



Determination of Fenvalerate residue in raisin via vortex-assisted surfactant-enhanced emulsification liquid–liquid microextraction (VSLLME) method by using HPLC system

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ABSTRACT

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In this project, ultra-trace amounts of *Fenvalerate* residue in raisin, were determined *via* vortex-assisted surfactant-enhanced emulsification liquid–liquid micro extraction (VSLLME) method and by using high performance liquid chromatography-photo diode array (HPLC-PDA) detector at 225nm. The main parameters relevant to this method were investigated and the optimum condition was established: 20 μ L chlorobenzene was used as extraction solvent, 0.9 mmol.L⁻¹ CTAB was selected as the surfactant, the extraction time was fixed at 60s, 2% sodium chloride was added and the extraction process was performed under the room temperature. Under the optimum condition, limit of detection (LOD) was 0.3 ng mL⁻¹. The relative standard deviation (RSD, n=6) was 2.87%. The linearity was obtained by five points in the concentration range of 0.3 to 100.0 ng mL⁻¹. Correlation coefficients (R²) was 0.9997, and the enrichment factor (EF) was 114. Finally, the proposed method has been successfully applied for determination of Fenvalerate in real samples. The recoveries of the target analyte in raisins samples were between 84.13% and 92.12%. It seems that the addition of a surfactant, which was used as an emulsifier, could enhance the rate of the mass-transfer from aqueous samples to the extraction solvent.

1. Introduction

Grapes are consumed both in fresh and in processed products such as wine, juice, jelly, seed extract, raisin, vinegar and seed oil. In 2016, the international organization of vine and wine (OIV) had reported that the grape production was estimated at 7.8 million tons (US) of which 39% was produced in Europe, 34% in Asia and 18% in the United States [1]. After the green revolution, the use of pesticides was increased for all products such as grain food, vegetables, fruits, cotton and tobacco [2]. Fenvalerate is an insecticide which is a mixture of four different isomers with various activities. For example, 2- α configuration, which often includes 23%, shows some especial insecticide activity. Fenvalerate has a

modest toxicity for the mammals that can be harmful for the central nervous system through prolonged exposure [3]. The residue levels of this insecticide are directly related to its application. Also, it is more toxic to bees and fishes, and does not significant effect on plants, while, it can stay active for a long time [4]. Since the presence of this pesticide could be harmful to human and the environment, its residues in food must be closely monitored [5].

In the recent years, chromatography methods have been used to measure pesticides (including Fenvalerate) in foods and beverages [6]. One of the most important tasks in determining the amounts of pesticide is the preparation of the sample. In this regard, various methods are used for preconcentration and preparation of those. As an

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instance, Yang et al. (2011) developed a vortex-assisted liquid-liquid micro extraction with a surfactant (VSLME), which its results showed that vortex mixing to disperse the extracted solvent is preferable compared to ultrasound (preventing the destruction of analyte) [5]. In addition, surfactants as an emulsifier can be used to maximize extraction efficiency and reduce time [7]. Gonzales et al. (2015) determined the amount of potassium carbonate in juice by Vortex-assisted surfactant-enhanced emulsification liquid-liquid micro extraction and the addition of ammonium perfluorooctanoate in the aqueous sample in combination with vortex agitation, which was observed to be a good extraction with short extraction time. Nur-Bahiyah et al. (2013) used the Vortex-assisted liquid-liquid micro extraction coupled with high performance liquid chromatography method for the determination of furfurals and patulin in fruit juices. The optimum extraction conditions for 5 mL sample were as follows: 1-hexanol as extraction solvent; volume of extracting was 200 μ L; 45 s vortex time; addition of 20 % salt. According to these conditions, the main advantages of this method are that this method requires a small amount of organic solvent and the extraction time is short [8]. High sensitivity, high accuracy and good selectivity of the analytical methods are required for measuring low concentrations. Using multi-residue method, due to the large number of pesticides in the market, is able to analyze a large number of pesticides in a run that is more effective and more commonly used.

In this project, ultra-trace amounts of Fenvalerate residue in raisin, were determined *via* vortex-assisted surfactant-enhanced emulsification liquid-liquid micro extraction (VSLME) method and by using HPLC-PDA detector at 225nm. The results showed that the proposed method is suitable for determination of Fenvalerate in real samples. The recovery factors of the target analyte in raisins samples were between 84.13% and 92.12%.

2. Experimental

2.1. Chemicals and reagents

Analytical standard Fenvalerate, Sodium dodecyl sulfate (SDS), Cetyltrimethylammonium bromide (CTAB), TritonTMX-100 and X-114 were supplied by Sigma-Aldrich (Saint-Quentin Fallavier, France). Magnesium sulfate anhydrous, Sodium chloride, acetonitrile and methanol (both HPLC grade) were purchased from Merck (Darmstadt, Germany).

2.2. Samples

Types of raisins were prepared randomly from East and West Azerbaijan (IR Iran) and from Bonab, Maragheh, Malekan, Miandoab and Urumieh cities, including Sun dry raisin, Golden raisin and Sultana raisin that they were kept until analysis in the refrigerator at 4 °C.

2.3. Instrumentation and chromatographic conditions

The liquid chromatographic system was Shimadzu® from Japanese public KK Company (Kyoto, Japan) composed of LC-20AD isocratic pump, whithermost oven and thermostat auto sampler, the loop volume is 20 μ L and a SPD-M20A UV-diode array detector. Data analysis was performed using CBM-20A software.

Separation was achieved at 35 °C using an octadecylsilyl (ODS) C18 analytical column (4.6 \times 100 mm, 3 μ m) and a security guard column C18 (4.6 mm) both purchased from waters®. Mobile phase consisted of Acetonitrile: H₂O (25:75) that flow rate was set at 1.0 mL/min. The auto sampler temperature was kept at +15 °C. The detection wavelength was set at 225 nm.

2.4. Preparation of solutions

Stock standard solution of Fenvalerate was prepared at 1 mg/lit in methanol, aliquoted and stored at room temperature. The calibration curve and the linear range determination of the standard methanol solutions were used for 10, 20, 30 and 50 ppb of Fenvalerate.

2.5. Optimizing the factors affecting in Fenvalerate extraction

The experimental variables were evaluated and optimized such as the type of solvent extraction, the solvent volume of the extractor, the type of surfactant, the concentration of surfactant, the time of the vortex, the effect of salting and the effect of extraction temperature. All experiments were performed with three times replications. Eventually the area of the peak was reported in Fenvalerate. The data were analyzed using Excel software.

2.6. Validation of the VSLME-HPLC Method for Fenvalerate Extraction

Standard methanol solutions (10, 20, 30 and 50 ppb) of Fenvalerate were used to plot the calibration curve and to determine the linear range of the method. In order to obtain a relative standard deviation, 6 repeated experiments were performed using samples at a concentration of 20 ng / ml of Fenvalerate in optimal conditions. A methanolic sample of 20 ng / ml was extracted from Fenvalerate and the peak area of the extract was compared with the direct calibration curve peak area to calculate the enrichment factor. The limit of detection (LOD) was calculated based on 3S/N and the limit of quantification (LOQ) based on 10S/N. The spiking was used at a concentration level of 20 ng / ml to evaluate the proposed method.

3. Results and discussion

This is a well-known fact that the HPLC system is one of the best instruments for analyzing the organic impurities

[9] which could be emerged during pharmaceutical, pesticide and other organic synthesis [10], or natural product extractions. Due to these, in the present project, we have used this instrument for analyzing the extracts. In following, we have presented our investigation about the effects of different parameters on the results of our extraction efficiency.

3.1. Effect of extraction solvent type

The extraction solvent volume has a significant effect on the extraction of analyte in the usage of the Vortex-assisted surfactant-enhanced-emulsification liquid-liquid micro extraction method. In general, the extractor solvent must have the following conditions:

1. The organic solvent density must be greater than the density of water.
2. There is little solubility in water.
- 3- Ability to extract the desired compounds.
4. It is compatible with the analytical system, such as gas chromatography and liquid chromatography.
- 5- High efficiency extraction for the desired analysis

According to the above conditions, as well as the nature of the analyte, and the use of reverse phase HPLC, chlorobenzene solvents, dichlorobenzene 1 and 2, and carbon tetrachloride have been investigated. After reviewing the results, the best result was the use of chlorobenzene solvent because chlorobenzene has a low vapor pressure (1.33 kPa), log k is relatively high (2.84), lighter than water and slightly solubility in water (0.4 millisecons) G / ml) and that is compatible with reverse phase high-performance liquid chromatography. Fig.1 shows the effect of extraction solvent volume.

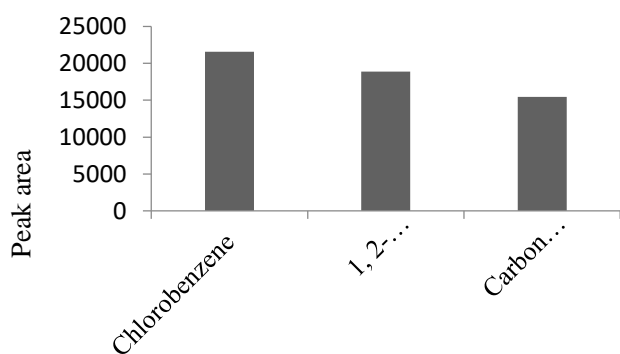


Fig 1. The effect of extraction solvent type on extraction efficiency by VSSLME-HPLC method. Extraction conditions: 20 μ l solvent, 0.9 mM CTAB, vortex time is 60 sec. with maximum speed, 2% salt and room temperature

3.2. Optimization of extraction conditions

3.2.1. Effect of the volume of solvent extraction:

Different volumes of chlorobenzene (20-60 μ L) were analyzed to investigate the effect of the extraction solvent volume on the efficiency of extraction by the VSSLME method. In the Fig.2 shown the extraction efficiency values in different volumes of extraction solvents. As seen, extraction efficiency decreases with extraction of

solvent extraction volume. This is due to the dilution of an extraction sample in an organic solvent. Therefore, the extraction solvent volume is selected 20 μ l for future analysis.

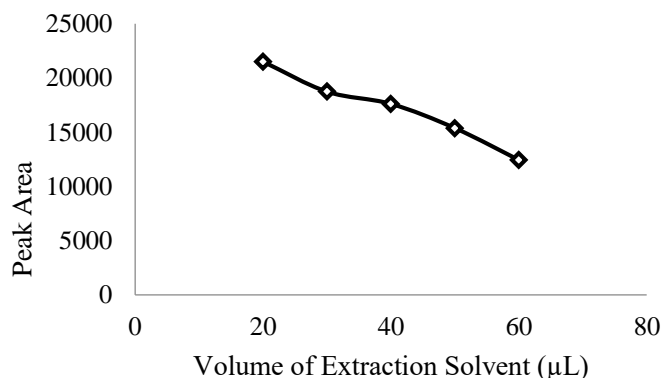


Fig 2. Effect of extraction solvent volume on extraction efficiency by VSSLME-HPLC, Extraction conditions: chlorobenzene extraction solvent, 0.9 mMCTAB, vortex time is 60 seconds with maximal speed, 2% sodium chloride salt and room temperature

3.2.2. Effect of Surfactant Type

In the VSSLME method, it is important to select the surfactant. In this method, the surfactant is an emulsifier and after the vortex, they emulsion the organic compounds in the aqueous medium. Four surfactants were evaluated to select the best surfactant for our analysis, including cationic surfactant (CTAB), anionic surfactant (SDS) and non-ionic surfactant (Triton X-100) and (Triton X-114). Fig.3 shown the effect of the type of surfactant. It is observed that extraction efficiency increases with the use of CTAB due to the presence of oxygen and nitrogen groups in the Fenvalerate structure that they can bonding with cationic surfactant. It therefore increases the extraction efficiency and enrichment factor, which plays a more effective role in the transition of the analyte from the aqueous phase to the organic phase. As a result, CTAB was selected as the appropriate emulsifier for further studies.

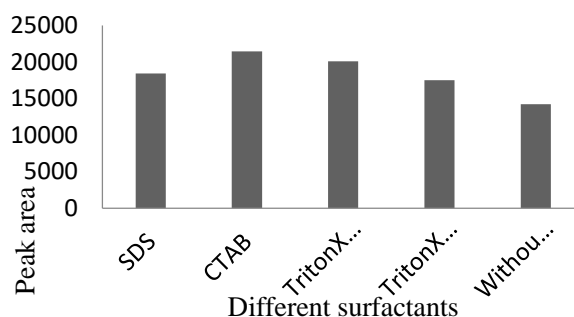


Fig 3. Effect of surfactant type on extraction efficiency by VSSLME-HPLC method. Extraction conditions: 20 μ l chlorobenzene solvent, 0.9 mM surfactant, vortex time is 60 seconds with maximum speed, 2% salt and room temperature

3.2.3. Effect of Surfactant Concentration

The effect of different concentrations of CTAB (0.5, 0.8, 0.9, 1, 1.2 and 1.5 mM) was investigated because the surfactant concentration is an effective factor in sample transfer and extraction efficiency. As shown in Fig.4, the extraction efficiency increases with increasing surfactant concentration below the critical point, but after that, the extraction efficiency decreases because of the concentration of the surfactant monomers and the formation of the micelles. They also can't function as well as their emulsifiers. Also, the part of analyte can also be placed in the micelles and reduce the extraction efficiency. Therefore, a concentration of 0.9 mMCTAB was selected for further studies.

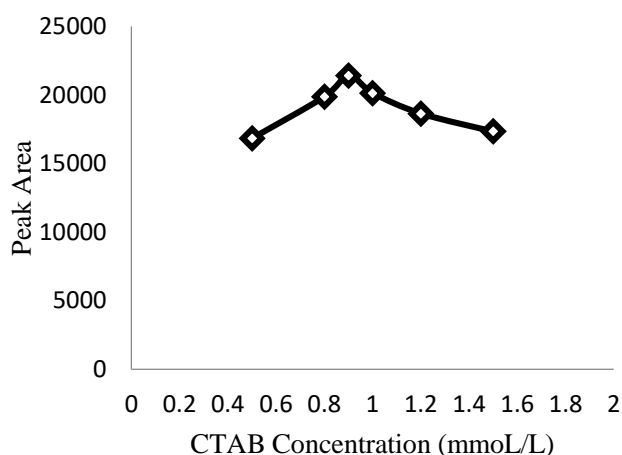


Fig 4. Effect of Surfactant Concentration on the efficiency of extraction by VSSLME-HPLC, Extraction Conditions: 20 μ L Solvent, CTAB Surfactant, Vortex time is 60 Seconds with Maximum Speed, 2% Sodium Chloride Sodium and room Temperature.

3.2.4. The effect of the vortex time

The extraction efficiency depends on the extraction time, because mass transfer is a time-dependent process, its speed decreases when the system reaches near equilibrium. Vortex-assisted surfactant-enhanced-emulsification liquid-liquid micro extraction is an equilibrium process, which then needs to be stable to achieve equilibrium. Therefore, the extraction time was investigated by considering the extraction efficiency. In this experiment, the extraction time was the time when the extraction solvent is completely dissolved in the aqueous solution of the sample. Extract solvent dispersion in the sample solution was related to the speed and duration of the vortex. The Vortex time was investigated at the maximum Vortex speed and different times (30, 60, 120 and 180 seconds). Also, can't be checked the time less than 30 seconds because the extraction solvent does not completely dissolve in aqueous solution. In the results, it was observed that in the range of 60-180 s there was no significant change in extraction efficiency due to the use of emulsion. So the time of 60 seconds were selected for future analysis.

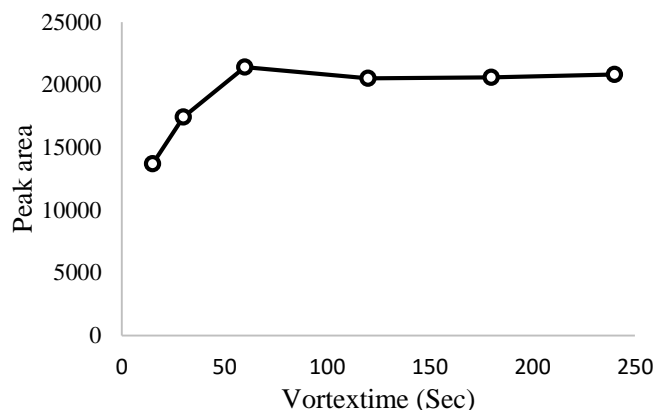


Fig 5. Effect of Vortex Time on Extraction Efficiency by VSSLME-HPLC Method, Extraction conditions: 20 μ l solvent, 0.9 mM, vortex with maximal rotational speed, 2% sodium chloride salt and room temperature

3.2.5. The effect of adding salt

Fig.6 shows the effect of ionic strength on extraction efficiency. It was observed that when the aqueous solution contains 2% (w / v) of sodium chloride, the extraction of the Fenvalerate is maximized. Sodium chloride is in ionic form in aqueous solution, which increases the ionic strength of the solvent, reduces the solubility of the Fenvalerate and improves the constant distribution of the analyte between the two organic and aqueous phases. So improving extraction efficiency is due to the effect of salting out.

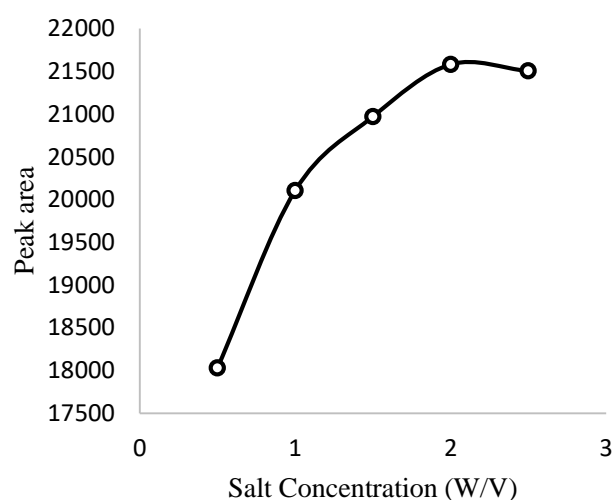


Fig 6. Effect of salt addition on extraction efficiency by VSSLME-HPLC method. Extraction conditions: 20 μ l chlorobenzene solvent, 0.9 mMCTAB, vortex time of 60 seconds with maximal rotational speed, sodium chloride salt and room temperature

3.2.6. Effect of temperature

Temperature is the driving force necessary for the complete diffusion of solvent in aqueous solution (an effective factor in the process of mass transfer and emulsifier). Therefore, the effect of extraction temperature was investigated in the range of 15 to 35 °C (15, 20, 25, 30 and 35). The results show that the extraction efficiency are not significant in the range of 15 to 22° C. Also, that is decreases at temperatures above 22 ° C. This is due to the reduction in the critical concentration of micelles by increasing the temperature from 22 ° C and the appearance of a cloudy state. This does not occur at room temperature, so the experiments were carried out at room temperature (22 ± 2 ° C).

3.3. Analytical performance

The optimal conditions for the proposed method were determined as follows: 20 µl of chlorobenzene as a solvent extraction, 0.9 mM-1 of CTAB cationic surfactant, vortex duration of 60 seconds with maximal rotational speed and 2% (w / v) of sodium chloride salt in Laboratory temperature. The Fenvalerate calibration curve in water has a linear and high correlation coefficient ($R^2 > 0/99$) at a concentration range of 0.3 to 3.0 ng / ml. The relative standard deviation (n=6), limit of detection, the limit of quantification was determined after increasing the specified values of Standard solution to raisin (20 ng / ml). Also, the enrichment factor was calculated from the extraction area (20 ng / ml of the aqueous sample) comparison with the area obtained from the direct injection of calibration curve. The results are shown in Table 1.

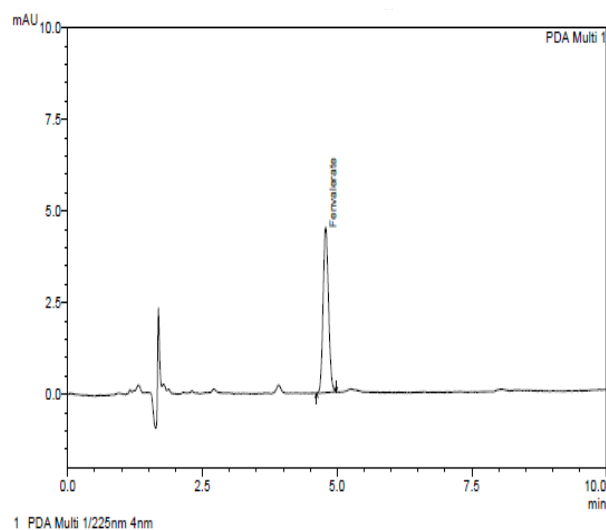


Fig 7. Calibration curve of VSSLME-HPLC method for measuring Fenvalerate in raisins

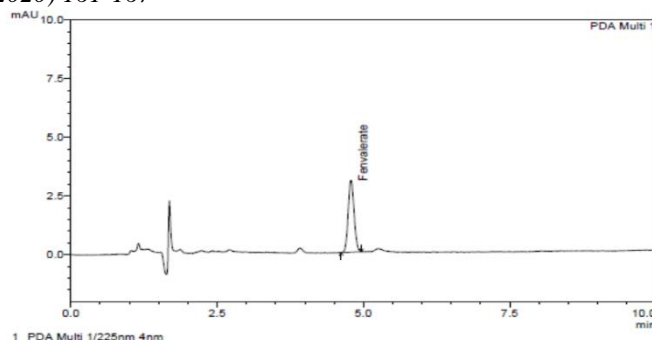


Fig 8. Chromatogram obtained from standard Fenvalerate solution extraction by VSSLME-HPLC method at a concentration of 20 ng / ml

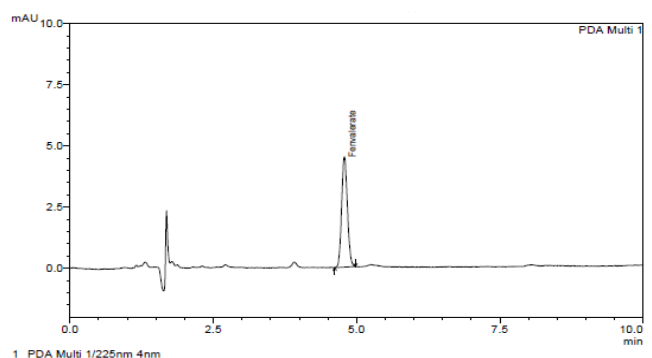


Fig 9. Chromatogram obtained from standard Fenvalerate solution extraction by VSSLME-HPLC method at a concentration of 30 ng / ml

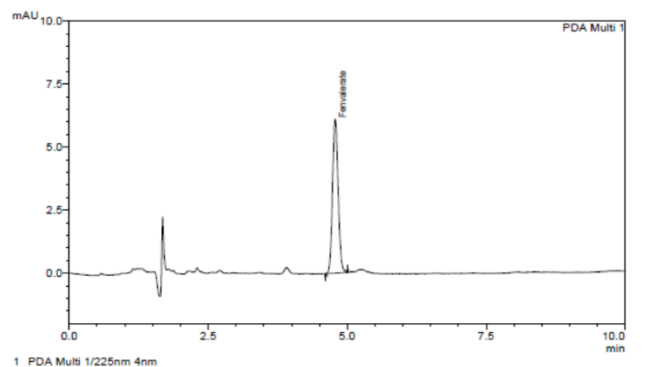


Fig 10. Chromatogram obtained from standard Fenvalerate solution extraction by VSSLME-HPLC method at a concentration of 40 ng / ml.

3.3.1. Results of measurement of Fenvalerate concentration in raisin samples

In order to evaluate the efficiency of our method in real samples, 12 species of raisin were prepared from East Azerbaijan and West Azerbaijan, including raisins Sun dry raisin, Golden raisin and Sultana raisin. Then they were analyzed by VSSLME-HPLC and finally were reported to have Fenvalerate values in them. The results of this analysis are presented in Table 2 and Table 3. The accuracy of the method was evaluated using the specified amount added to the selected samples (20 ng / ml).

Table 1. Validity values of the VSLLME-HPLC method

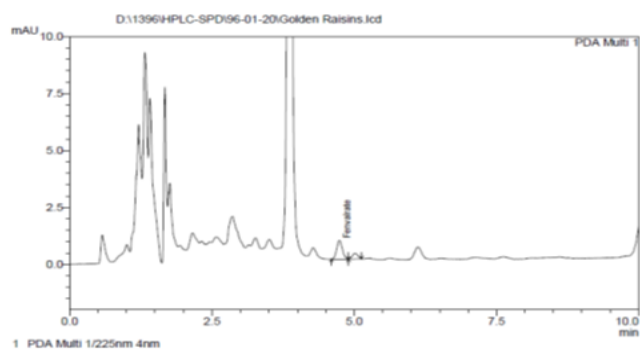
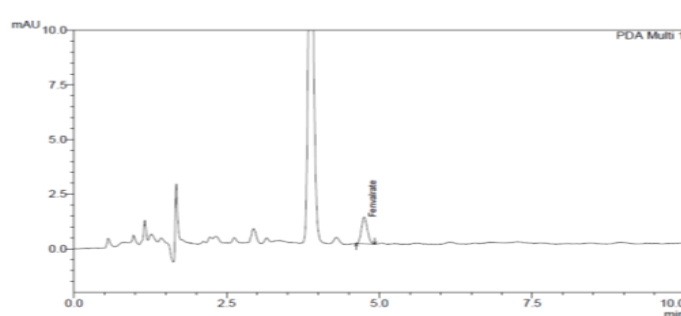
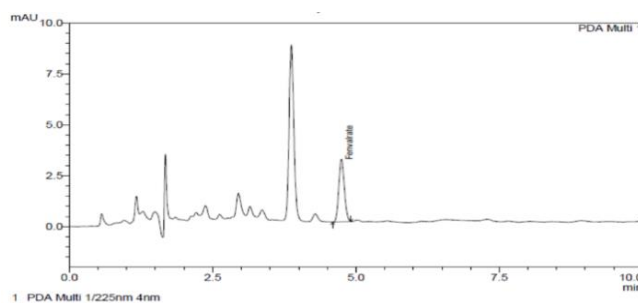
sample	Enrichment factor	limit of quantification (ng mL ⁻¹)	limit of detection (ng mL ⁻¹)	Relative standard deviation (%)	correlation coefficient
Fenvalerate	114	1.1	0.3	2.87	0.9997

Table 2. Study of the extraction recovery of Fenvalerate from raisin samples in East Azerbaijan by VSLLME-HPLC method

5- Sample	4- Fenvalerate concentration(ng mL ⁻¹)	3- Amount of added (ng mL ⁻¹)	1- Result (ng mL ⁻¹)
9- Golden raisins	8- 26.21±1.15	7- 20	6- 38.88±2.42
13- Golden raisins	12- 17.41±1.51	11- 20	10- 34.25±2.15
17- Sultana raisin	16- 19.35±1.22	15- 20	14- 36.17±1.28
21- Sultana raisin	20- 22.81±1.14	19- 20	18- 38.20±2.41
25- Sun dry raisin	24- 52.78±2.08	23- 20	22- 67.05±2.28
29- Sun dry raisin	28- 49.32±2.27	27- 20	26- 62.46±2.11

Table 3. Study of the extraction recovery of Fenvalerate from raisin samples in West Azerbaijan by VSLLME-HPLC method

Sample	Fenvalerate concentration(ng mL ⁻¹)	Amount of added (ng mL ⁻¹)	Result (ng mL ⁻¹)
Golden raisins	15.41±0.28	20	32.38±1.39
Golden raisins	10.60±0.56	20	27.48±1.46
Sultana raisin	14.82±0.32	20	31.20±1.09
Sultana raisin	18.96±1.04	20	35.34±1.76
Sun dry raisin	29.8±0.88	20	45.01±1.58
Sun dry raisin	36.26±0.41	20	51.48±1.09

**Fig 11.** Chromatogram obtained from standard Fenvalerate solution extraction in Golden raisin samples of East Azerbaijan by VSLLME-HPLC method.**Fig 12.** Chromatogram obtained from standard Fenvalerate solution extraction in Sultana raisin samples of East Azerbaijan by VSLLME-HPLC method**Fig 13.** Chromatogram obtained from standard Fenvalerate solution extraction in Sun dry raisin samples of East Azerbaijan by VSLLME-HPLC method

4. Conclusion

In the present study, extraction of the trace amounts of Fenvalerate was performed using a sensitive, precise and simple method called VLLSME. In our proposed method, the selectivity and enrichment factor of the work was increased, and the use of organic solvents was decreased. Also, the extraction time was significantly reduced. Moreover, the combination of this method with HPLC, led to a very good limit of detection (LOD at ng/ml). Optimization of the effective parameters on the micro-extraction process was investigated and its optimum conditions were obtained. This method can be used to identify and determine the very low concentrations of Fenvalerate in raisins. The amount of pesticide residues in the Golden-raisin and Sultana raisin samples is slightly lower than that of Sun dryraisin, due to the presence of sulfur and sodium bicarbonate in Golden raisin and Sultana raisin, which has a slight effect on reducing the remaining Fenvalerate content. Also, the results showed that the Sulfur and alkaline substances such as sodium bicarbonate in Golden raisins and Sultana raisin cause hydrolysis of ester chain of the Fenvalerate.

Conflict of interests

The authors declare that there is no conflict of interest.

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