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RESEARCH ARTICLE

# A new method for identifying methadone in urine samples by trapping it in dendrimer

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**ABSTRACT**

This article used an accurate urine sample (US) from a person with daily use of methadone (MTD), whose presence was confirmed through the diffusion liquid-liquid microextraction method followed by the gas chromatography-mass spectroscopy (GC-MS) technique. At a temperature of 40 0C, keep the sample. The 2nd generation polyamidoamine dendrimer (2-GPD), which was previously synthesized, was released in the US Samples were taken from the container at different intervals.

It injected the model into the high-performance liquid chromatography ultraviolet (HPLC-UV) machine, filtering and extraction by diffusion liquid-liquid microextraction. Examining the HPLC-UV absorption of the samples taken from the US shows that with time MTD molecules migrate from the US into the cavities of the 2-GPD, and the ultraviolet absorption of MTD decreases. The finding confirms the suitability of 2-GPD as an agent for the extraction.

The identification of MTD was reported in the actual US Monitoring and controlling the consumption of various drugs, such as MTD, is a challenging clinical and legal toxicology issue. Examining medications in a model such as the US always requires high accuracy. These methods include. The pre-purification stage is based on precision instruments.

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## INTRODUCTION

Addiction has become a global challenge, and the number of addicts is increasing daily [1, 2]. MTD hydrochloride, abbreviated as MTD with the brand name Dolophine, is an industrial drug often used as a maintenance treatment to curb opiate addiction, such as opium, heroin, and morphine, sometimes to relieve severe pain [3]. The duration of the effect of MTD is much longer than that of heroin. The impact of a single dose is approximately 24 hours. In contrast, in the case of heroin, the duration of the effect may be about two hours. As a result, a person needs fewer times a day. This consumption differs according to people's consumption and body metabolism [4, 5]. MTD differs from morphine in terms of molecular structure but is similar to morphine in terms of analgesic properties. Studies show that 10 to 70 mg of MTD has the same effect as 10 mg of morphine [6]. Under the supervision of addiction treatment, specialists made detoxification and physical withdrawal from drugs with MTD. During the treatment, whenever the addicted person has hangover symptoms, a certain amount of MTD is given to him by a doctor specializing in this field. In other words, the doctor uses MTD to keep the person's hangover tolerable. After a few weeks of the treatment or stabilization of the person's withdrawal, consumption gradually decreases and finally reaches zero [7]. MTD is generally consumed as tablets, syrup, and ampoules. MTD consumption

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can control hangovers in heroin addicts for up to 24 hours. MTD enters and leaves the body system at a controlled rate and remains in the body for longer than heroin or any other drug.

Injectable MTD is used in the hospital to control severe pain. MTD tablets are also available in white and brown colors that do not differ in color [8].

Examining biological samples such as the US is essential in forensic medicine. For this purpose, preparation by different chemical methods has a unique role. In this study, the identification of MTD in biological samples has been made in a new way using 2-GPD. Examining biological samples such as the US is essential in forensic medicine. For this purpose, preparation by different chemical methods has a unique role.

The identification of MTD in the US today is made through the extraction of diffusion liquid. It was the help of the GC-MS confirmation method. The ultraviolet absorption of MTD can be seen in the HPLC device and with the help of the DAD detector. We used a small amount of US, about 1 mL, in this method. Research confirmed the gradual migration of MTD into the cavities of the 2-GPD. For the first time, the 2-GPD introduced a new identification method of MTD. We used an accurate US. The HPLC method is less expensive than the GC-MS method. **Scheme 1** shows a new way of identifying MTD in the US by trapping it in a dendrimer.

## MATERIAL AND METHODS

Tetraethyl orthosilicate and ethylenediamine were purchased from Merck, Germany. Glucuronidase enzyme and lysosomes were purchased from Sigma-Aldrich, Germany.

*Preparation of 2-GPD*

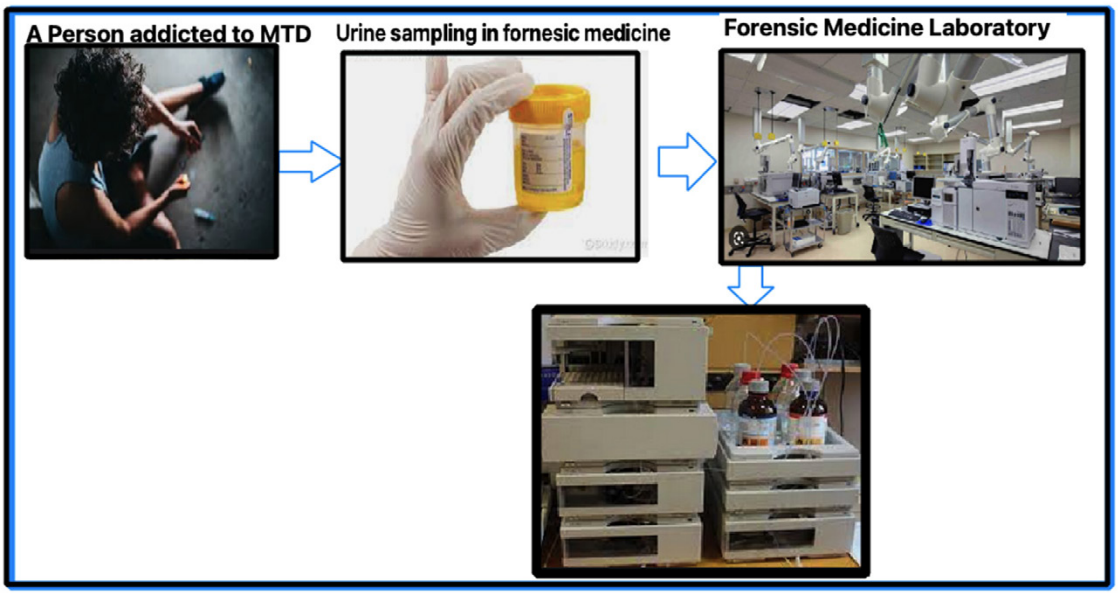
All the information about the preparation is given in the supplement. These are shown in the previously published article [9].

*Preparation of accurate US*

A 55-year-old man took the U.S., a long history. The opium was withdrawn with MTD syrup and with a daily dose. Detoxification for opium withdrawal with MTD generally takes 2-3 weeks. Arbitrary and excessive use of MTD to get rid of addiction not only does not get rid of opium addiction, but with the occurrence of MTD addiction and its complications, it will cause new problems for the patient.

*Identification of MTD in the US by liquid-liquid diffusion extraction technique followed by GC-MS confirmatory method*

Initially, a unique US strip confirmed the possible presence of MTD in the US. Then, to extract MTD, the liquid-liquid diffusion extraction method was used as follows. Centrifuge 7 mL of a US for 5 min at 3000 rpm. Adjust the US pH




Scheme 1. A new way of identifying methadone in the US is trapping it in a 2-GPD.

to 10 with 1 M sodium hydroxide. Mix 2 mL of acetonitrile with 500 µL of chloroform and add a syringe and pressure to the US Centrifuge of the sample for 5 minutes at 3000 rpm.

Dry the chloroform phase under a stream of $N_2$. Dissolve the sample in 100 µL of $CH_3OH$ and inject it into the GC-MS.

GC-MS model 6850 gas chromatography and M.S. detector model 5975 are from Agilent company. The software of the device is of Agilent Chemstation type. The carrier gas is helium with a purity of 99.9999%, flow 1.5 ml $min^{-1}$, inlet temperature 25$^0$C, and injection volume 1 µL. After identifying MTD in the US, keep this sample in the refrigerator until the test is performed.

*Release of 2-GPD in an MTD-containing accurate U.S.*

2 mg of 2-GPD was added to 1 mL of a precise US containing MTD, diluted, and six hours at 37$^0$C incubated the model. Then, an example of the solution was injected every two hours into the HPLC device. As expected, the ultraviolet absorption of MTD in the US decreased with time. It indicated the absorption of MTD in the cavities of the 2-GPD. Since MTD is used in treating drug addiction on the one hand, and the other hand, its arbitrary use causes addiction to it, identifying MTD in the US of people is essential from the perspective of judicial and family courts. Determining MTD in the US is mainly accompanied by HPLC and GC-MS confirmation methods, considered reliable techniques. In this research, the presence of MTD in the US was confirmed by trapping MTD in 2-GPD cavities through a side and new method to identify MTD. Ultraviolet absorption of MTD is checked using the HPLC technique. The passage of time and decrease in ultraviolet absorption shows the absorption of MTD in dendrimer cavities and proves the presence of MTD in the U.S.

*Instrument analysis*

*HPLC*

The HPLC of this device, model 2800, is from the KNAUER company in Germany. Samples were performed in Eurosphere 100 column with normal pressure (150 MPa) and flow rate = 1 ml/min. The mobile phase ratio (phosphate buffer pH=2.3: acetonitrile) was (63:37).

*GC-MS*

GC-MS model 6850 gas chromatography and M.S. detector model 5975 are from Agilent company. The software of the device is of Agilent Chemstation type. The column of the device is of capillary type with a length of 29 meters, an inlet temperature of 250 °C, and an injection volume of 1 µL.

## RESULT AND DISCUSSION

*FT-IR spectrum of synthesized 2-GPD*

The peaks are shown in **Fig.** (**1a-d)** belong to the following: 1032 $cm^{-1}$ is related to the stretching vibration of O-C groups 1-peak. Two peaks at 1645 and 1546 $cm^{-1}$ are assigned to C=O stretching vibrations of amide I (and N-H bending or C-N stretching vibrations) of amide II (internal structure of nano dendrimer. Lower energy vibrations of methylene groups, scattering, and the asymmetric H-C-H shape change states are seen in peaks 1462, 2942, and 365.41 $cm^{-1}$, respectively. Peaks in 2828 and 3407 $cm^{-1}$ in the bands. It is given that the C-H stretch ratio is 3280 $cm^{-1}$ is allocated to the stretching state of primary amine and amide groups, respectively.

The carbon and nitrogen bond is one of organic chemistry's most widely used reactions. Whole

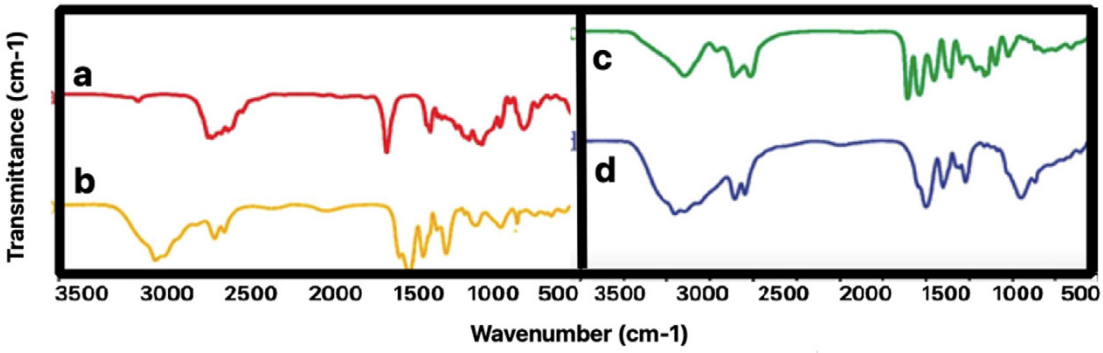



Fig. 1. FT-IR spectrum of the second-generation depleted 2-GPD synthesized (a-d) 2-GPD-0.5-2.

generations of 2-GPD contain ten amines on the surface and 20 amines in the core. Esfand and Tomalia [21] explained the history of the discovery of dendrimers. Esmaeili and Mousavi [7] have used dendrimers to make an oral insulin drug, a nanoparticle compound covered with polymer-2-GPD, and a chitosan drug is replaced inside their cavities. FT-IR data were consistent with the figures presented in the paper. **Fig. 1** shows the FT-IR spectrum of the second-generation depleted 2-GPD (**Fig. 1a-d**).

As you can see in **Fig. 1**, the absorption band appearing at 1740 $cm^{-1}$ corresponds to C=O, and the absorption bands appearing at 1700 $cm^{-1}$ and 1200 $cm^{-1}$ are related to the C-O-C stretching vibration, which confirms the presence of the ester group. The absorption band appearing at 11330 $cm^{-1}$ is connected to a tertiary amine. The absorption bands 3363 $cm^{-1}$ and 3281 $cm^{-1}$ correspond to $NH_2$, and the absorption bands 1200 $cm^{-1}$ and 1161 $cm^{-1}$ correspond to the first and third types of amine stretching vibration. The liquid-liquid diffusion microextraction method is used to confirm the identification of MTD from the preparation method (Dispersive Liquid-Liquid Microextraction). All figures are shown in the previously published article [9].

*Analysis of HPLC-UV absorbance of actual U.S.'s containing MTD*

First, 1 ml of US was centrifuged and smoothed the piece with the help of a 0.45 µL needle filter. So,10 mL of distilled water was mixed, and its pH was adjusted to 10 mL using 1 M sodium hydroxide. 2.5 mL methanol and 300 µL of $CHCl_3$ were added to the US with the help of a 2.5 mL syringe and pressure. The piece was centrifuged for 5 min at 3000 rpm. Precipitated chloroform was collected and transferred to a clean vial.

The model was evaporated under $N_2$ flow. 30 µL of methanol was added to the vial and injected into the high-performance liquid chromatography device under column $C_{18}$ (25 cm X 4.6 mm X5 µmm).

The detector includes the limit of detection (LOD) for MTD equal to 10 mg $mL^{-1}$ in this method. Analysis of HPLC-UV absorbance of actual U.S.'s containing MTD under 2-GPD tapir HPLC-UV absorbance is the standard sample of MTD. **Fig. 2a** shows an HPLC-UV plot of the MTD standard.

*HPLC-UV absorption of actual U.S.'s containing MTD*

The MTD index was observed in the HPLC-UV device at 5 min. After searching a library similar to the MTD standard, a clear peak of MTD was observed in the U.S.

**Fig. 2b,** HPLC-UV peak intensity related to MTD in the US containing 2-GPD shows that it is less intense than the absorption standard of MTD (**Fig. 2a**) at an inhibition time of 4.74 min.

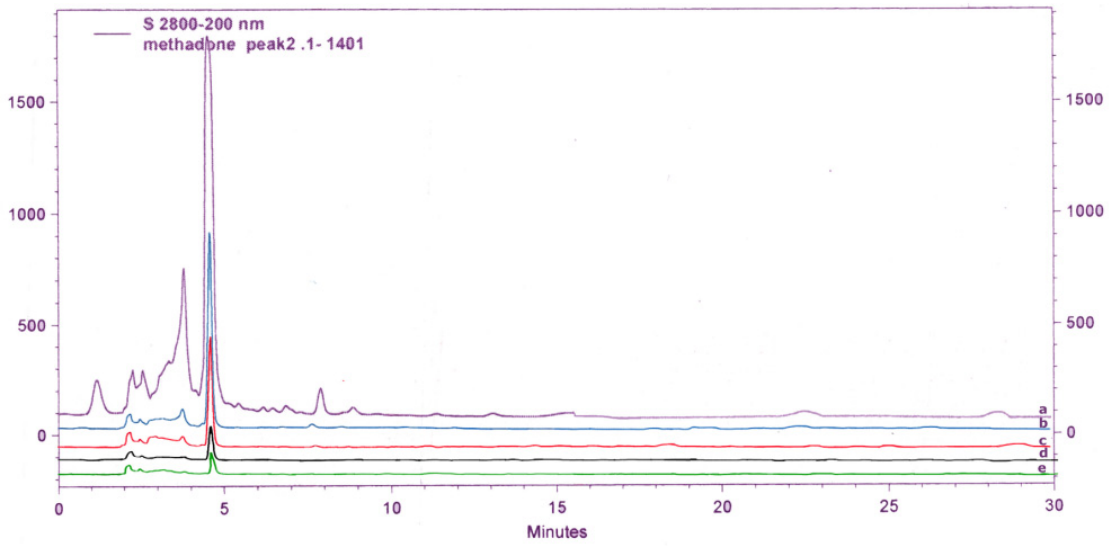



Fig. 2. HPLC UV absorbance of US (a) HPLC-UV plot of MTD standard, (b) HPLC-UV plot of authentic US containing MTD, (c) HPLC-UV chart related to accurate US containing 2-GPD after two h, (d) HPLC-UV chart related to accurate US containing 2-GPD after five h, (e) HPLC-UV chart related to accurate US containing 2-GPD after 12 h.

A clear peak at the inhibition time of 4.74 minutes, confirmed through a library search with a similarity coefficient of 99.98%, indicates the presence of MTD.

MTD has a long elimination half-life (1 to 2 days). Mainly by hepatic metabolism via N-dimethylation to pharmacologically inactive 2-ethylidene-1,5-dymethyl-3,3-diphenylpyrrolidine (EDDP). In a previous study [10], a simple, rapid, and efficient method for preconcentration of MTD using dispersive liquid microextraction (DLLME) followed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was developed. The extraction method is based on rapidly injecting a mixture of extraction solvents and dispersants into an aqueous solution to form a turbid ternary solvent system (aqueous solution: extraction solvent: dispersion solvent). This method successfully determined MTD in human urine, plasma, saliva, and sweat samples.

*HPLC-UV absorbance of US containing MTD after the release of 2-GPD incubation, and two hours*

In this step, 2-GPD, synthesized in the previous step, was added to the accurate US containing MTD; the piece was incubated for 12 h for two hours. The MTD index peak was observed at 5 min inhibition time.

The inhibition time of MTD in this method is 4.74 min (R.T. = 4.74 min). Given that in identifying narcotics and drugs, a US is considered a preferable sample; on the other hand, the title of analyte in it is difficult due to its complex matrix. The diffusion liquid-liquid microextraction method is suitable for removing the inhibiting factors and providing optimal identification conditions. Finally, with the help of the HPLC-UV technique peak of MTD was observed. A sample was injected into the HPLC-UV device from the accurate US of a person addicted to MTD. The rise of MTD was seen at an inhibition time of 4.74 minutes. It was observed. Then, with the addition of 2-GPD, the sample was incubated for 12 hours at 37°C. Then placed at ambient temperature, and after that, a sample was injected into high-performance liquid chromatography-Ultraviolet. The ultraviolet absorbance of the sample showed that It reduces the absorption of MTD. It confirms the substitution of MTD in the cavities of the 2-GPD.

In previous studies, they developed and confirmed the identification method of MTD hydrochloride in oral solutions [11]. In another study, they investigated MTD and tramadol in the vitreous fluid with the help of the diffusion liquid-liquid microextraction method preparation method and the ultra-HPLC identification method performed by the ultra-HPLC [12]. **Fig. 2c** shows the intensity of the HPLC-UV peak related to MTD. It is the US containing 2-GPD. After two hours of retention at ambient temperature, observed at the inhibition time of 4.74 min, it is compared to the standard absorption rate. MTD, as well as authentic US containing MTD, is less intense.

Since the urine sample has a complex matrix, detecting drugs in it due to their polar structure requires specific preparation and separation because the drugs mostly overlap with the peaks of the structural compounds of urine. Therefore, methods that separate the drug are essential to selectively remove urine from the environment. In a previous study [13], sol-gel/copper nanocomposites by electrochemical deposition have been introduced as a new, simple, and one-step technique for the preparation of solid phase microextraction (SPME) coating for the extraction of MTD (a synthetic narcotic) in urine samples. Scanning electron microscopy revealed the porous surface structure of the sol-gel/copper nanocomposite coating. Direct immersion SPME was used following HPLC-UV determination. The results show that the new nanocomposite fibers have relatively high extraction efficiency. Another study used dispersive liquid-liquid microextraction based on freezing floating organic droplets for MTD extraction and determination by high-performance U.V. liquid chromatography. Due to the use of an organic solvent with low density and low melting point and proper technique, there is no need for a micro syringe or fiber to support the organic microdroplet. In addition, the extractant droplet can be easily collected by solidifying it at low temperatures. It used the proposed method to determine MTD in serum and urine samples of an addicted person undergoing treatment with MTD [14].

*HPLC-UV absorption of MTD-containing US after the release of 2-GPD incubation and five hours*

After incubation for five hours, dendrimer and another example were injected into the HPLC-UV at the specific inhibition time. The MTD index peak was observed. As you can see, the ultraviolet absorption of MTD is decreasing.

Considering the intensity of ultraviolet

absorption of MTD in the FigureFigure above, you can see that with time. Free MTD is being replaced in the 2-GPD cavities. It shows that our proposed method is being carried out successfully.

In a previous study, the layer-by-layer method investigated MTD encapsulation (MTD) in polymer layers. $Fe_3O_4$ nanoparticles were synthesized by polyethylene glycol and modified with folic acid. MTD was conjugated to nanoparticles, and polymer layers surrounded this nanosystem. MTD percentage loaded, in vitro release, and investigated kinetic factors [5, 6].

Another study in 2022 investigated the substitution of morphine in 2-GPD cavities with the help of the beta-glucuronidase enzyme, which introduced this technique as a new method for identifying morphine in actual office samples [9, 15].

As shown in **Fig. 2d,** the intensity of the HPLC-UV peak related to MTD in the US containing 2-GPD is less intense than the absorbance level in **Fig. 2c**. Cavities of 2-GPD indicate an increase in the gradual substitution of MTD.

**Fig. 2e** shows the intensity of the HPLC-UV peak related to MTD in the US containing 2-GPD, which is less intense than the absorbance of graph **Fig. 2d.** The migration of MTD into dendrimer cavities happens slowly. The proof of this can be seen through the reduction of MTD absorption. The more MTD migration into the holes increases, the more absorption shows a more significant decrease [14].

*HPLC-UV absorption of MTD-containing US after the release of 2-GPD incubation and 12 hours*

For 12 hours included, a sample of authentic US containing MTD and dendrimer was. Another example was injected into the HPLC-UV. At the specific inhibition time, the peak of the MTD index was observed. The U.V. absorption of MTD was significant.

The accurate administration sample containing MTD was previously proven to have MTD through the diffusion liquid-liquid microextraction GC-MS method, 2-GPD. It was previously synthesized and was added after incubation at specific time intervals, a sample of it from inside (**Fig. 3A-D**). After filtering with a syringe head filter and extracting by the diffusion liquid-liquid microextraction method, it was injected into the high-performance liquid chromatography device. The -ultraviolet absorption of the sample was checked by its DAD detector. The MTD's ultraviolet absorption decreased over time, which confirms the substitution.

The indicator peak in the high-performance liquid chromatography at the inhibition time is 4.74

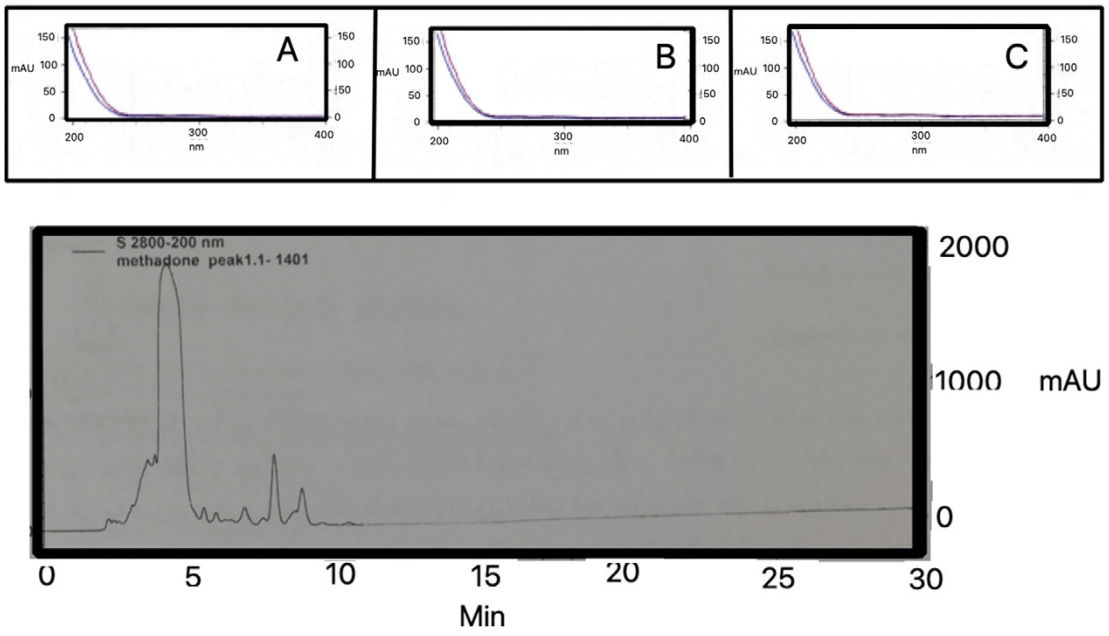



Fig. 3A-D. Searching the index peak in HPLC-UV confirms the presence of MTD.

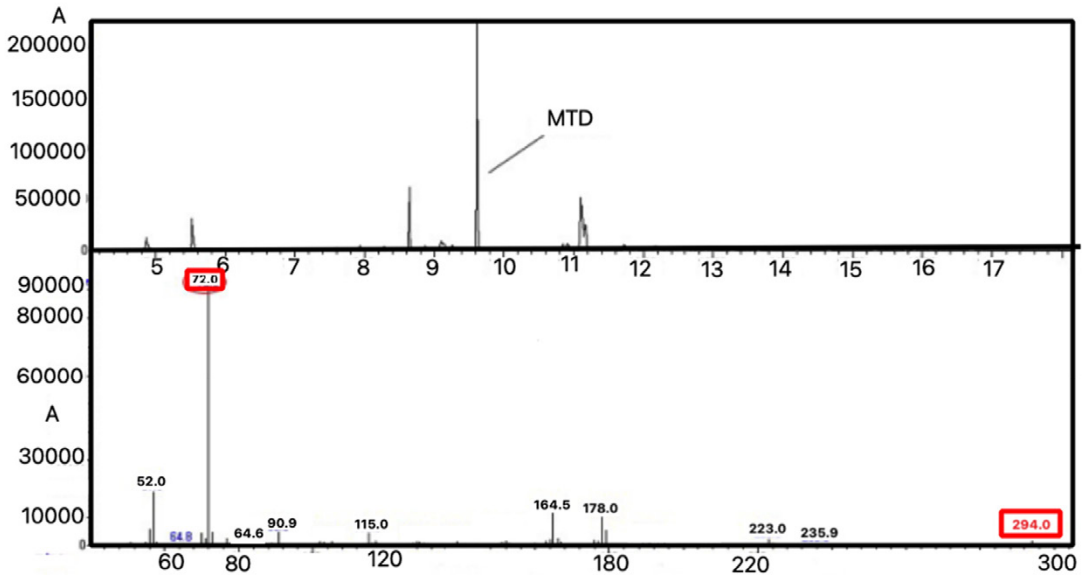



Fig. 4. GC-MS plot of the US from a living addict in withdrawal from MTD.

min, which confirms the presence of MTD with a high similarity percentage of 99.96%. Therefore, it ensures that the indicator peak is the same MTD [14, 16].

The volume of the dispersion solvent is one of the essential factors that should be used for MTD extraction and determined by chromatography. Changing the magnitude of the dispersion solvent can lead to changes such as changes in the importance of the collected organic phase, the size of the droplets, and the polarity of the aqueous phase. All these factors are influential on the efficiency of microextraction. Therefore, it is necessary to investigate and optimize the effect of the volume of the spreading solvent. Determination of MTD in serum and the US successfully used the proposed method. The subject was an addict who was being treated for MTD [10].

A simple, rapid, and efficient method for MTD preconcentration used diffusion liquid-liquid microextraction. Followed by HPLC-UV was developed. The extraction method is based on rapidly injecting a mixture of extraction solvents and dispersants into an aqueous solution to form a turbid ternary solvent system (aqueous solution: extraction solvent: dispersion solvent). This method successfully determines MTD in human US., plasma, saliva, and sweat samples [13, 17-22]. Electrochemical sol-gel nanocomposites are a technique to prepare microextraction coatings for MTD extraction. It investigated the porous surface structure of sol-gel. The results show that the new US nanocomposites have high extraction efficiency.

**Fig. 4** shows the GC-MS chromatogram of MTD indicator peaks. Indicative peaks are observed at 72, 86, and 73, confirming the presence of the MTD in one's US. The US was stored in a refrigerator at 4 °C for the following experiments [23, 24].

The MTD is widely used to treat heroin addiction and is often encountered in forensic samples. In GC-MS, MTD mainly produces a 72 m/z ion, which is not characteristic enough for identification. Molecular ion determination, achieved using chemical ionization, provides diagnostic information and better drug identification. The method is sensitive and diagnostic. It is commonly used in the laboratory [25, 26].

A GC-MS method has been proposed to determine the MTD in the US and plasma by liquid-liquid extraction with predefine as an internal standard. The linear concentration range is 0.05-10 μg $mL^{-1}$ for US and 0.05-1 μg $mL^{-1}$ for plasma. Those correlation coefficients are excellent [27]. After about 12 hours replaced, a large part of MTD in the dendrimer pits and the significant reduction of MTD's UV-Vis absorption indicates this. Examining and comparing the intensity of U.V. absorption with time shows the success of this method.

*Scanning Electron Microscopy (SEM) 2-GPD*

Morphology and size determination of 2-GPD nanoparticles were studied using SEM (TESCAN model, VEGA/XMU, Czech Republic). **Fig. 5** shows SEM morphology for 2-GPD.

In 2019, Esmaili and Khodayi [6, 28] investigated dendrimer encapsulation in polymer layers based on magnetic nanoparticles. In this study, encapsulation of MTD in polymer layers was done by layer-by-layer method. Nanoparticles ($Fe_3O_4$, NPs) were synthesized by polyethylene glycol and modified with folic acid. MTD was conjugated to nanoparticles, and polymer layers surrounded this nanosystem. MTD percentage loaded, in vitro release. The average size of prepared nanoparticles was 23 nm. The capsule surface potential changed from +39 to -27 mV for chitosan and alginate. In this study, a nanosystem was designed to reduce the release rate of MTD. The laboratory study showed that the release of MTD is sufficiently slow and controllable. With this nanosystem designed, addicts can consume these nanomaterials only twice a week.

Based on histological studies, drug injection can reduce liver damage. Most of the nanoparticles with spherical morphology were dispersed on the porous surfaces, which is the advantage of higher absorption. Based on the SEM data, the nanoparticles were spherical, but their characters were passable due to 2-GPD. In a study conducted on

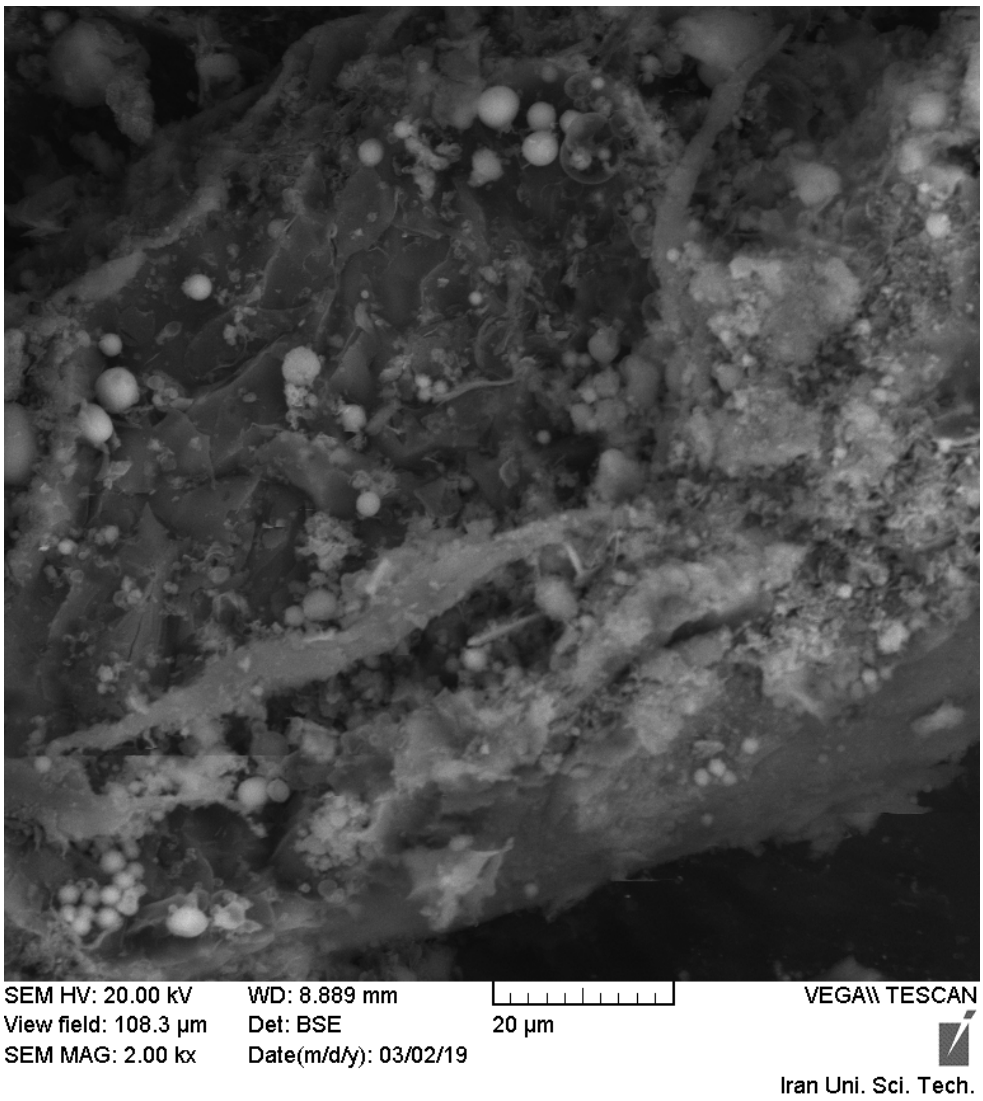



Fig. 5. SEM morphology for 2-GPD.

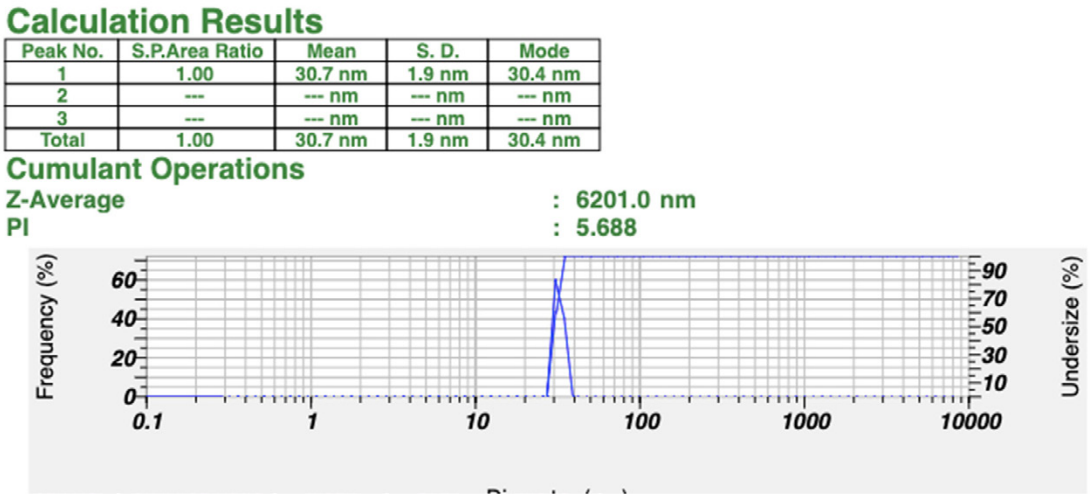



Fig. 6. The optimal curve of the sample of this nanocarrier loaded with the nanosome in the extract of the plant by the DLS nanosized; that is, it indicates the shape in which the optimal mean condition of the sample of particles with a size of nanometers has been reported.

2-GPD, the surface contained SEM micrographs of compartments within the 2-GPD structure [15, 29], which supported our findings. Amand and Esmaeili [30] analyzed the properties of nanoparticles made of hybrid polymers containing anticoagulants. The morphology (average size of the formed spheres) shows that the produced nanocapsules ranged from 136 to 183 nm in diameter, with an average of 176 nm. Comparing the current results with other research leads that the plant extract is added to the nanoparticles system and not used in the particles for force regeneration.

*GC-MS assay*

Qualitative analysis of MTD in GCMS was performed as a full scan under GC-MS and the mentioned conditions in the urine sample and based on a NIST library search.

After standard injection of MTD, which has an inhibition time of Run time=11.69 min and based on a library search with m/z indices of 72, 86, 73, 294, an accurate urine sample containing MTD was injected into the device, which in this case, in addition to MTD index peak and at the same MTD inhibition time, the MTD metabolite EDDP peak index was observed. The inhibition time for MTD metabolite is 11.15 minutes, and the index peak is 277, 276 (**Fig. 4**). In one study [16, 27, 31], MTD, along with buprenorphine, was the most common drug used to treat opioid dependence. This study aimed to analyze MTD and its primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine (EDDP), in the urine and plasma of opioid addicts. The study group included drug users voluntarily admitted to a detoxification center. This study was conducted to identify whether urine or plasma provides better results for the proposed method. This method can be applied in clinical laboratories to rapidly determine MTD levels in urine instead of plasma and monitor MTD substitution therapy. In another study [23], MTD is a strong sedative widely used to treat heroin addiction and is often encountered in forensic samples. In gas chromatography electron impact (E.I.)/mass spectrometry (GC-MS) mode, MTD predominantly produces a single m/z 72 ion, which is characteristic enough for identification. Molecular ion determination, which can be obtained using chemical ionization (CI), provides diagnostic information and better drug identification. This method is sensitive and diagnostic and is now routinely used in our laboratory.

*XRD determination*

X-ray diffraction has elucidated dendrimers' structure and phase behavior with different end-chain surface topologies. This theorem includes the 2-GPD block and statistical dendrimers with aliphatic and mesogenic terminals. Groups and homo-dendrimers of generations 1 and 2, which contain only mesogenic end groups, exhibit a monolayer nematicon phase. The exact structure of the layered arrangement was determined by X-ray

reflection of thin layers on it. A second-generation statistical dendrimer substrate does not show any mesogenic phase.

*DLS determination*

Nanoparticles have attracted attention in the last decade due to their unique physical properties and good chemical stability, which can lead to diverse potential applications. In this work, dendrimers with amine-terminating groups were synthesized and characterized by spectroscopy. These dendrimers were investigated as templates for the preparation of nanoparticles. Nanoparticles with a diameter of about nanometers were obtained in the presence of G2.0-G5.0 dendrimers. The size of the produced particles decreased with the increase of dendrimer generations. Higher-generation dendrimers have a rigid structure with many end groups on the surface and may play a decisive role [32, 33].

Dynamic Light Scattering (DLS) is a physical method often used to measure and size the distribution of particles in solutions and suspensions. DLS dispersion size is shown in Fig. 6. It shows the optimal sample size of particles. DLS results showed that the particle size is equal to 30.7. According to the results. As a result, it can be said that the size of the resulting particles is much smaller, and its ability is much more effective in the size of caries; previous studies also confirm this issue [34].

## CONCLUSION

Monitoring and controlling the consumption of various drugs, such as MTD, is a challenging clinical and legal toxicology issue. Examining medications in a sample such as the US always requires high accuracy. It is sensitive due to the complexity of the matrix. Microextraction methods such as LLME and SPE is essential because of minimizing matrix interference and maximizes analyte concentration. The use of unique tools such as gas chromatography or L.C. with mass spectroscopy and the preferred technique of LC-MS/MS is also of interest today. In addition, dendrimer macromolecules as host molecules to replace the desired analyte, including various drugs, can open the way for their extraction and identification technique.

## CONFLICT OF INTEREST

There is no conflict of interest

## REFERENCE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