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Development of an easy and rapid analytical method for the extraction and preconcentration of chloroquine phosphate from human biofluids prior to GC–MS analysis

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ABSTRACT

A vortex assisted spraying based fine droplet formation liquid phase microextraction (VA-SFDF-LPME) method was developed to determine chloroquine phosphate at trace levels in human serum, urine and saliva samples by gas chromatography–mass spectrometry (GC–MS) with single quadrupole mass analyzer. In the first part, several liquid phase microextraction (LPME) and magnetic solid phase extraction (MSPE) methods were compared to each other in order to observe their extraction ability for the analyte. VA-SFDF-LPME method was selected as an efficient and easy extraction method due to its higher extraction efficiency. Optimization studies were carried out for the parameters such as extraction solvent type, sodium hydroxide volume/concentration, sample volume, spraying number and mixing type/period. Tukey's method based on post hoc test was applied to all experimental data for the selection of optimum values. Optimum extraction parameters were found to be 12 mL initial sample volume, two sprays of dichloromethane, 0.75 mL of 60 g/kg sodium hydroxide and 15 s vortex. Under the optimum conditions, limit of detection and quantification (LOD and LOQ) were calculated as 2.8 and 9.2 μg/kg, respectively. Detection power of the GC–MS system was increased by approximately 317 folds with the developed extraction/preconcentration method. The applicability and accuracy of the proposed method was evaluated by spiking experiments and percent recovery results for human urine, serum and saliva samples were found in the range of 90.9% and 114.0% with low standard deviation values (1.9–9.4).

1. Introduction

The coronavirus (COVID-19) disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread worldwide and ruined the health system especially in developing countries (Sunkari, Korboe, Abu, & Kizildeniz, 2020). The disease infects people by the direct contact of infected individuals, contaminated surfaces/wastes, airborne/respiratory droplets and fecal-oral routes (Heller, Mota, & Greco, 2020; Kitajima et al., 2020; Patrício Silva et al., 2021). Up to now, millions of people have been infected by the COVID-19 disease and death toll is still on the rise (Chandra, Verma, Singh, Jain, & Netam, 2021; COVID-19 Map - Johns Hopkins Coronavirus Resource Center, 2020). There are several antiviral drugs to fight against COVID-19 and its symptoms. Chloroquine with its antiviral property is known as one of candidate drugs used in the treatment of the disease (Costanzo, De Giglio, & Roviello, 2020). According to in vitro studies, SARS-CoV-2 was inhibited by using chloroquine chemical (Devaux, Rolain, Colson, & Raoult, 2020; Li, Geng, Peng, Meng, & Lu, 2020; Wang et al., 2020). On the other hand, chloroquine has various adverse effects on human bodies such as retinal toxicity, cardiotoxicity, neurotoxicity, myotoxicity and hypokalemia (Mubagwa, 2020). For these reasons, it is necessary to develop an analytical method for accurate and sensitive determination of chloroquine at trace levels in biological samples to obtain comprehensive evaluation on its detrimental effects and consequences.

In general, gas chromatography and liquid chromatography have been used to separate drugs and non-retained compounds from each other and equipped with proper detector for their instrumental detection (Kar, 2005). Chloroquine has been qualified/quantified by high performance liquid chromatography-ultraviolet detection (HPLC-UV) (Cheomung & Na-Bangchang, 2011), high performance liquid chromatography-fluorescence detection (HPLC-FL) (Samanidou, Evaggelopoulou, & Papadoyannis, 2005), gas chromatography (GC)-nitrogen

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sensitive detector (Churchill, Mount, & Schwartz, 1983; Viala, Deturmeny, Estadieu, Durand, & Cano, 1981), differential pulse voltammetry (DPV) (Mashhadizadeh & Akbarian, 2009), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Boonprasert, Sri-In, Pongnarin, Chatsiricharoenkul, & Chandranipapongse, 2012; Singhal et al., 2007), laser induced fluorescence (Amador-Hernández, Fernández-Romero, & Luque De Castro, 2001), liquid chromatography/ion trap mass spectrometry (LC-IT-MS), liquid chromatography/time of flight mass spectrometry (LC-TOF-MS) and nuclear magnetic resonance (NMR) spectroscopy (Dongre et al., 2009). Additionally, there is no many studies in literature for the determination of chloroquine by using GC instruments. However, GC systems have higher resolution and peak capacity for complex matrices than LC systems. Moreover, GC hyphenated with MS are named as powerful measurement method for the volatile and semi-volatile compounds (McEwen & McKay, 2005).

Extraction methods are frequently used to eliminate or decrease matrix effects and preconcentrate the analyte into detectable concentrations (Marchi, Rudaz, & Veuthey, 2009). Several offline extraction and microextraction methods such as solid phase microextraction (SPME) (Beale et al., 2018; Kudlejova, Risticevic, & Vuckovic, 2012; Lord & Pawliszyn, 2000; Risticevic, Lord, Górecki, Arthur, & Pawliszyn, 2010), headspace solid phase microextraction (HS-SPME) (Miekisch, Fuchs, Kamysek, Neumann, & Schubert, 2008), dispersive liquid-liquid microextraction (DLLME) (Jain & Singh, 2016), magnetic solid phase extraction (MSPE) (kui Li & ping Shi, 2019), switchable-hydrophilicity solvent liquid-liquid microextraction (SHS-LLME) (Ahmar, Nejati-Yazdinejad, Najafi, & Hasheminasab, 2018; Alshana, Hassan, Al-Nidawi, Yilmaz, & Soylak, 2020; Shahvandi, Banitaba, & Ahmar, 2018), hollow-fiber liquid-phase microextraction (HF-LPME) (de Bairros et al., 2015), hollow-fiber with drop-to-drop solvent microextraction (HF-DDSME) (Tapadia, Shrivas, & Upadhyay, 2011), ionic liquid-dispersive liquid-liquid microextraction combined with micro-solid phase extraction (IL-DLLME-μ-SPE) (Ge & Lee, 2013), ultrasound-enhanced air-assisted liquid-liquid microextraction (USE-AALLME) (Barfi et al., 2015) and molecularly imprinted solid-phase microextraction (MISPME) (Ansari & Karimi, 2017) have been employed to extract and preconcentrate different drugs prior to their qualification/quantification with proper instruments. In DLLME method, a dispersive solvent is used to efficiently disperse the extraction solvent into the aqueous solution (Assadi, Farajzadeh, & Bidari, 2012; Przyjazny, 2019; Sajid, 2018). However, the usage of dispersive solvent in the microextraction procedure causes to high solvent consumption and waste. For this reason, air-assisted liquid phase microextraction methods have been introduced to decrease or eliminate the dispersive solvent (Campillo, Gavazov, Viñas, Hagarova, & Andruch, 2020; Farajzadeh, Mohebbi, Pazhohan, Nemati, & Afshar Mogaddam, 2020). One study published by Dikmen et al. presented spraying based fine droplet formation liquid phase microextraction (SFDF-LPME) as a rapid and simple microextraction method for the extraction and preconcentration of a pesticide without dispersive solvent (Dikmen et al., 2020).

The object of this study was to develop a sensitive analytical method for trace determination of chloroquine phosphate in human urine, saliva and serum samples. In the determination, vortex assisted spraying based fine droplet formation liquid phase microextraction prior to GC–MS system was employed to preconcentrate the analyte. Influential parameters on the VA-SFDF-LPME method was fully optimized by univariate optimization approach. After the analytical performance studies, spiking experiments were performed in human urine, serum and saliva samples in an effort to ascertain the accuracy and applicability of the proposed method.

2. Experimental section

2.1. Chemicals and reagents

Chloroquine phosphate (99.4%) was supplied from Abdi İbrahim pharmaceutical company (İstanbul, Turkey). Dichloromethane, 1,2-dichloroethane and ammonium hydroxide were purchased from Merck (Darmstadt, Germany). Chloroform, acetonitrile and ethanol were obtained from Isolab Laborgeräte GmbH Chemicals (Eschau, Germany). Sodium hydroxide (98%) was supplied from Ak Kimya (Yalova, Turkey). Ultrapure water produced by Elga Flex 3 Water Purification System (High Wycombe, United Kingdom) was used during all sample/standard preparation and cleaning processes.

2.2. Instrumentation

Chromatographic separation of the analyte was performed on an Agilent 6890 gas chromatograph equipped with an HP5MS column (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness). An Agilent 5973 mass selective detector with a single quadrupole was interfaced to GC system for the qualitative/quantitative determination of chloroquine. The analytical conditions of the GC–MS system were as follows: inlet temperature 290 °C; helium as the carrier gas at 3.0 mL/min; injection volume, 1.0 μL; injection mode, splitless; ionization voltage, 70 eV; MS source temperature, 230 °C; MS quadrupole temperature, 150 °C; transfer line temperature, 280 °C. A ramp temperature program consisting of an initial 120 °C was increased to 260 °C (60 °C/min) and held for 4.0 min. The second ramp was to 300 °C at the rate of 60 °C/min. Qualifier/quantifier ions and retention time for chloroquine were 319/86 *m/z* and 5.58 min, respectively.

An analytical balance (OHAUS PA214C) with a resolution of 0.1 mg was used throughout all sample/standard preparations. A vortex mixer supplied from IsoLab Laborgeräte GmbH (Germany) was used for all mixing purposes. A centrifuge (Hettich-EBA20) was used to achieve distinct phase separation in the developed microextraction method.

2.3. Microextraction procedure

Sodium hydroxide (0.75 mL, 60 g/kg) was added into 12.0 mL sample/standard solution in order to remove phosphate ion found in the analyte structure. A spray bottle containing dichloromethane as the extraction solvent was connected to the centrifuge tube by a screw cap with a center hole. Next, the centrifuge tube was overturn and the extraction solvent was sprayed into the aqueous solution two times. The sprayed aqueous solution was then vortexed for 15.0 s to assist the analyte mass transfer from the aqueous phase to the extraction solvent. In order to facilitate the organic phase separation, the aqueous solution was centrifuged at 3461*g* for two minutes. The organic phase was transferred into a clean vial with the help of a microliter pipette and sent to the GC–MS system by an automatic liquid sampler. The repeatability of spray system was tested by gravimetric measurements. For this purpose, dichloromethane was sprayed two times with the help of spray system into three separate empty tubes. The mean value of dichloromethane sprayed was calculated as 0.281 ± 0.06 g corresponding to 211.6 ± 4.2 μL that was converted by using the density of dichloromethane.

In the experiments, all standard and sample solutions were prepared by gravimetric approach that has more accuracy and precision than the volumetric one.

2.4. Human urine, serum, and saliva samples

All biological samples were obtained from volunteers in our research laboratory. Protein precipitation was applied to all samples before applying the developed microextraction method.

In spiking experiments, human urine sample (2.33 g) was firstly spiked to desired concentration and alkalified by 0.95 g concentrated ammonium hydroxide. Acetonitrile (3.30 g) was added into the urine sample to precipitate protein ingredient in the sample and then diluted to 7.0 g with ultrapure water. After the protein precipitation, centrifugation process at 4420*g* for 5.0 min was applied to the sample for the

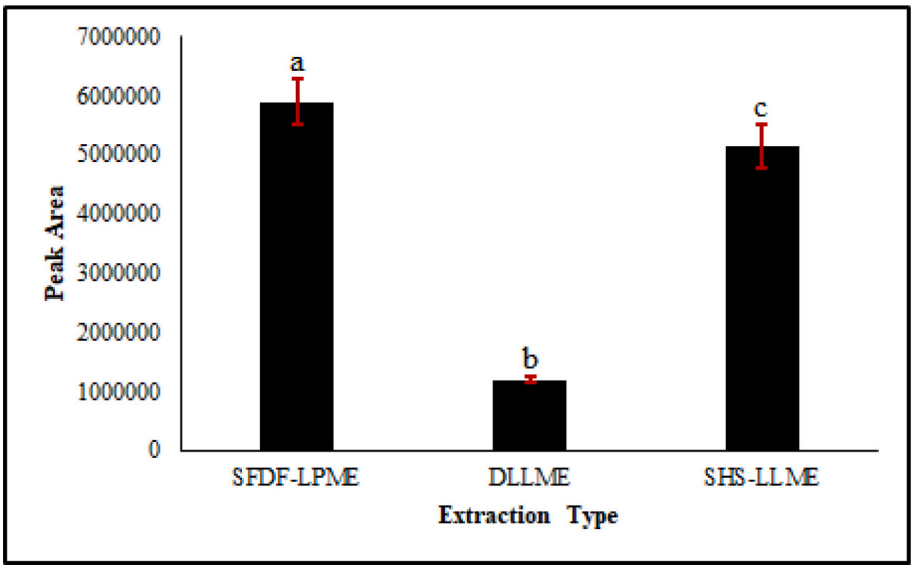

Fig. 1. Selection of microextraction method.

separation of supernatant phase. Following that 6.0 g of supernatant was transferred into a clean 50 mL tube and diluted to 40.0 g with ultrapure water.

Human serum and saliva samples (2.33 g) were separately weighed and spiked to the desired concentration. Acetonitrile (4.20 g) as the reagent for the protein precipitation was added to the spiked sample. After diluting to 7.0 *g* with ultrapure water, centrifugation at 4420*g* was held for 5.0 min. The supernatant phase (6.0 g) was completed to 40 g with ultrapure water. After performing the protein precipitation of all samples, the developed microextraction procedure given in Section 2.3 was applied to the diluted samples.

2.5. Statistical test

In the optimization studies, all parameters were investigated in three replicates and optimum values were selected in terms of peak area, standard deviation value and statistical evaluation by post hoc comparison with analysis of variance (ANOVA). JASP 0.9.1.0 software was used throughout all statistical analyses. The statistical significance was assessed by Tukey's honestly significant differences (HSD) test with 5.0% significance. Letters (a,b,c,d) on the figures represents significant differences according to p_{tukey} value at 95% confidence interval. If two data were statistically similar, same letters were written to demonstrate their similarity.

2.6. Experimental conditions for the extraction comparison

SFDF-LPME, DLLME and SHS-LPME methods were individually applied to 8.0 mL standard solutions (1.0 mg/kg) after adding 0.50 mL of 80 g/kg sodium hydroxide. In the DLLME method, an organic solvent mixture including 200 μL chloroform and 2.0 mL ethanol was injected into the solution. Another experiment was performed by the SHS-LPME method. 1.0 mL of protonated *N*,*N*-dimethyl benzylamine was added into the aqueous solution and then 1.0 mL of 80 g/kg sodium hydroxide was pipetted into the solution. The synthesis of protonated *N*,*N*-dimethylbenzylamine was reported in our previous study (Erarpat et al., 2019). One standard solution was extracted by the SFDF-LPME method consisting of two sprays of chloroform implemented by the spray system detailed in Section 2.3. All solutions were subjected to vortex mixing for 15.0 s and centrifugation process for 2.0 min. The organic phases were injected into the GC–MS system.

MSPE method was also tested to observe its extraction efficiency for the selected analyte. Fe_3O_4, amidosulfonic acid coated Fe_3O_4, salicylic acid coated Fe_3O_4, stearic acid coated Fe_3O_4, cobalt, nickel nanoparticles (10.0 mg) and Fe_3O_4/reduced graphene oxide nanocomposite (10.0 mg) were tested to extract the analyte from aqueous sample. Each nanoparticle was attentively poured into the aqueous solution and then vortex mixing was applied for 30 s in order to collect the analyte onto the nanoparticle surface. After separating the nanoparticles by an external magnetic field, analyte elution was carried out by 200 μL of chloroform.

3. Results and discussion

Optimization studies were performed to achieve high signal to noise ratio, low matrix effects and high preconcentration factor. For this purpose, important parameters such as type of microextraction method, type of extraction solvent, spraying number, initial sample volume, mixing type/period, concentration/volume of sodium hydroxide were meticulously investigated by univariate optimization approach. Chloroquine concentration in the optimization of microextraction method, extraction solvent, spraying number was 1.0 mg/kg while the other optimizations were performed using 0.5 mg/kg chloroquine standard solution.

3.1. Comparison of different microextraction methods with SFDF-LPME

In this study, SFDF-LPME, DLLME and SHS-LPME methods were tried to compare their ability to preconcentrate the analyte from the aqueous solution. All experiments in Section 2.6 were done for the selected liquid phase microextraction methods. According to the results demonstrated in Fig. 1, the SFDF-LPME method is the best liquid based microextraction method among the tested ones. In addition, this method had different results according to ANOVA results at 95% confidence level. It is also clear that it had several benefits such as simplicity, cheapness, low solvent consumption and easy operability if compared to the SHS-LLME method having the second highest peak area.

MSPE was also tried to test its performance on the extraction/preconcentration of the analyte. However, no detectable signal was obtained for all type of nanoparticles. This result proved that the SFDF-LPME method was an efficient microextraction method for target analyte due to its high peak areas.

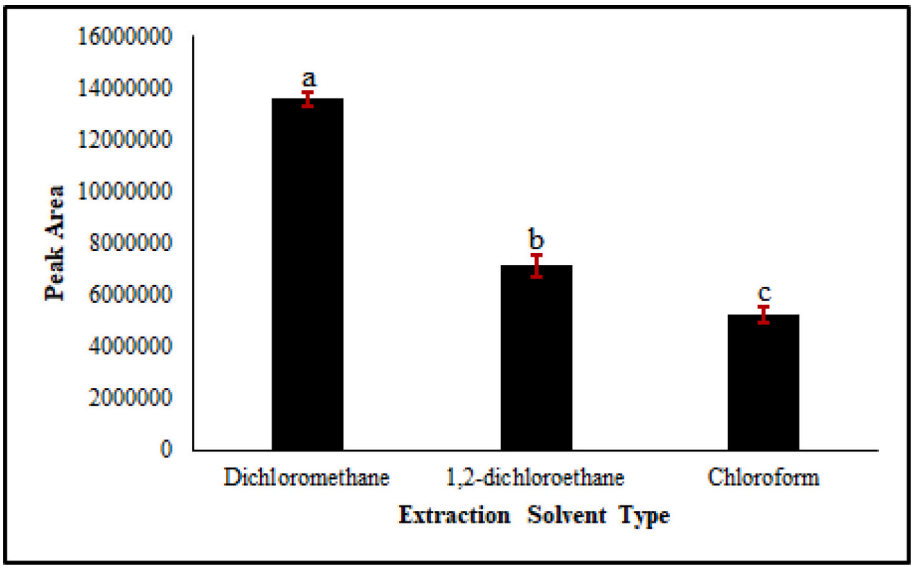

Fig. 2. Optimization of extraction solvent type.

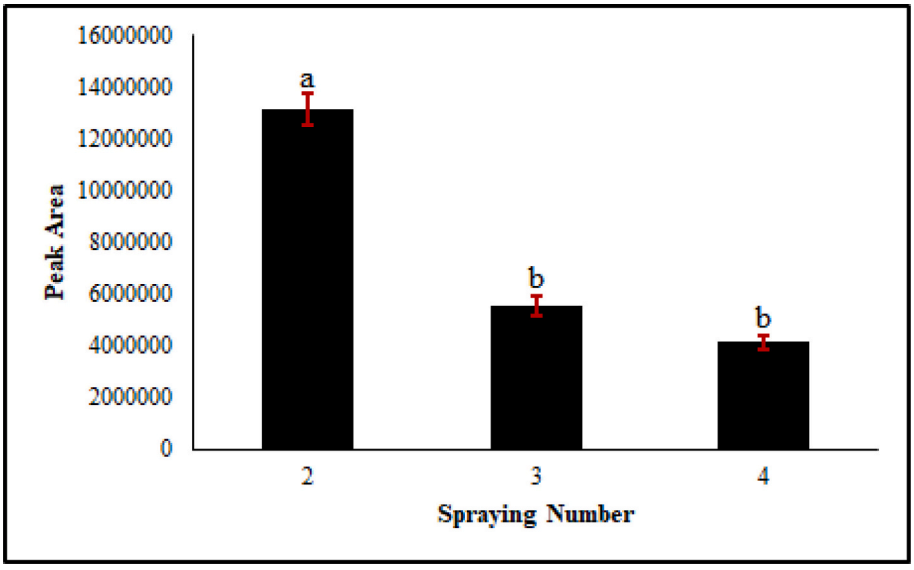

Fig. 3. Optimization of spray number.

3.2. *Optimization studies for the VA-SFDF-LPME method*

First attempt to optimize the VA-SFDF-LPME method was the selection of extraction solvent. It is important to choose an ideal extraction solvent for the liquid based microextraction methods because it should have different density, water immiscibility and be capable of the preconcentration of the analyte (Psillakis & Kalogerakis, 2002). Three halogenated solvents (chloroform, dichloromethane and 1,2-dichloroethane) were selected for this optimization. As can be seen in Fig. 2, all halogenated solvents gave different peak areas with low standard deviation values (≤10%). Dichloromethane was chosen as the optimum extraction solvent due to its maximum peak areas. Its experimental results were also statistically different from chloroform and 1,2-dichloroethane at 95% confidence interval.

In literature, DLLME is one the most used preconcentration methods for the determination of several organic and inorganic analytes. The key point in this method is to distribute the extraction solvent through the aqueous solution with the aid of a dispersive solvent that possess miscibility in both water and organic phases (Rutkowska, Płotka-Wasylka, Sajid, & Andruch, 2019). In spite of its high extraction efficiency and rapid equilibrium between the extraction solvent and aqueous phase, the requirement for the dispersive solvent results in competition with the extraction solvent for the analyte(s) and decreases the extraction outputs (Przyjazny, 2019). In this study, the selected extraction solvent was introduced into the aqueous solution by the spray system. Hence, dispersion of the extraction solvent into the aqueous solution was achieved without a dispersive solvent. In this optimization, spraying number was investigated between one and three sprays (Fig. 3) because of its effect on the preconcentration factor. According to the integrated peak areas, the best results were obtained when two sprays were applied to the aqueous solution. There was also a gradual decrease in the peak areas resulted from the inverse relationship between the volume of extraction solvent and preconcentration factor. Therefore, two sprays were used for the subsequent experiments.

Mixing is an effective way to enhance the mass transfer of the analyte from the aqueous phase to the extraction solvent. For this purpose, mechanical shaker, vortex and ultrasonication bath were examined to obtain high signal to noise ratios for the analyte. Vortex had slightly

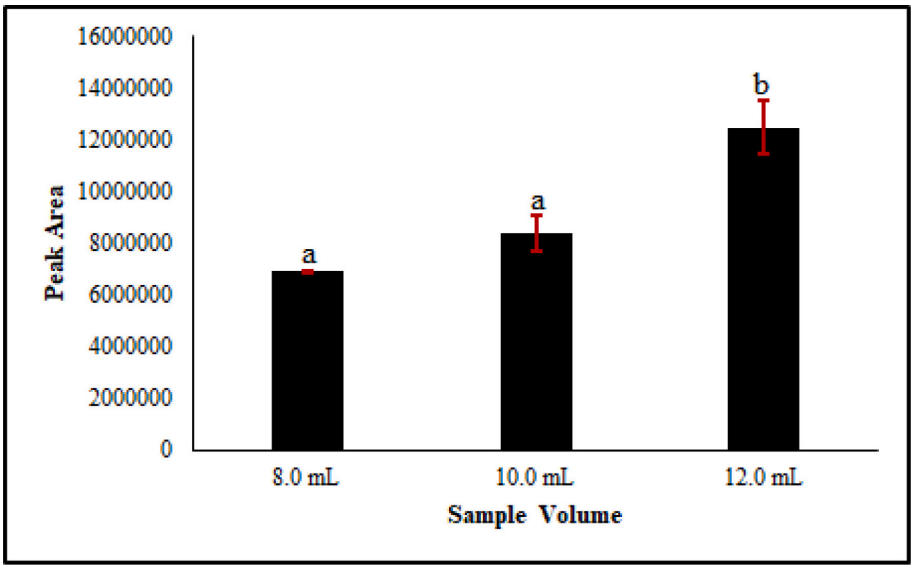

Fig. 4. Optimization of initial sample volume.

higher peak heights than other mixing types. Its repeatability was also the lowest one when standard deviation values were considered. Hence, mixing with vortex was selected as the optimum one. Further experiments were done for the selection of vortex period. The tested periods were 15, 30, 45, 60 s. In addition, one experiment was performed without vortex mixing to make a comparison with other periods. The optimum period was determined as 15 s due to its high signals, low standard deviation value and statistically significant difference from other periods.

It is well known that initial volume of the sample is a critical parameter to achieve better results in the extraction of trace analytes from the aqueous samples (Soylak & Uzcan, 2020). Three different initial volumes of chloroquine standard solution (8.0, 10.0 and 12.0 mL) were tested to increase the preconcentration factor of the analyte. As expected, the highest results were recorded for 12.0 mL of sample volume (Fig. 4) which was also the maximum volume to avoid leaking from the screw cap of the centrifuge tube. Therefore, 12.0 mL was used as the initial sample volume for the further studies.

In this study, sodium hydroxide was used to remove phosphate ion from the analyte since there was no signal obtained from the analyte extraction without sodium hydroxide. However, sodium hydroxide concentration and volume can positively or negatively affect the analyte extraction and purification. Several concentrations of sodium hydroxide in the range of 20 and 80 g/kg were investigated to assess its effects on the developed method. Similar results were recorded for 20 and 40 g/kg of sodium hydroxide while 60 and 80 g/kg gave approximately 18% higher signals than the lower concentration values. Based on the peak areas, 60 g/kg was chosen as the optimum value to eliminate phosphate ions from the analyte.

Sodium hydroxide volume was also adjusted to its optimum value because its higher volumes could lead to excess dilution of the sample. 12.0 mL sample solutions with different volumes of sodium hydroxide between 0.25 and 1.0 mL were individually tested to determine the optimum volume. According to the ANOVA results, all volumes had similar results at 95% confidence interval. Although 0.25 mL had the highest peak areas, 0.75 mL was chosen as the optimum one because 0.25 mL gave higher standard deviation value.

3.3. Analytical figures of merit and recovery studies

A series of standard solutions containing different concentrations of the analyte was gravimetrically prepared and analyzed by the GC–MS system. The linearity of the analyte was achieved between 3.8 and 77.3 mg/kg with 0.9991 coefficient of determination value. Limit of detection and quantitation (LOD and LOQ) for the aqueous standard solution were found to be 0.9 and 2.9 mg/kg, respectively. The developed VA-SFDF-LPME-GC–MS method was also evaluated in terms of linearity,

Table 1
Analytical figures of merit and comparison of the developed method with other published studies.

Method	LOD[a]	LOQ[b]	Linear range	Coefficient of determination, R^2	Reference
GC-MS[c]	0.9 mg/kg	2.9 mg/kg	3.8–77.3 mg/kg	0.9991	This work
VA-SFDF-LPME-GC-MS[d]	2.8 μg/kg	9.2 μg/kg	9.9–1003.9 μg/kg	0.9996	This work
LC-MS/MS[e]	–	2.56 ng/mL	2.56–1220 ng/mL	–	(Kaewkhao et al., 2019)
LC-MS/MS[e]	–	20 ng/mL	20–5000 ng/mL	–	(Gallay et al., 2018)
HPLC-UV/VIS[f]	50 ng/mL	150 ng/mL	150–2500 ng/mL	–	(Lejeune et al., 2007)
LLLME-HPLC-UV[g]	0.3 μg/L	1.0 μg/L	1.0–200 μg/L	0.9995	(Daneshfar, Khezeli, & Manafi, 2009)
GC-NSD[h]	5.0 ng/mL	–	–	0.9999	(Churchill et al., 1983)

[a] LOD: Limit of detection.
[b] LOQ: Limit of quantification.
[c] GC-MS: Gas chromatography-mass spectrometry.
[d] VA-SFDF-LPME-GC-MS: Vortex assisted spraying based fine droplet formation liquid phase microextraction-gas chromatography–mass spectrometry.
[e] LC-MS/MS: Liquid chromatography/tandem mass spectrometry.
[f] HPLC: High performance liquid chromatography-UV/VIS detection.
[g] LLLME-HPLC-UV: Single drop liquid-liquid-liquid microextraction-high performance liquid chromatography-UV detection.
[h] GC-NSD: Gas chromatography-nitrogen selective detection.

Table 2
Percent recovery results obtained for human urine, serum and saliva samples.

Sample	Concentration, μg/kg	External calibration method, Recovery% ± SD[a]	Matrix matching method, Recovery% ± SD[a]
Human serum	57.8	44.8 ± 2.8	90.9 ± 5.6
	112.5	50.9 ± 1.3	107.2 ± 2.8
	190.8	50.8 ± 2.6	106.3 ± 5.4
	271.2	49.1 ± 1.2	102.0 ± 2.4
Human urine	53.0	74.3 ± 1.7	93.7 ± 2.1
	110.5	75.5 ± 2.5	114.0 ± 3.8
	188.2	68.8 ± 1.7	106.3 ± 2.6
	268.7	64.9 ± 1.2	101.5 ± 1.9
Human saliva	53.5	36.2 ± 1.7	97.9 ± 4.6
	110.8	44.0 ± 3.8	102.4 ± 8.8
	188.7	46.0 ± 3.1	101.5 ± 6.9
	268.8	47.3 ± 4.4	102.3 ± 9.4

[a] Uncertainties (±): Standard deviation for n = 3.

LOD and LOQ values. Under the optimum conditions, linear range, LOD and LOQ for the aqueous standard solution were calculated as 9.9–1003.9, 2.8 and 9.2 μg/kg, respectively. If all dilution processes in Section 2.4 is used, LOD/LOQ values for human serum, urine and saliva samples were calculated as 141.6/471.9, 106.6/355.4 and 144.0/480.0 μg/kg, respectively. The analytical results are given in Table 1.

The developed method enhanced the detection power of GC–MS system by 317 times for target analyte calculated by dividing LOD value of GC–MS to that of VA-SFDF-LPME-GC–MS. This result indicates that trace levels of the analyte were preconcentrated and detected by the proposed VA-SFDF-LPME-GC–MS system. In Table 1, some liquid chromatographic methods such as high performance liquid chromatography (HPLC) (Lejeune et al., 2007), liquid chromatography/tandem mass spectrometry (LC-MS/MS) (Gallay et al., 2018), single drop liquid-liquid-liquid microextraction combined with isocratic high performance liquid chromatography with ultraviolet detection (LLLME-HPLC) (Daneshfar et al., 2009) were presented in literature for the determination of chloroquine at trace levels. However, there are limited number of gas chromatographic method to qualify and quantify chloroquine in biological samples. According to the limit of detection values in Table 1, the presented VA-SFDF-LPME-GC–MS method reached very low limit of detection by the help of a simple and rapid microextraction method without expensive and time-consuming methods. In addition, the developed microextraction method can be combined with more sensitive instruments like GC–MS/MS and LC-MS/MS systems to decrease LOD and LOQ values for the analyte.

The optimized VA-SFDF-LPME-GC–MS method was developed using aqueous standard solutions, but it should be evaluated in terms of its applicability to real samples including human urine, serum and saliva. For this reason, recovery experiments were performed to verify applicability and accuracy of the method. First, all samples were treated and preconcentrated according to the procedure detailed in Sections 2.3 and 2.4, respectively. However, all samples gave chromatographic signals below the detection limit. Next, the developed method was applied to the spiked samples. Percent recovery results calculated via external calibration method showed negative matrix effects on the analyte for the samples. In such cases, matrix matching calibration method can be used to compensate the matrix effects because of the similar matrices of calibration standard solutions and real samples. For this purpose, each sample was spiked at five different concentrations and calibration plots for each sample were obtained to quantify the analyte in the spiked samples. Table 2 demonstrates the details of recovery studies.

As given in Table 2, satisfactory recovery results were obtained for human serum (90.9%–107.2%), urine (93.7%–114%) and saliva (97.9%–102.4%) samples with repeatable signals when the matrix matching method was carried out. These results confirmed the method applicability and accuracy for the selected biological samples. Chromatograms obtained from GC–MS for the spiked samples and their blank measurements are given in Fig. 5.

4. Conclusion

In this study, chloroquine was isolated and preconcentrated from the selected biological samples by using a rapid and simple VA-SFDF-LPME-GC–MS method. In addition, several LPME and MSPE methods were

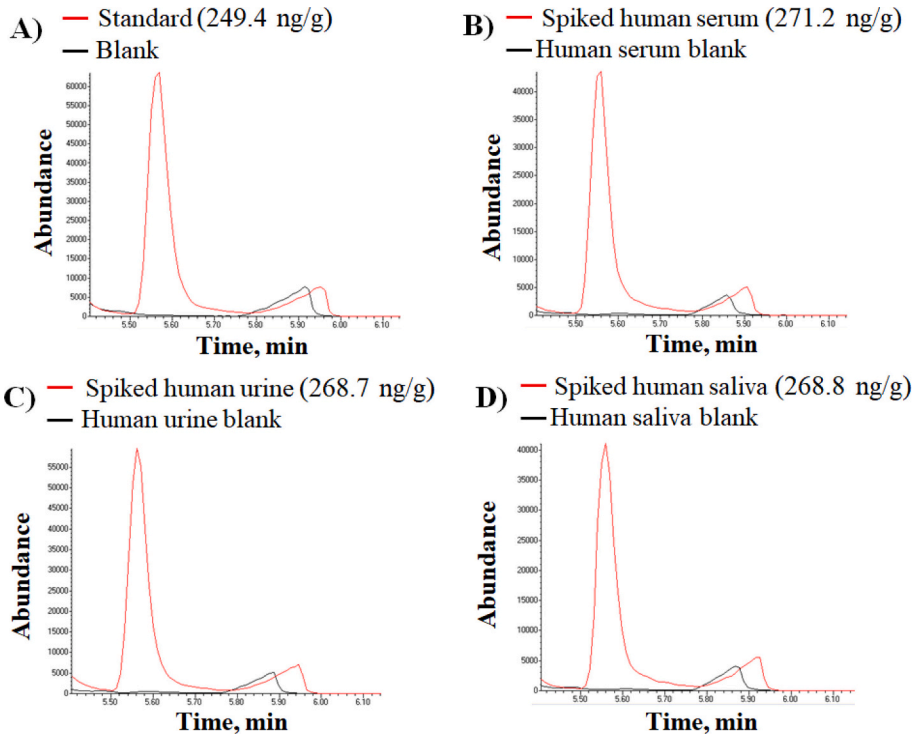

Fig. 5. Chromatograms belonging to standard solution with its blank as ultrapure water (A), human serum (B), urine (C) and saliva (D).

investigated to get low limit of detection for the analyte. After the univariate optimization studies for the proposed method, LOD/LOQ values were recorded as 2.8/9.2 μg/kg. The LOD value of conventional GC–MS system was enhanced by about 317 folds. The developed method was successfully applied to the spiked biological samples to check the method applicability and accuracy. Satisfactory recovery results for the VA-SFDF-LPME-GC–MS system were found in the range of 90.9%–114.0%. Further, the developed microextraction process is finished within 45 s for one sample with low volume organic solvent. It can be concluded that the proposed microextraction method was sensitive and accurate since LOD and LOQ values were at ppb levels and percent recovery results for the biological samples were close to 100%. The developed method can be applied for the preconcentration/extraction of variety of analytes.

Declaration of Competing Interest

None.

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