REPUBLICAN CENTER OF FORENSIC EXAMINATIONS NAMED AFTER X. S. SULAYMONOVA UNDER THE MINISTRY OF JUSTICE OF THE REPUBLIC OF UZBEKISTAN

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EXPERT OPINION NO. 29/18(10912)8.1M/ 16326

Tashkent

"24" November 2022

On November 14, 2022, the Republican Center of Forensic Examinations Named after X. S. Sulaymonova under the Ministry of Justice received a letter from the CEO of the RS Success Agro JV LLC R. Sh. Ibragimova under No. 2022-11-11/271/1 dated November 11, 2022, with a request to: 1) determine the composition of the oil submitted for examination; 2) determine the presence or absence of tetrahydrocannabinol in the composition of the oil sample submitted for testing.

Based on the request, a contract was drawn up under No. 243/2022 dated Nov 14, 2022, and a payment was made on November 18, 2022.

The examination was conducted by the specialist of the Laboratory of Forensic Examination of Materials, Substances and Products (KIMVI) of the Republican Center of Forensic Examinations (RCSE), the chief expert N. V. Korableva.

Note: 1. The specialists are not responsible for the information, objects and/or samples provided by the customer. Therewith, the examination findings relate to the object and/or sample provided by the customer (according to Paragraph 19 of the Instruction on the Procedure to Conduct a Forensic Examination in Forensic Expert Institutions of the Ministry of Justice of the Republic of Uzbekistan and Paragraph 7.8.2.2 of the Management System Manual (QPCM) of the RCSE developed as per the requirements of ISO 17025:2017).

2. In the course of examining physical evidence and examination objects, the measures and measuring instruments used in the research methodology undergo periodic metrological verification (calibration) in accordance with the approved annual plan /2/

The following was submitted for examination: one glass receptacle – a dark glass bottle with a polymer screw cap and a colored label bearing the inscription "HEMP SEED OIL 250ml". The label indicated the manufacturing company RS Success Agro JV LLC, the nutritional and energy value (Photo 1,2). The bottle contained an oily liquid of yellow-greenish color, Object 1 (hereinafter the expert's designation).

The appearance of the investigated Object 1 is shown in Photo 1,2.

The examination used the following literature:

1. Oʻzbekiston Respublikasi Adliya vazirligining sud ekspertiza muassasalarida sud ekspertizasini oʻtkazish tartibi toʻgʻrisidagi YOʻRIQNOMA, Mar 02, 2011, No. 2202.

2. QOΠ 5.10(7.8) (4) Reporting of results, approved by the Director of RCSE, dated Feb 04, 2022.

3. Recommended guidelines for quality assurance and good laboratory practices. Manual for use by national laboratories. UN, 2000.

3. SUPELCO, BF3-methanol, product specification and typical procedure/Sigma-aldrich, USA, 1998.

4. Savitsky A. N., Kuznetsov D. I., Beltsova T. F., Semenova L. I. Kriminalisticheskoie issledovanie pischevykh zhyrov [Forensic examination of edible fats]. Moscow, 1980.

5. HPLC Application Note: Multicomponent analyses of fats and oils using diode-array detection. Rainer Schuster, Hewlett-Packard, FRG.

6. "Kodeks Alimentarius" ZHIRY, MASLA I PROIZVODNYE PRODUKTY, Standart Kodeksa Dlia Poimenovannykh Rastitelnyh Masel [Codex Alimentarius. Fats, oils and related products. Codex Standard for Named Vegetable Oils]. Moscow, 2007.

7. Recommended methods for the identification and analysis of cannabis and cannabis products (revised and updated edition of the MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES, UN, New York, 2010.

Examination methods: The questions asked were solved by conducting a chromatographymass spectrometry study.

Preparation of samples for analysis: aliquots of 10 ml each were taken from Object 1 submitted for examination into cone flasks, 50 ml of ethyl alcohol were added, and extraction was performed using ultrasonic treatment for 10 minutes, then the samples were poured into separation funnels and left for phase separation for 2 hours. After separation, the alcohol layer was poured into porcelain plates, evaporated to dryness under a hood at room temperature, added 1 ml ethyl alcohol each, and used for further analysis.

The prepared samples of Object 1 were analyzed using a chromatography–mass spectrometer SHIMADZU GCMS-QP2020 with a capillary column $30 \text{ m} \times 0.25 \text{ mm}$ in size containing 5% solution of phenylmethylsiloxane in dimethylsiloxane, the carrier gas being helium. Conditions of analysis: the gas flow rate in column was 2.2 ml/min, the injector temperature 250 °C, the programmed column thermostat temperature from 150 to 280 °C, the temperature rise rate 10 °C/min, the exposure 10 min, the linear velocity 56.3 cm/s, the common gas stream flow rate 118.2 ml/min, the linear pressure rate control 185.9 kPa, the sample size of 1 µl, and the split ratio 1:50.

The peaks were identified by comparing the peak mass spectra of the examined Object 1 with those available in the mass spectra libraries NIST17, Forensic Toxicologies, DD2019, NISTFULL.L, Wiley275.L, SWDRUG.3.11L, CAYMANSPECTRA.L, and PMW_TOX3.L.

Analysis of the resulting chromatograms and mass spectra shows that:

- one peak was identified in the extract of oily liquid (Object 1), with a retention time of 18.83 minutes, fragment ions with a m/z of 314, 246, 231, 193, 121, 91, 77, corresponding to cannabidiol (CBD).

No peaks characteristic of the narcotic substance, tetrahydrocannabinol (THC), were identified in the submitted oily liquid (Object 1).

To determine the composition of the sample of oily liquid submitted for examination (Object 1), its fatty acid composition was determined by chromatography-mass spectrometry analysis of fatty acid methyl esters.

Preparation of samples for analysis: aliquots of 0.20 ml each were taken in duplicate from Object 1 submitted for examination into penicillin flasks for the methylation process, 1 ml of oftoluene and an alkylating solution (trifluoroborate in 10% methanol) were added into each flask. Methylation was conducted by heating in water bath at a temperature of 60 °C for 10 minutes, then the flasks were cooled down, added 1 ml of distilled water, stirred up, and, after phase separation, the upper organic layer was taken for analysis.

Chromatographic conditions: the analysis was conducted on the chromato-mass spectrometer Agilent Technology GC/MS AT 5973M using a capillary column of 30 m \times 0.25 mm in size with 5% phenylmethylsiloxane at the injector temperature of 280 °C, the MS source temperature being 230 °C, the MS quadrupole temperature 180 °C, the column thermostat temperature programmed from 170 to 280 °C, the temperature rise rate 10 °C/min, the sample size 1 µl, and no flow split.

Analysis of the obtained chromatograms and mass spectra of the methylated solutions of the

submitted oily liquid (Object 1) indicates that peaks were found in the examined Object 1, corresponding to methyl esters of saturated (palmitic, stearic) and unsaturated (linoleic, oleic) fatty acids characteristic of vegetable oils.

Thus, the analysis has determined that the oily liquid submitted for examination (Object 1) contains unsaturated and saturated fatty acids characteristic of vegetable oils.

Summary of examination findings:

The examinations conducted have determined that the oily liquid submitted for examination in one glass bottle made of dark glass bearing a label with the inscription "HEMP SEED OIL 250 ml" contains unsaturated and saturated fatty acids characteristic of vegetable oils, as well as trace amounts of the cannabinoid characteristic of plant genus Cannabis – cannabidiol (CBD).

No traces of the narcotic drug tetrahydrocannabinol were found in the composition of the oily liquid submitted for examination (Object 1), as determined by chromatography-mass spectrometry analysis.

Answers to the questions asked as a matter of the inquiry:

1. The oily liquid submitted for examination in one glass bottle made of dark glass bearing a label with the inscription "HEMP SEED OIL 250 ml" contains unsaturated (palmitic, stearic) and saturated (linoleic, oleic) fatty acids characteristic of vegetable oils, as well as trace amounts of the cannabinoid characteristic of plant genus Cannabis – cannabidiol (CBD).

2. There is no narcotic drug tetrahydrocannabinol in the composition of the oily liquid submitted for examination, with a label reading "HEMP SEED OIL 250ml".

 RCSE Specialist
 [signature]
 N. V. Korableva

[Stamp: O'ZBEKISTON RESPUBLIKASI ADLIYA VAZIRLIGI X. SULAYMONOVA NOMIDAGI RESPUBLIKA SUD EKSPERTIZASI MARKAZI * // XULOSALAR UCHUN]

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Photo 1-3 General view of Object 1 submitted for examination.

Specialist: *[signature]* N. V. Korableva