Recently, it has been demonstrated that Diethyldithiocarbamic acid (DDTC) and N-methyl-N-trimethylsilyltrifluoroaceta mide (MSTFA) compounds can be used for derivatization of HN2 to be further analyzed (8,9). The resultant adducts might be subjected to both HPLC and GC-MS techniques (10,11,15). In this paper, we present and describe the gas choromatography-mass spectrometry (GC-MS) technique for the determination of HN2 which has been extracted from contaminated soil samples.

MATERIALS AND METHOD

a. Materials: N–methyl-N-trimethylsilyltrifluoacetamide (MTSFA) (SIGMA, M7891), containing 1% trimethylchlorosilone and Nitrogen mustard (hydrochloride salt) were purchased from SIGMA Chemical Co. Organic solvents like hexane, methanol, ethanol and the other reagents were from MERCK Co. In this study, deionized and bidistilled water was used.

b. Precautionary Process: Since nitrogen mustard is harmful and hazardous substance, we have taken all required measures for protecting ourselves. All of preparations concerning HN2 were performed in a Laminar Flow Cabinet. During the study, we used individual protective equipment against toxic effects of HN2 (12,13). Contaminated materials were treated with the solution containing 5M NaOH / Ethanol (1:10; v/v) and removed from the working area with nylon packages (12).

c. Sample Preparation: The soil weighted 1 gr was spiked with different concentrations of HN2 standart solutions prepared by using extraction solvents, and allowed to stand for 60 min at 4 0C prior to solvent extraction (14). Spiked soil samples were separately extracted with hexane, methanol, dichloromethane and isopropyl alcohol - Chloroform mixture (9:1, v/v) by using ultrasonic vibration (SONICATOR, Model 350) and then centrifuged at 3000 rpm for 20 min (Eppendorf centrifuge 5810 R). During the extraction by isopropyl alcohol / chloroform, potassium carbonate was utilized for an additional effectivity for the extraction. After centrifugation, organic phase containing HN2 was removed and concentrated separately to 0.5 ml by the evaporation in a centrifugal vacuum evaporator (Speed Vac - Plus-SC. 110 a) joined refrigerated Vapor Trap (RVI 4104) at 10 0C.

d. Derivatization Procedure: Trimethylsilyl derivatization was performed following solvent extraction and evaporation of the samples. To the dry residue, 20 ml of N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was added. The samples were redisolved by vortex mixing for 10 s and allowed to react for 30 min at room temperature (15,16,17).

e. Gas Chromatography-Mass Spectrometry (Gc-Ms): GC-MS analysis of derivatized HN2 was carried out on Hewlett-Packard 5971-GCD Plus instrument coupled to a mass spectrometer via an all-glass jet separator. GC-MS was performed by employing a high performance capillary column (Hewlett-Packard) with HP-1 (Crosslinked methyl siloxane), 30 m x 0.25 mm I.D., film thickness 0.25 μm. The oven temperature program was that beginning from 40 0C as an initial temperature for 2.0 min, then increased at 10 0C / min to a final temperature 280 ^{0}C and held for 10 min. Helium was used as the carrier gas at a flow rate of 0.7 ml / min.

Detector mass range was 10-450 m/z. We used the splitless injection as 1 mL, in addition, injector temperature was 250^{0}C. Electron impact mass spectrometer running conditions are as follows. Mass range 40-500 m/z, electron energy 70 eV, source temperature 200 ^{0}C, source pressure 2 x 10-6 Torr, accelerating voltage 6 kV and the temperature of the interface 250 ^{0}C.

RESULTS AND DISCUSSION

In this study, HN2 spiked to the soil samples was extracted by using with different solvents with the aid of ultrasonic vibration, centrifugation and centrifugal vacuum evaporation. The HN2 extracts derivatized by MSTFA were introduced to GC-MS instrument (16,17). It was pointed out that the HN2 samples being at the same concentrations were extracted with different solvents to observe the solvent efficiency. Based on GC-MS chromatograms, the recovery efficiencies of organic solvents used for HN2 extraction were evaluated. The recoveries in dichloromethane and isopropyl alcohol / chloroform extractions were high. In both extractions, the recovery efficiency was 80% more than the expected value. We could not obtain good recovery value in the other solvent extractions. The derivatization agent used hereby was MSTFA which would have a better efficiency for derivatization of organic compounds to be detected by GC-MS technique than the other derivatization agents, according to the resources (8,9,15,17,18). According to the program, we evaluated the intra-day and inter-day precision expressing as CV. For intra-day precision, we firstly extracted the four soil samples spiked with HN2 in four different concentrations on four different days. For inter-day precision, we also extracted the four samples having the HN2 with the same concentrations in one day. After extraction, all samples were derivatized by MSTFA and then introduced into GC-MS. According to this results obtained from GC-MS chromatograms (Figure 1, Figure 2), the intra-day precision was determined as 3.1 to 6.5 % CV being ranged for dichloromethane and isopropyl alcohol / chloroform extractions. The inter-day precision was obtained as 3.5-6.5 % for the same extractions mentioned above and the recovery was also found between 87.1 and 95.4 %. These results are illustrated in Table-I. Calibration results between 1 and 100 mg/ml were shown in Figure 3. Detection limit was 1 mg/ml, linearity range was found from 1 to 100 mg/ml. With respect to the previous studies performed, no studies including different extraction procedures for the determination of the method with highest recovery have been met.

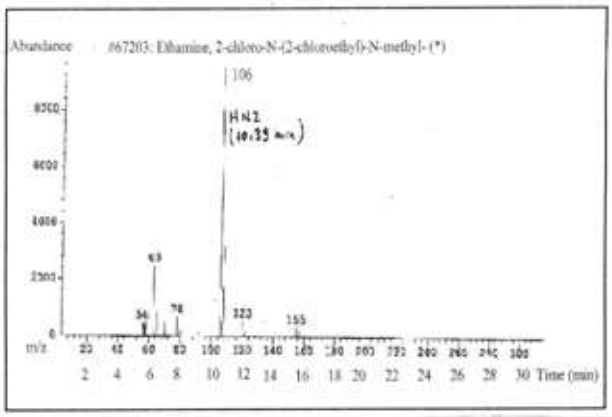

Figure 1. GC-MS spectrum of isopropyl alcohol / chloroform extract of nitrogen mustard (HN2). N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) has been used for derivatization of HN2.

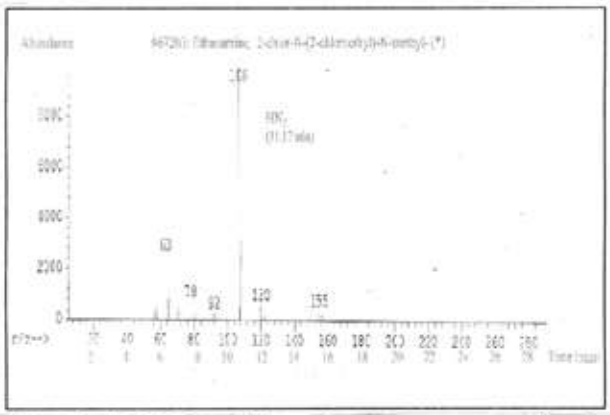

Figure 2. GC-MS analysis of nitrogen mustard after derivatization with N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA). Mass spectrum of dichloromethan extract of nitrogen mustard is shown above.

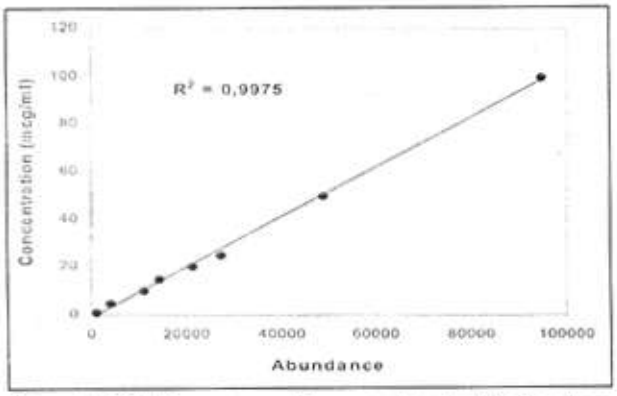

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Table I: Intra-day and inter-day precision and recovery results of the modified and optimized GCMS methods.

Concentration (µg/ml)	Recovery (%)	Precision (%)	
		intra-day	inter-day
1	87.1 ± 4.9	6.5 ± 2.7	6.5 ± 2.6
5	89.0 ± 4.2	3.6 ± 1.7	5.1 ± 1.6
10	92.0 ± 3.5	3.1 ± 0.9	3.5 ± 2.7
25	95.4 ± 3.2	4.6 ± 0.9	6.1 ± 1.8

In this study, our target was to detect and confirm HN2 as trimethylsilyl derivatives by using GC-MS technique. Only while applying this method, on the other hand, we have aimed that we would be able to determine the most available sample handling for HN2. For this reason, very kind of extraction methods were tested to determine the most available removing process of HN2 with high recovery. The results that we obtained from this study demonstrate that the application of GC-MS running with regard to our operating conditions would provide unequivocal identification of trimethylsilyl derivatives of HN2. We conclude that a procedure based on effective extraction followed by MSTFA derivatizaton and capillary column GC-MS confirmation may be recommended for the assessment of HN2 at the high sensitivity.

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