# Investigation of extraneous peak reveals ethylene oxide in food supplements

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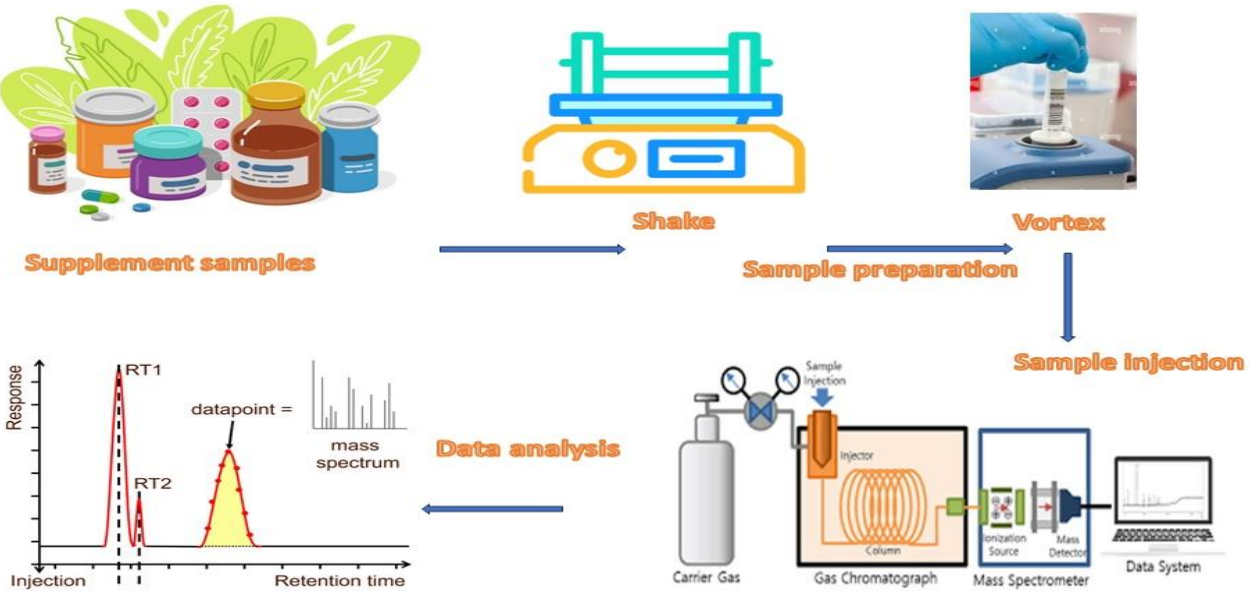
## Graphical abstract




The global food supplement market is growing rapidly and so as the concerns on their quality, safety, and product efficacy. Product monitoring is challenging due to lenient regulation across the globe when compared to pharmaceutical counterparts. As part of quality and safety compliance of food supplements, products are being tested randomly in the Emirate of Abu Dhabi, UAE. This study is the part of routine monitoring of supplements during which an extraneous peak was observed and characterized. Analysis was performed using a Gas chromatography coupled to mass spectrometry and headspace extraction sampler. The extraneous peak with signal to noise ratio (S/N) of 140 was identified as ethylene oxide. Since traces were observed in active product formulation, it is worthwhile to monitor the levels in a higher number of supplements to elucidate its significance.

Key words: supplement; ethylene oxide; GC-MS-MS; contaminants; extraneous peak; maximum residue limit

## Introduction

Food is the basic sustenance for life and food supplements are substances intended to complement dietary requirements having nutritional or physiological effects. In general, food supplements comprise of nutritive products such as vitamins, minerals, amino acids, fatty acids, and fibers that are essential for the maintenance of good health. The Food, Drug, and Cosmetic Act of Food and drug administration (FDA) defines a dietary supplement as a product, taken orally, containing a dietary ingredient intended to supplement the diet [1].

The global food supplement market has grown over 140 billion USD in recent times and is estimated to have a compound annual growth rate (CAGR) of around 9.0% by 2028 [2]. Thus, increasing use of dietary supplements claiming health benefits raises concerns on their quality and safety in addition to product efficacy. Also, product monitoring is challenging due to lenient regulation across the globe when compared to pharmaceutical counterparts [3].

In the United Arab Emirates (UAE), ethnically diverse group of people hailing from different cultural and traditional background live and use of different forms of dietary supplement is not uncommon [4]. According to one study, more than half of the studied population had taken dietary supplements at some point [2].

From the regulatory perspective, dietary supplements having classification as “foods” by the Ministry of Health is controlled by food control authority while the borderline products are submitted to the Ministry of Health for classification [5]. Food supplements with specific intention to enhance nutritional elements such as

vitamins, minerals, fatty acids, amino acids, enzymes, prebiotics, probiotics, collagen, dietary fibers, melatonin, propolis, pollen, herbs or food herbal extracts are covered by UAE.S GSO 2571:2021. As part of quality and safety compliance of food supplements, products are being tested randomly to elucidate their status. This study is the part of routine monitoring of supplements during which an extraneous peak was observed and characterized.

**Materials and Methods:**

*Sample preparation*

The samples were homogenized using a mechanical shaker for 10 minutes and 1 gram of the homogenate was added to 50 ml of diluent (acetonitrile) and vortexed for 5 minutes. The mixtures were centrifuged at 5000 rpm for 5 minutes. An aliquot of 5 ml from the centrifuged supernatant was transferred to HS vial and analyzed using GC-MS/MS equipped with dynamic head space sampler.

*Standard preparation*

Reference standard (RESTEK # 30548) was used to create calibration curves. The final concentration of ethylene oxide matrix matched standards solutions was prepared at 5, 10, 20, 50, 100 and 200 ng/ml levels in acetonitrile.

*Analytical condition:*

All analyses were performed using a GC-2010 PLUS system coupled to mass spectrometry and headspace extraction sampler “HS” (Auto sampler AOC-6000 Plus-Shimadzu, Japan). The residual analytes were separated on a Rxi-624 Sil MS capillary column. Helium gas was used as the carrier having the initial flow rate of 1.5 mL/min followed by a constant linear velocity of 44 cm/s. Since

splitless injection is ideal for trace analysis for very low analyte concentrations, it was used for 0.5 min followed by split mode (5:1) The oven temperature gradient program was as follows: hold an initial temperature of 35 °C for 2 min, ramp to 235 °C at 20 °C/min, and finally hold for 2 min. The column was reconditioned at 200 °C for 2 min to eliminate all impurities co-extracted from the matrix and minimize carry-over effects. The temperatures of the injection port and MS transfer line were 200 and 230 °C, respectively. The GC instrument was directly interfaced to a GCMSTQ8040 triple quadrupole mass spectrometer (Shimadzu). The MS analyses were conducted in positive electron ionization (EI) mode with a filament current at 60 µA. An ionization energy at 70 eV was applied. The ion source temperature was set at 230 °C. For ion fragmentation, argon (grade 5.0) was used as the collision-induced dissociation (CID) gas. Quantifications were performed using multiple reaction monitoring (MRM) transitions. The specific transitions and the optimum collision energies (CEs) for all compounds are listed in Table 1. The data handling and system operations was carried out by GCMS Solution software (version 4.52; Shimadzu).

**Results and Discussion:**

In general, nutritional supplement product ingredients are very diverse in their chemical composition and often do not match label claims [6]. Accidental contamination and economically motivated substances in the supplements raise concerns on the product quality especially with herbal and botanical ingredients, [7].

*Investigation of extraneous peak*

The current investigation of dietary supplement was part of routine monitoring for both chemical and microbiological parameters as per UAE.S GSO 2571:2021. In addition, a forensic screening for volatile organics was also employed with mass ranging from 40-500.

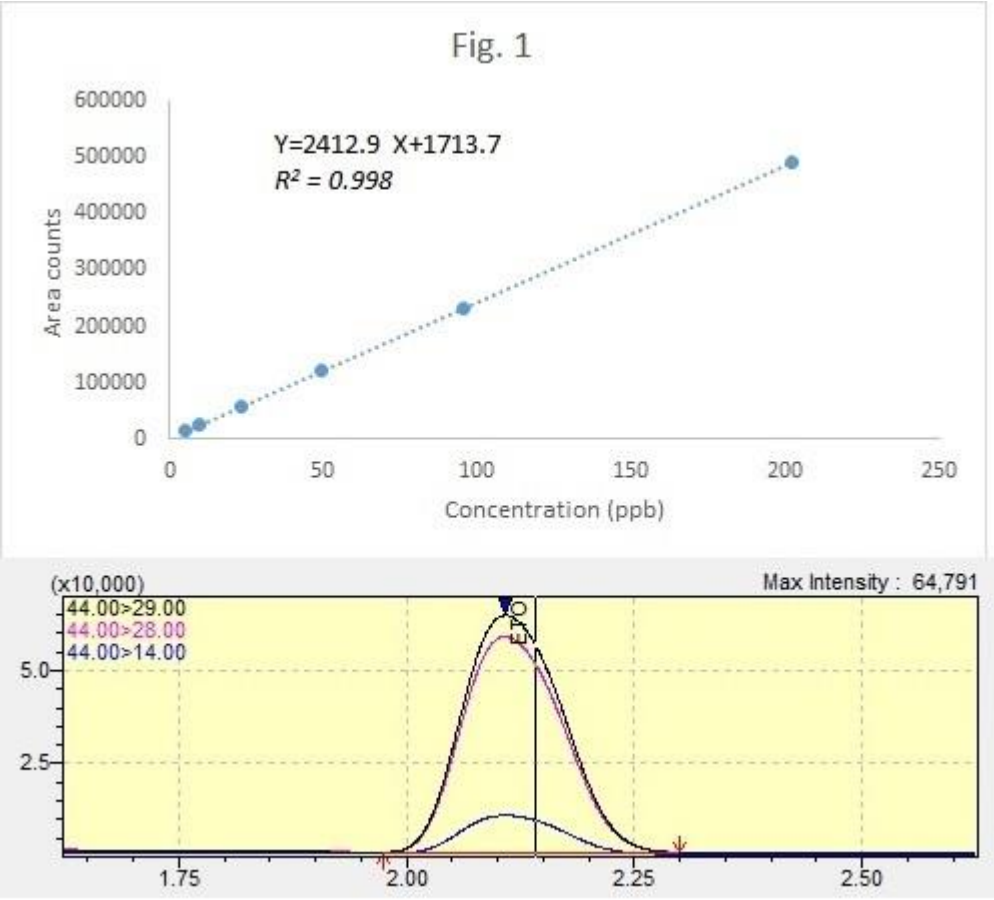



**Fig.1.calibration curve of ethylene oxide standards and chromatogram exhibiting the retention time.**

The forensic screening revealed an extraneous peak with signal to noise ratio (S/N) of 140 which does not match with any of the solvents used. Upon further scrutiny, this peak was identified as ethylene oxide based on the mass number obtained by GC-MS with EI source (Fig. 1). Analyte identification criteria was based on retention time (RT), and the relative MRM transition intensities which

should not vary by more than ± 1% and ± 20% respectively, relative to a spiked control sample [8]. It is a disinfectant and effective fumigant used to protect against microorganisms and hence used in the sterilization of tools in medicine or in food industries to extend the nutritional value and shelf life. [9].

Of the 4 tested samples, 2 of them were found to contain 23 and 44 ppb of ethylene oxide. The maximum residue limit (MRL) for food supplements is set at 0.1 mg/kg (100ppb) including the capsules used in supplement formulations. [10]. Though the levels are within the prescribed limits and the traces could attribute to the sterilization of gelatin capsule, we used only the active medicinal compound from the capsule and hence it is worthwhile to monitor the levels in a higher number of supplements to elucidate its significance.

In conclusion, the present study has identified the extraneous peak in nutritional supplement products as ethylene oxide demonstrating the need to extend the study to a higher number of samples.

## Acknowledgments

The authors gratefully acknowledge the support and encouragement of the Abu Dhabi Quality and Conformity Council. Gratitude is also extended to the management of Central Testing Laboratories (CTL) for invaluable support.

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