ORIGINAL ARTICLE

# Evaluation of Methamphetamine, Methadone, Tramadol, Diazinon, Phosalone, Trichlorfon, Mancozeb, and Penconazole in Hand-made Alcoholic Beverages in North Khorasan Province, Iran

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**Abstract**

***Background:*** Unrecorded alcoholic beverage consumption is a health problem in Iran. Extensive use of pesticides in agricultural products may lead to the presence of residues of these compounds in hand-made alcoholic beverages. There are claims that the producer of hand-made alcoholic beverages adds some drugs to increase efficiency. The objective of this study was to assess the presence of methamphetamine, methadone, tramadol, diazinon, phosalone, trichlorfon, mancozeb, and penconazole in homemade alcoholic drinks**.**

***Methods:*** Thirty hand-made alcoholic beverages were analyzed in this study. Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and liquid-liquid extraction (LLE) were selected for samples' pretreatment. Gas chromatography-mass spectrometry (GC-MS) was used to identify diazinon, phosalone, trichlorfon, mancozeb, and penconazole and high-performance liquid chromatography (HPLC) was used to identify tramadol, methadone, and methamphetamine.

***Results:*** This study showed that diazinon was detected in three samples (10% of the total samples) at 10.8 to 16.5 µg/L and Penconazole was detected in two samples (6% of the whole samples) at a range of 78.5 to 810.6 µg/L. However, Mancozeb, trichlorfon, and phosalone were not in alcoholic beverages. In addition, methadone, tramadol, and methamphetamine were not detected in any samples.

***Conclusion:*** The results of our research showed that hand-made alcoholic beverages contain pesticide residues, and the use of these beverages causes diseases in the body.

**Keywords**: Alcoholic beverages, Diazinon, Mancozeb, Methamphetamine, Methadone

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## INTRODUCTION

Alcoholic beverages are made by fermenting sugars and converting them to ethanol, the source of which is a variety of fruits and agricultural products [1]. According to the World Health Organization (WHO), unregistered alcohol is defined as homemade or informally produced alcohol, such as smuggled alcohol and alcohol obtained through cross-border shopping (which is recorded in a different jurisdiction) [2]. According to a report by the WHO, "about 2.3 billion people are currently consuming alcohol. Total per capita alcohol consumption in the world over 15 years of age increased from 5.5 liters of pure alcohol in 2005 to 6.4 liters in 2010 and remained at 6.4 liters in 2016". A quarter (25.5%) of all the alcoholic drinks consumed worldwide are unregistered alcohol [3]. According to studies, any alcoholic beverage can contain one or more other compounds in addition to ethanol [4]. Many claim that consuming unrecorded alcohol due to toxins other than ethanol can cause harm [5]. The growth of grapevines may be infected by parasites treated with insecticides and fungicides. Pesticide residues may be found on grapes or other processed products [6]. According to the European Pesticide Action Network (EPAN), in eight countries, 100% of the regular wines contained pesticides [7]. Contamination of food products with pesticide residues can be harmful to health. Short-term effects such as headache and nausea and chronic effects such as cancer, reproductive damage, and endocrine disorders are caused by pesticides as side effects. [8]. Some drugs (for example, Benzodiazepines) are consumed in large amounts for nontherapeutic purposes with added to alcoholic drinks during the crimes. At rave parties and crime, it was discovered that the mixtures of alcoholic beverages and these drugs have a synergistic effect. [9]. A national epidemiology survey in Iran, performed on 7840 people aged 15 to 64, found that 5.7% of people had consumed alcohol at least once in the past 12 months [10]. The production, distribution, and consumption of alcohol in Iran are not allowed for religious reasons, so alcohol addiction is not a concern. Despite these bans,

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alcoholic beverages are made and sold illegally in Iran [5]. This study evaluates the residual pesticides of diazinon, phosalone, trichlorfon, mancozeb, penconazole and methamphetamine, methadone, and tramadol in hand-made alcoholic beverages in North Khorasan province, Iran.

## METHODS

### Standards and Reagents

All standards and reagents were of analytical grade, and the solvents were of HPLC grade. Diazinon, phosalone, trichlorfon, mancozeb, and penconazole standard were purchased from AriaShimi (Zahedan, Iran). The purities of the standard pesticides were from 95% to 98%. Stock standard solutions of 50 mg/L were prepared in acetonitrile and stored at -20 C°. Working standard solutions were prepared by diluting the stock standard solution with acetonitrile and stored at -20 C°. A working solution of TPP (Triphenyl phosphate) for the internal standard, acetonitrile, methanol, water, anhydrous sodium chloride, sodium citrate, and magnesium sulfate, obtained from Merck company. Primary, secondary amine (PSA) sorbent was obtained from Sigma-Aldrich company. Standard methamphetamine, tramadol, and methadone were provided by Tehran Daroo Company (Tehran, Iran).

### Apparatus

An Agilent 7890B GC system (Agilent Technologies, USA) with a 5977 Series MS. HP-5 MS analytical column (30 × 0.25 mm × 0.25 mm film thickness) was used for gas chromatography (GC), with helium (99.9999%) as the carrier gas at a flow rate of 1.0 ml min-1. The column temperature was initially at 80 °C (hold for 1 min), Increased to 185°C (hold for 10 min) at the rate of 15°C min-1, and then increased to 270 °C. The temperature of the injector port was 270°C, and 1 ml was injected in spitless mode. The total running time was 28 min.

An HPLC from KNAUER AZURA (with serial Number FBE181100004) four-solvent with C-18 chromatographic column Lichrospher-RP8ec (5 ~ m, 250 x 4.6 mm) and DAD detector (with serial Number FOJ174500001) was used. The mobile phase consisted of 63 to 37 potassium dihydrogen, phosphate buffers, and acetonitrile at a rate of 1 ml min-1.

### Sample Preparation

### QuEChERS Extraction

Thirty hand-made beverage samples were taken from the Forensic Medicine Laboratory (North Khorasan, Iran). The procedure of Guzsvány et al. was followed with some modifications described below for the extraction and clean-up [11]. The extraction vials contained 10.0 mL of the beverage samples. Anhydrous NaCl (1 g), anhydrous MgSO4 (4 g), sodium citrate (1 g), and a mixture of trisodium citrate and disodium citrate (0.5 g) were added from separate prepackaged pouches. Additionally, 5 mL of acetonitrile was also added to the vials. The vials were then closed and mixed on a vortex mixer for 1 minute. The sample was vortexed for 2 min, then centrifuged for 10 min at 5000 RPM. 1 ml of the upper layer was carried into a 2.0 ml microcentrifuge tube containing 50 mg PSA and 300 mg MgSO4. The 50 microliters of 0.2 mg/L TTP were added to the tube, and the sample was vortexed for 1 min and then centrifuged for 5 min at 5000 rpm. Finally, 2 µl of the upper solution was injected into GC-MS [12].

### Liquid-liquid Extraction

The extraction was carried out according to Clarke et al. 2004 [13]. Briefly, 1 ml of the sample was placed into a 12 ml centrifuge tube. 2 ml of saturated sodium borate (pH to 12) was then added. The sample was shacked for 1 min, and then 8ml of 1-Chlorobutane was added to the tube and was mixed for 10 min and centrifuged for 10 min. Next, the organic layer was transferred to the second tube. Then 3 ml of 0.1 mol/l sulfuric acids added and was mixed for 5 min and centrifuged for 5 min. After that, the organic layer aspirates and is discarded. The aqueous layer was extracted with 0.5 ml of 2 mol/L sodium hydroxide and 3 ml of 1-Chlorobutane. Then organic layer was transferred to a 3 ml centrifuge tube and evaporated at 60° just to dryness. Dry residues were reconstituted in 100 µL 1- chlorobutane, and 2 µL injected into GC-MS. The extraction yields of methamphetamine, methadone, and tramadol with 1-chlorobutane in this method are 0.7, 0.95, and 1respectively [14].

## RESULTS & DISCUSSION

The organoleptic properties of alcoholic beverages can vary depending on the type of fruit and climatic conditions of fruit growth [15]. Organoleptic properties refer to the sensory characteristics of a substance that can be perceived by our senses, such as taste, smell, color, appearance, and texture. Total samples were analyzed for organoleptic properties and compared with the standard of wine-related samples. The four organoleptic properties in this study, including pH, appearance, color, and odor, were recorded and shown in Table 1 [16]. Approximately 33.2% of samples had a pH between 3 and 4, 56.8% had a pH between 4 and 5, and 10% had a pH greater than 5. The standard properties for pH suggest that the expected range should fall between 2.9 and 4.6. The appearance property describes how the substance looks, 23.4% of samples had a clear appearance, while 76.6% were opaque. About 41.9% of samples had an alcohol-like odor, 10% had a vinegar-like odor, and the remaining 48.1% were odorless. The table 1 provides information about the color of the substance. Approximately 13.2% of samples had a reddish color, 40% were brown, 16.8% were colorless, and 30% were yellow. Magdalena Gajek et al. evaluated the influence of organoleptic properties (producer, type of wine, and origin) on the levels of some elements, such as the concentration of Cu, Co, Cr, and Cd. They reported that red wines contained higher values of the mean of B, Ba, Cr, Mn, Cu, Sr, and Zn, and white wines, higher levels of elements such as Li, Ag, Be, Bi, Cd, Co, Ti, and K were had. The results also showed higher pH levels for red wines due to the second fermentation process typically carried out for this type of wine (malolactic fermentation) [29].

The results of the apparatus analysis of 30 samples are as follows. Out of 30 samples, in 25 (83.3%) samples, none of

**Table 1. Organoleptic properties and frequency of alcoholic beverages in the North Khorasan province, Iran. Total samples (30) were analyzed for the organoleptic test. Organoleptic properties were compared with the standard properties of the wine-related samples.**

| Organoleptic properties | | Frequency of sample (%) | Standard properties |
|---|---|---|---|
| PH | <3 | None | 2.9-4.6 |
| | 3-4 | 33.2 | |
| | 4-5 | 56.8 | |
| | >5 | 10 | |
| Odor | Alcohol | 41.9 | Fruit<br>Floral<br>Herbal<br>mineral |
| | Vinegar | 10 | |
| | Odorless | 48.1 | |
| Appearance | clear | 23.4 | Clear |
| | opaque | 76.6 | |
| color | Redish | 13.2 | Red<br>White (deeper gold, yellow, brown) |
| | Brown | 40 | |
| | Colorless | 16.8 | |
| | Yellow | 30 | |

the residues of the pesticides were found. Diazinon residue was found in 3 samples at 12.7 ± 1.8 µg/L. Two samples contained the penconazole residues at 444.75 ± 365.85 µg/L. Mancozeb, trichlorfon, and phosalone were not found in alcoholic samples. Also, methadone, tramadol, and methamphetamine were not found in any alcohol samples. Therefore, 16.6% of the studied samples contained pesticide remnants. Al Nasir et al., in an evaluation of sixty homemade wines, describe that organochlorine (OCP) pesticide residues were detected in 73% of the wine samples. Still, no organophosphorus (OPP) pesticide residue was detected due to the generally shorter half-life of these pesticides [17].

Dubravka Vitali Čepo et al. reported that total pesticide concentrations and the average number of pesticides per sample in organic products were significantly lower than in conventional products [30]. Some chromatographic techniques, such as thin-layer chromatography, Gas Chromatography/Mass Spectrometry (GC–MS), and High-Performance Liquid Chromatography/Diode Array (HPLC–DAD), have been used to analysis of poisons in beverages [18]. These methods have a very high sensitivity and accuracy, so we also used the High-Performance Liquid Chromatography/Diode Array (HPLC–DAD) to identify the methadone, tramadol, and methamphetamine in the hand-made alcoholic beverages. Mohammad Reza Ghadirzadeh et al. (2019) reported that in 15% of alcoholic drinks, at least one sample containing one of the drugs tramadol, methadone, diazepam, oxazepam, flurazepam, and alprazolam. Still, tramadol was the most abundant [19]. In alcoholic beverages, there are different elements like organic acids, sugars, and both colored and non-colored polyphenols. These components can either complicate or hinder the accurate analysis of pesticides. As a result, numerous analytical approaches have been created in recent years to detect and measure pesticides in alcoholic beverages [20]. Various sample preparation methods reduce the sample matrix complexity in identifying pesticide residues in the beverage matrices [21]. Among all the methods used for extraction and separation of pesticide residues from biological and non-biological matrices, the QuEChERS method has a very high sensitivity to extracting residues of pesticides [22-25]. The method known as QuEChERS (quick, easy, cheap, effective, rugged, and safe) was initially introduced by Anastassiades et al. for analyzing pesticide residues in fruits and vegetables that contain a lot of water [26]. This method was also used to analyze pesticide residues in wine [27]. Jiang et al. described a QuEChERS method for determining 77 pesticides in wine. They reported that the recoveries of all pesticides were in the range of 70–110%, and the limits of quantification (LOQs) for the 77 pesticides were in the range of 0.003 to 0.05 mg/L, also (%relative standard deviation (RSD) was a range of 4 to 9 [17]. In addition, the QuEChERS method is cost-effective and environmentally friendly with minimal solvent use. We also used the QuEChERS to extract the pesticide residues in the hand-made alcoholic beverages. Between 2012 and 2014, a study examining the use of pesticides in Iranian agriculture found that approximately 14,000 tons of pesticides are utilized each year in Iran. Out of the total volume of all pesticides sold, 43% of it was herbicides. Insecticides and acaricides together made up 37% of the volume, while fungicides accounted for 19% [28]. The results of a study conducted in Iran reveal that pesticide residues can be found in grape products like fruit juices and alcoholic beverages. These findings support the results of the current study [28-30]. The RSD (relative standard deviation) was performed by spiking blank samples five times at 50 and 200 µg /L concentrations and RSD lower than 5% at both concentration levels**.** Limits of detection (LOQs) values, defined as the lowest concentration of the analyte, were found in the range 2.3–48 µg /L for diazinon, phosalone, trichlorfon, mancozeb, and penconazole and in the range 295–386.4 µg/L for methamphetamine, methadone, and tramadol, respectively. LOQs and LODs values are shown in tables 2 and 3.

The study primarily analyzed alcoholic beverages in a specific region, which limits the generalizability of the findings to other regions within the country.

**Table 2. Limits of quantification (LOQs) and Limits of detection (LODs) of diazinon, phosalone, trichlorfon, mancozeb, and penconazole**

| Compound | LOD (µg /L] | LOQ (µg /L) |
|---|---|---|
| Diazinon | 0.15 | 2.3 |
| Mancozeb | 3.2 | 48 |
| Penconazole | 0.23 | 3.91 |
| Phosalone | 0.36 | 6.12 |
| Trichlorfon | 0.18 | 3.42 |

**Table 3. Limits of quantification (LOQs)and Limits of detection (LODs) of methamphetamine, methadone, tramadol**

| Compound | LOD (ug/L) | LOQ (ug/L) |
|---|---|---|
| Methamphetamine | 108 | 334.8 |
| Methadone | 118 | 295 |
| Tramadol | 138 | 386.4 |

## CONCLUSION

The widespread use of pesticides in agricultural products such as grapes causes the presence of pesticide residues in alcoholic beverages. Out of 30 alcoholic beverages analyzed, five samples contained residues of pesticides. The results of our research showed that hand-made alcoholic beverages contain residues of pesticides and the continuous use of these beverages causes diseases caused by their accumulation in the body.

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