Development of a Nanocluster-Based Platform for Determination of Sofosbuvir

Zahra Karimzadeh1,2, Abolghasem Jouyban1,3, Elaheh Rahimpour1,4,*

1 Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
2 Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
3 Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

* Corresponding author. E-mail: rahimpour_e@yahoo.com
Abstract

**Background:** Sofosbuvir is a potent direct-acting antivirus agent that has been listed as a promising medicine for the treatment of all genotypes of hepatitis C virus. As antiviral drugs could be metabolized to their associated compounds and toxicologically and pharmacologically interfere with the parent drugs, identifying the therapeutic range of drugs would be notable. **Methods:** In the current study, copper nanoclusters (Cu NCs) are synthesized during the reduction of copper nitrate with hydrazine hydrate in a protected media and used as a nanoprobe for the determination of sofosbuvir in plasma samples. Herein, synchronous fluorescence spectroscopy (SFS) is used for monitoring of fluorescence variation of nanoprobe owing to the excessive benefits compared with the traditional fluorescence. **Results:** SFS peak of Cu NCs has appeared at 355 nm with Δλ=80 nm which is decreased in the presence of sofosbuvir. To optimize the reaction factors, a response surface methodology is used and in the optimized conditions, a linear concentration-response plot is obtained in a range of 0.05-6.0 µg mL\(^{-1}\) with a limit of detection of 0.0147 µg mL\(^{-1}\). **Conclusion:** The developed method also reveals good repeatability and selectivity for sofosbuvir in plasma samples.

**Keywords:** Sofosbuvir; Copper nanoclusters; Synchronous fluorescence spectroscopy; Response surface method; Quenching
Introduction

Hepatitis C virus (HCV) responsible for chronic and acute liver infection is one of the significant worldwide health problems. The severity of this single-stranded RNA virus can be serious or mild enduring for weeks or a whole lifetime.\(^1\) Until recently, HCV was treated by ribavirin and interferon therapy that could cause serious-lethal adverse effects and high pill burden.\(^2\) Regarding various drawbacks of the previous therapies, more effective and tolerated therapy called direct-acting antivirus agents (DDA) has been recently established for the treatment of HCV. Sofosbuvir is a potent DDA that was listed as a promising medicine for the treatment of all genotypes of HCV. Owing to their numerous merits including high efficacy, few side effects, a high rate, and a short period of cure, sofosbuvir has been extensively adopted as an important drug for HCV treatment.\(^3\) Sofosbuvir acts by inhibiting the NS5B nucleotide polymerase through interfering with the RNA replication process\(^4\) and reach to maximum plasma concentration in about 0.5 to 2 h with a maximal concentration of 567 µg.L\(^{-1}\) as a therapeutic level.\(^5\)

As antiviral drugs could be metabolized to their associated compounds and toxicologically and pharmacologically interfere with the parent drugs, identifying the therapeutic range of drugs would be notable. Furthermore, an enhancement in the efficacy of therapy and a decrease in the side effect and care costs would be occurred by knowing the concentration of drugs in the biological samples.\(^6,7\)

Therefore, developing a sensitive, selective, fast, easy, and eco-friendly method for the determination of sofosbuvir in biological fluids would be highly encouraging. So far, there are some published techniques for sofosbuvir determination alone or with other metabolites such as reverse phase high performance liquid chromatography (HPLC)-UV,\(^8,9\) UPLC-mass spectrophotometry (MS)-MS,\(^10,11\) thin-layer chromatography (TLC),\(^12-14\) voltammetry\(^15\) and spectrophotometry.\(^7,16-18\) Despite the advantages of each method for employment in clinical courses, most of the previous techniques need more time, expensive instrumentation, extensive extraction procedures, and a high rate of solvent consumption. In this regard, developing a reproducible, stable, reliable, and fast sensor for the analytical determination of drugs has aroused massive attention by virtue of their importance in the biological samples.

Recently, nanoclusters (NCs) as a group of nanomaterial have ignited a surge of unprecedented interest in various fields including bioassays, sensing, and single-molecule optoelectronics due to their small particle size. The high sensitivity, stability, efficiency, easy operation, fast, and low-cost features of NCs offer proper conditions for being used in fluorescent sensing protocols.\(^19,20\) Metal NCs especially Cu NCs have been extensively adopted as fluorescent probes for sensing due to their not only abundant, available, and inexpensive source; but also, their good fluorescence properties.\(^21,22\) Thus, the use of Cu NCs advocates great potential for clinical applications. In the present study, a Cu NCs-based probe is developed for the determination of sofosbuvir in plasma samples. Herein, synchronous fluorescence spectroscopy (SFS) is used for monitoring of fluorescence variation of nanoprobe owing to the excessive benefits compared with traditional fluorescence. SFS is an attractive multidimensional fluorescence, simple, and sensitive technique for biomolecules quantitative determination which includes scanning by emission and excitation monochromators simultaneously.\(^23\) The narrow-sharp spectrum obtained in SFS results in the simplification of the emission spectra, a decrease of interferences, and enhancement of the resolution and selectivity of the spectra.\(^24\) The reaction conditions are optimized by response surface methodology (RSM) and the proposed method is fully validated for the determination of sofosbuvir in plasma samples.
Experimental

Reagents and solutions

Citric acid ($C_6H_8O_7$•H$_2$O) and cetyltrimethyl ammonium bromide (CTAB, $C_{19}H_{42}BrN$) received from Merck and hydrazine hydrate ($H_4N_2$•H$_2$O) and copper nitrate ($Cu\ (NO_3)_2$•3H$_2$O) purchased from Sigma-Aldrich in analytical grade are used as reagents for Cu NCs preparation. A solution of 1000 mg$L^{-1}$ of sofosbuvir (Khorshid Kadous Pharmaceutical Co., Iran) is prepared by dissolving it in a proper amount of methanol and working solutions are freshly prepared daily by diluting the stock solution with deionized water.

Apparatus and software

A FP-750 spectrofluorometer (Jasco Corp., Japan) at synchronous mode with $\Delta \lambda = 80$ nm, medium sensitivity, 10 nm slit width in emission and 5 nm slit width in the excitation paths at room temperature is applied for recording the SFS spectrum. For pH adjustments, a digital pH-meter model 744 (Metrohm Ltd., Switzerland) and for characterization of nanoparticles, a CM30 Transmission electron microscopy (Philips, The Netherlands, www.philips.com) are used. Furthermore, MINITAB (Minitab Inc. Release 17.0) software is employed for ANOVA analysis and experimental design.

Synthesis of Cu NCs

The fluorescent Cu NCs are prepared by the reduction of copper nitrate using hydrazine in the presence of CTAB and citric acid.$^{25}$ For this purpose, first, 0.05 mL citric acid of 0.1 mol L$^{-1}$ (0.5 mmol L$^{-1}$ in 10 mL) and 0.0182 g of CTAB are inserted into the 10 mL of 2 mol L$^{-1}$ aqueous solution of hydrazine hydrate and a colorless solution is obtained which is stirred for 30 minutes. Besides, another solution is prepared by inserting 0.05 mL citric acid of 0.1 mol L$^{-1}$ and 0.0182 g of CTAB, and 0.0482 g of Cu (NO$_3$)$_2$•3H$_2$O into the deionized water following the stirring process. Then, the as-prepared solutions are mixed and stirred at room temperature for 3 h. The color change to reddish-brown indicates the synthesis of Cu NCs. The prepared Cu NCs are kept at 4 °C in a dark place for usage.

Samples preparation

Human plasma samples are used for assaying the sofosbuvir. In this regard, 500 μL of human plasma sample is poured into a 2.0 mL microtubes and spiked with different concentrations of sofosbuvir. For protein precipitation, about 500 μL of acetonitrile is added to the desired plasma sample and vortexed for 2 min. The tube contents are centrifuged at 10000 rpm for 5 min and clear supernatant is transferred to a clean microtube.

General procedure

The analysis procedure is made in a 2 mL microtube by a batch method. 50 μL of 0.1 mol.L$^{-1}$ phosphate buffer (pH 7.0) is added into the microtube containing prepared plasma (~ 800 μL obtained from the previous section) and finally, 20 μL of Cu NPs is added to it. After incubation of the sample for 10 min, the SFS spectrum is recorded by the simultaneous scanning of both $\lambda_{ex}$ and $\lambda_{em}$ at a constant $\Delta \lambda$ of 80 nm.

Results and discussion
**Characterization of Cu-NCs**

The size and shape of the prepared NCs are characterized using TEM. According to Fig. 1, the morphology of the Cu NCs is almost spherical and uniform in shape and size with an average diameter of less than 6 nm.

***Fig. 1***

**Detection mechanism discussion**

The quenching mechanism for a SFS probe arises from two way: (i) interaction with nanoparticles surface and a change in the nature of nanoparticles which is characterized by a decrease in the intensity of the SFS peak of nanoparticles; and (ii) the inner filter effect of the analyte which is the re-absorption of excited or emitted light by a quencher. In both cases, the SFS signal of nanoprobe is decreased proportionally to analyte concentration increase. In the present study, a strong SFS emission at 355 nm is achieved at a ∆λ of 80 nm; on the other hand, the simultaneous scanning of both λ_ex and λ_em at a constant ∆λ of 80 nm gives a maximum emission intensity at 355 nm which shows that maximum excitation intensity is observed in 80 nm lower than this wavelength (i.e. 275 nm). According to the maximum absorbance of sofosbuvir which is observed at 260 nm, the excitation spectrum of Cu NCs is completely overlapped by the UV absorption of sofosbuvir (Fig. 2) and interprets their SFS spectrum and consequently quench the SFS intensity of NCs. As can be seen in Fig. 3, Cu NCs show a strong SFS peak centered at 355 nm with ∆λ=80 that quenched with sofosbuvir adding and shows a linear relationship with its concentration in the range of 0.05-6.0 µg mL⁻¹.

***Figs. 2 and 3***

**Optimization of reaction conditions by an experimental design**

The relation between variables and response functions is investigated by central composite design (CCD) which considers the interactions between the independent parameters. These parameters include pH, reaction time, the concentration of phosphate buffer, and the volume of Cu NCs. Five levels of +α (maximum), +1, 0 (central) -1, and −α (minimum) are established for the above-mentioned parameters. The difference between SFS intensity in the presence and absence of sofosbuvir is considered as analytical response and a plasma sample spiked with 2.0 µg.mL⁻¹ sofosbuvir is used for all measurements. The corresponding tables and data are given in electronic supporting material (ESM). Each parameter effect on the response is plotted by three-dimensional (3D) graphs and shown in Fig. 4. As can be seen in Fig. 4a, the maximum intensity for the analytical response is observed at pH 7.0 which can be supplied with 0.05 µL of 0.1 mol.L⁻¹ phosphate buffer (0.005 mol.L⁻¹). On the other hand, a better result in the response signal is obtained as the Cu NCs volume raised to 20.0 µL and increase more than this value leads to a decrease in the response due to self-quenching phenomena (Fig. 4b). Furthermore, fluorescence intensity increases with an increase in time and remains relatively constant after 10 min (Fig. 4c). According to these results, the optimum values are pH of 7.0, buffer phosphate concentration of 0.005 mol.L⁻¹, Cu NCs volume of 20 µL, and reaction time of 10 min. The obtained equation for the dependency of SFS response to the investigated parameters in an uncoded unit is shown as:
$$Y = -368.099 + 99.162 X_1 + 5708.804 X_2 + 9.459 X_3 - 8.411 X_1^2 - 0.108 X_3^2 - 0.401 X_4^2 + 1.400 X_1 X_4 - 276.190 X_2 X_3 - 1.179 X_2 X_4 - 0.031 X_3 X_4 \quad (1)$$

Where $X_1$, $X_2$, $X_3$, and $X_4$ stand for pH, buffer concentration, Cu NCs volume, and reaction time, respectively. Sign and coefficient values represent the positive/negative effect of each parameter and their interactions.

**Interference study**

The selectivity of the Cu NCs based nanoprobe to sofosbuvir is assessed by adding different concentrations of other over-the-counter or co-administrated drugs in a solution with a constant concentration of sofosbuvir (1.0 µg.mL$^{-1}$) and recording the signal variation. The results are listed in Table 1. The results demonstrate that the presence of all investigated drugs (except for caffeine) does not affect the response of the nanoprobe. Although, the tolerance limit for ibuprofen, nicotinamide, and losartan is reported to be 10 times which means 10.0 µg.mL$^{-1}$ for each drug. It should be noted that the therapeutic level in plasma for all of them is reported to be higher than 10 µg.mL$^{-1}$. So it can be concluded that the developed nanoprobe has acceptable selectivity for sofosbuvir determination in plasma samples.

**Analytical figures of merit**

In the optimum conditions obtained by CCD, a linear relationship between sofosbuvir concentration and SFS response is observed in the range of 0.05–6.0 µg.mL$^{-1}$ with a regression coefficient of 0.9972 (Fig. 5). $\Delta I_{SFS}$ (difference of SFS intensity in the presence and absence of sofosbuvir) is considered as an analytical signal in these calculations. The limit of detection (LOD) and limit of quantification (LOQ) for this method are reported to be 0.0147 and 0.044 µg.mL$^{-1}$, respectively. LOD and LOQ are defined as $3S_b/m$ and $10S_b/m$ where $S_b$ is the blank standard deviation and $m$ is the slope for the calibration curve. $S_b$ is obtained by measuring six blank samples. To evaluate the precision of the developed methodology, the analysis is repeated on the same and different days for a constant concentration of sofosbuvir (2.0 µg.mL$^{-1}$). The relative standard deviations (RSDs%) for inter-day and intra-day are calculated as 0.6 % and 1.8 %, respectively that points out the good repeatability and reproducibility of the validated method. A comparison of the current method with some other reported methods in the literature is summarized in Table 2. As can be seen, the analytical characterization of the validated method is good and comparable with that of other reported methods.

**Analytical application**

The validate method is successfully applied for the analysis of spiked plasma samples with sofosbuvir. In order to investigate the method’s accuracy, recovery tests are performed by spiking the proper
concentrations of sofosbuvir. The obtained results are given in Table 3. The recoveries ranged from 96.23% to 105.95%. Numerical analyses of these results by Student’s t-test shows that there is no significant difference between added and found values.

***Table 3***

**Conclusions**

In the current study, an inexpensive, eco-friendly, and simple SFS approach based on Cu NCs is developed for the determination of sofosbuvir. The validated nanoparticle-based technique has been exposed to be promising for sofosbuvir detection in plasma samples with desired features such as high sensitivity, fast response time, easy operation, and low interfering substances. This method is successfully used for the sofosbuvir determination in plasma samples with recoveries ranged from 96.23% to 105.95%. This is the first effort on the nanoparticle-based optical probe for the quantification of sofosbuvir levels and it can be a highly in-demand method in clinical experiments.

**Acknowledgments**

This work was supported by Research Affairs of Tabriz University of Medical Sciences, under grant number 65403. The authors would like to thank khorshid kadous pharmaceutical co and Dr. H. Poustchi, Digestive Diseases Research Institute, Tehran University of Medical Sciences for gifting sofosbuvir powder.

**Authors’ contributions**

Zahra Karimzadeh: The acquisition, analysis, drafting the work, Abolghasem Jouyban: Design of the work, interpretation of data for the work, Elaheh Rahimpour: design of the work, interpretation of data for the work.
References

1. Spearman CW, Dusheiko GM, Hellard M, Sonderup M, Hepatitis C, Lancet (London, England), 2019;394:1451-1466.

2. Rafiq SM, Banik GR, Khan S, Rashid H, Khandaker G, Current burden of hepatitis C virus infection among injecting drug users: A mini systematic review of prevalence studies, Infect. Disord. Drug Targets. 2014;14:93-100.

3. Gentile I, Maraolo AE, Buonomo AR, Zappulo E, Borgia G, The discovery of sofosbuvir: a revolution for therapy of chronic hepatitis C, Expert Opin. Drug Discov. 2015;10:1363-1377. Doi: 10.1517/17460441.2015.1094051

4. Bhatia HK, Singh H, Grewal N, Natt NK, Sofosbuvir: A novel treatment option for chronic hepatitis C infection, J. Pharmacol. Pharmacother. 2014;5:278-284. Doi: 10.4103/0976-500X.142464

5. https://go.drugbank.com/drugs/DB08934

6. Marshall AD, Grebely J, Dore GJ, Treloar C, Barriers and facilitators to engaging in hepatitis C management and DAA therapy among general practitioners and drug and alcohol specialists-The practitioner experience, Drug Alcohol Depend. 2020;206,107705. Doi: 10.1016/j.drugalcdep.2019.107705

7. El-Wekil MM, Mahmoud AM, Marzouk AA, Alkahtani SA, Ali R, A novel molecularly imprinted sensing platform based on MWCNTs/AuNPs decorated 3D starfish like hollow nickel skeleton as a highly conductive nanocomposite for selective and ultrasensitive analysis of a novel pan-genotypic inhibitor velpatasvir in body fluids, J. Mol. Liq. 2018; 271, 105-111. Doi: https://doi.org/10.1016/j.molliq.2018.08.105

8. Ahmed A, Mahtab T, Saleem S, Akula S, Khan MD, Validation of RP-HPLC method for determination of sofosbuvir in bulk and pharmaceutical dosage forms, Int. J. Pharm. Sci. Nanotech. 2020;13,4826-4830. Doi: 10.37285/ijpsn.2020.13.2.4

9. Zaman B, Siddique F, Hassan W. RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies, Chromatographia 2016;79:1605-1613. Doi: 10.1007/s10337-016-3179-9

10. Gandhi BM, Rao AL, Rao JV. UPLC-MS/MS method for determination of sofosbuvir in human plasma, Ann. Pharm. Fr. 2017; 75, 257-266. Doi: 10.1080/10408347.2021.1923456

11. El-Yazbi AF, Comparative validation of the determination of sofosbuvir in pharmaceuticals by several inexpensive ecofriendly chromatographic, electrophoretic, and spectrophotometric methods, J. AOAC Int. 2017; 100:1000-1007. Doi: 10.5740/jaoacint.16-0295

12. Fares MY, Abdelwahab NS, Abdelrahman MM, Abdel-Rahman HM, Determination of sofosbuvir with two co-administered drugs; paracetamol and DL-methionine by two chromatographic methods. Application to a pharmacokinetic study, Bioanalysis, 2019;11 : 349-364. Doi: 10.4155/bio-2018-0191
13. El-Gizawy SM, El-Shaboury SR, Atia NN, Abo-Zeid MN. New, simple and sensitive HPTLC method for simultaneous determination of anti-hepatitis C sofosbuvir and ledipasvir in rabbit plasma, J. Chromatogr. B 2018;1092:432-439. Doi: 10.1016/j.jchromb.2018.06.033

14. Mansour FR. A new innovative spectrophotometric method for the simultaneous determination of sofosbuvir and ledipasvir, Spectrochim. Acta A 2018;188:626-632. Doi: 10.1016/j.saa.2017.07.066

15. Mahmoud AM, El-Wekil MM, Mahnashi MH, Ali MFB., Alkahtani SA. Modification of N,S co-doped graphene quantum dots with p-aminothiophenol-functionalized gold nanoparticles for molecular imprint-based voltammetric determination of the antiviral drug sofosbuvir, Microchim. Acta 2019;186:617. Doi: 10.1007/s00604-019-3647-7

16. Mansour FR, A new innovative spectrophotometric method for the simultaneous determination of sofosbuvir and ledipasvir, Spectrochim. Acta. A 2018;188:626-632. Doi: 10.1016/j.saa.2017.07.066

17. Abo-Talib NF, El-Ghobashy MR, Tammam MH. Spectrophotometric methods for simultaneous determination of sofosbuvir and ledipasvir (HARVONI tablet): comparative study with two generic products. J. AOAC Int. 2017; 100:976-984. Doi: 10.5740/jaoacint.16-0330

18. Rezk MR, Monir HH, Marzouk HM. Spectrophotometric assessment of the brand new antiviral combination: Sofosbuvir and velpatasvir in their pure forms and pharmaceutical formulation, Spectrochim. Acta A 2019;213:159-166. Doi: 10.1016/j.saa.2019.01.058

19. Gill R, Zayats M, Willner I.J. Semiconductor quantum dots for bioanalysis, 2008;47:7602-7625. Doi: 10.1002/anie.200800169

20. Alivisatos P. The use of nanocrystals in biological detection, Nat. Biotechnol. 2004;22 : 47-52. Doi: 10.1038/nbt927

21. Wang C, Shu S, Yao Y, Song Q. A fluorescent biosensor of lysozyme-stabilized copper nanoclusters for the selective detection of glucose, RSC Adv. 2015;5:101599-101606. Doi: 10.1039/C5RA19421K

22. Zhang M, Qiao J, Zhang S, Qi L. Copper nanoclusters as probes for turn-on fluorescence sensing of L-lysine, Talanta 2018;182:595-599. Doi: 10.1016/j.talanta.2018.02.035

23. Wang L, Liang AN, Chen HQ, Liu Y, Qian BB, Fu J. Ultrasensitive determination of silver ion based on synchronous fluorescence spectroscopy with nanoparticles, Anal. Chimica Acta, 2008;616:170-176. Doi: 10.1016/j.aca.2008.04.027

24. Patra D, Mishra AK, Recent developments in multi-component synchronous fluorescence scan analysis, Trends Anal. Chem. 2002; 21 :787-798. Doi: 10.1016/S0165-9936(02)01201-3

25. Ghosh SK, Rahman DS, Ali AL, Kalita A. Surface plasmon tunability and emission sensitivity of ultrasmall fluorescent copper nanoclusters, Plasmonics 2013;8:1457-1468. Doi: 10.1007/s11468-013-9559-1

26. Panigrahi SK, Mishra A.K., Inner filter effect in fluorescence spectroscopy: as a problem and as a solution, J. Photochem. Photobiol. C. 2019;41:100318. Doi: 10.1016/j.jphotochemrev.2019.100318
27. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics, Crit. Care, 2012; 16:1-4. Doi: 10.1186/cc11441
Figure 1. TEM image of the Cu NCs (A) and particle size distribution curve (B).
Figure 2. Absorbance spectrum of the Cu NCs and sofosbuvir.
Figure 3. SFS spectrum of the Cu NCs in the absence (a) and presence of sofosbuvir in the concentration range of 0.05-6.0 µg mL\(^{-1}\) (b-f). Conditions: pH 7, Cu NCs volume= 20.0 µL, [phosphate buffer]=0.005 mol L\(^{-1}\), and time = 10 min. \(\lambda_{\text{max}}= 355\) nm with \(\Delta \lambda=80\).
Figure 4. The response surface of the analytical signals as a function of (a) pH and [Buffer], (b) pH and Cu NCs volume, (c) pH and time, respectively.
Fig. 5. The calibration curve for response of proposed nanoprobe toward sofosbuvir
Table 1. Tolerance limits of some co-administrated and over-the-counter drugs in the determination of 1.0 µg mL\(^{-1}\) of sofosbuvir.

| Interfering substances                                                                 | Tolerance limit (Times) |
|---------------------------------------------------------------------------------------|-------------------------|
| Clonazepam, amoxicillin, aspirin                                                       | 100                     |
| Diazepam, oxazepam, celecoxib, diltiazem, daclatasivir, naproxen, chlordiazepoxide, paracetamol, pantoprazole, | 20                      |
| Ibuprofen, nicotinamide, losartan, caffeine                                            | 10                      |
Table 2. Comparison of analytical characteristics of the presented method with other reported techniques for determination of sofosbuvir.

| Method                  | Sample                     | LOD (µg mL\(^{-1}\)) | Linear range (µg mL\(^{-1}\)) | Reference |
|-------------------------|-----------------------------|------------------------|-------------------------------|-----------|
| RP-HPLC\(^a\)-UV        | Pharmaceutical formulation  | 0.02                   | 40-240                        | [7]       |
| RP-HPLC-UV              | Pharmaceutical formulation  | 2.7                    | 9.0-56.0                      | [8]       |
| Spectrophotometery      | Pharmaceutical formulation  | 5.6                    | 20.4–207.5                    | [6]       |
| Spectrophotometery      | Pharmaceutical formulation  | 1.21                   | 5.0–90.0                      | [17]      |
| Voltametry              | plasma                      | 0.0003                 | 0.001–0.4                     | [14]      |
| TLC\(^b\)               | Pharmaceutical formulation  | 0.8\(^c\)              | 2.5–24.0\(^c\)               | [13]      |
| Cu NCs-based SFS        | plasma                      | 0.44                   | 0.05–6.0                      | This work |

\(^a\) Reverse phase high performance liquid chromatography  
\(^b\) Thin-layer chromatography  
\(^c\) Unit is µg/band
Table 3. Determination of sofosbuvir in spiked plasma samples.

| Sample | Added (µg mL\(^{-1}\)) | Found ± SD (µg mL\(^{-1}\)) | Recovery (%)\(^a\) | t-test \(^b\) |
|--------|---------------------|--------------------------|-------------------|-------------|
| Plasma 1 | 0.50 | 0.51 ± 0.01 | 103.30 | 1.73 |
|     | 1.00 | 1.05 ± 0.04 | 105.95 | 2.16 |
|     | 2.00 | 1.98 ± 0.04 | 99.00 | 0.87 |
|     | 4.00 | 4.03 ± 0.16 | 100.81 | 0.32 |
|     | 6.00 | 6.15 ± 0.25 | 102.64 | 1.04 |
| Plasma 2 | 0.50 | 0.51 ± 0.01 | 103.30 | 1.73 |
|     | 1.00 | 1.05 ± 0.03 | 105.03 | 2.88 |
|     | 2.00 | 1.92 ± 0.04 | 96.23 | 3.46 |
|     | 4.00 | 4.08 ± 0.09 | 102.19 | 1.54 |
|     | 6.00 | 6.22 ± 0.19 | 103.71 | 2.00 |

\(^a\) Mean of three determination ± standard deviation

\(^b\) \(t\)-critical = 4.3 for \(n=3\) and \(P = 0.05\).