Rapid, Accurate, and Quantitative Detection of Propranolol in Multiple Human Biofluids via Surface-Enhanced Raman Scattering

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Supporting Information

ABSTRACT: There has been an increasing demand for rapid and sensitive techniques for the identification and quantification of pharmaceutical compounds in human biofluids during the past few decades, and surface-enhanced Raman scattering (SERS) is one of a number of physicochemical techniques with the potential to meet these demands. In this study we have developed a SERS-based analytical approach for the assessment of human biofluids in combination with chemometrics. This novel approach has enabled the detection and quantification of the β-blocker propranolol spiked into human serum, plasma, and urine at physiologically relevant concentrations. A range of multivariate statistical analysis techniques, including principal component analysis (PCA), principal component–discriminant function analysis (PC-DFA) and partial least-squares regression (PLSR) were employed to investigate the relationship between the full SERS spectral data and the level of propranolol. The SERS spectra when combined with PCA and PC-DFA demonstrated clear differentiation of neat biofluids and biofluids spiked with varying concentrations of propranolol ranging from 0 to 120 μM, and clear trends in ordination scores space could be correlated with the level of propranolol. Since PCA and PC-DFA are categorical classifiers, PLSR modeling was subsequently used to provide accurate propranolol quantification within all biofluids with high prediction accuracy (expressed as root-mean-square error of predictions) of 0.58, 9.68, and 1.69 for serum, plasma, and urine respectively, and these models also had excellent linearity for the training and test sets between 0 and 120 μM. The limit of detection as calculated from the area under the naphthalene ring vibration from propranolol was 133.1 ng/mL (0.45 μM), 156.8 ng/mL (0.53 μM), and 168.6 ng/mL (0.57 μM) for serum, plasma, and urine, respectively. This result shows a consistent signal irrespective of biofluid, and all are well within the expected physiological level of this drug during therapy. The results of this study demonstrate the potential of SERS application as a diagnostic screening method, following further validation and optimization to improve detection of pharmaceutical compounds and quantification in human biofluids, which may open up new exciting opportunities for future use in various biomedical and forensic applications.

In recent years, the demand for analytical techniques that can provide fast, reliable, and accurate quantitative measurements of drug molecules within biofluids has increased widely. There is a vast amount of patient-specific information present in biofluids, and thus they provide vital medical information that can be utilized for diagnostic purposes, such as by providing patient-specific baselines of biomolecules, determination of pharmaceutical dosage, pharmacodynamics, and pharmacokinetics, as well as monitoring drug compliance and response. Propranolol, which is a β-adrenergic blocker (more commonly termed a beta-blocker), is used for the treatment and/or prevention of various disorders including hypertension, anxiety, convulsions, arrhythmias, migraine, and angina pectoris. The detection of propranolol in human body fluids such as serum, plasma, and urine is commonly achieved via the use of gas chromatography (GC), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE). However, these techniques are time-consuming, can require long pretreatment steps to enrich the sample for the target analyte, incur the use of expensive reagents, and involve high instrumental costs. Moreover, professionally trained personnel with considerable background knowledge and expertise are required to operate and evaluate the results from such instruments. These drawbacks can be said to have (at least in part) been the driving force behind the optimization and development of novel analytical methods and protocols for rapid, inexpensive, and on-site detection and monitoring of such drugs in various biological fluids.

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Raman spectroscopy is a vibrational spectroscopic physicochemical technique that is based on the interaction of light with the chemical bonds within a sample. In Raman spectroscopy, light is inelastically scattered upon interaction with molecules that undergo polarization and thus provides a highly specific molecular fingerprint that can be used for the identification and quantification of various analytes. Raman spectroscopy is considered to have exceptional potential for use in the analysis of biofluids for a number of reasons, one of which is that water, as the major component of all biofluids, is a very weak Raman scatterer. Previous studies have reported the highest possible signal intensity and reproducibility. Therefore, it is crucial to optimize the experimental conditions between the aggregation agents and the metal substrates used.

Surface-enhanced Raman scattering (SERS) is another technique for overcoming this known limitation. This technique builds on either the adsorption or close proximity of an analyte to a suitably roughened metal surface. However, SERS enhancement effect can differ significantly between different analytes, and the degree of aggregation can also vary between the aggregation agents and the metal substrates used. Therefore, it is crucial to optimize the experimental conditions thoroughly according to the analyte of interest to achieve the highest possible signal intensity and reproducibility.

SERS has received much attention recently as an ideal technique for successfully measuring and identifying illicit drugs, including fractional factorial design for the optimization of the parameters which were needed for the quantitative detection of mephedrone. Alharbi and colleagues also demonstrated the application of SERS for the quantitative detection of tramadol in artificial urine, while Dong et al. used this approach for the quantitative detection of methamphetamine in human urine. Furthermore, Yang et al. detected low levels of cotinine and benzoylcegonine in saliva with 8.8 and 29 ppb detection levels, respectively. Very recently, Raman spectroscopy (in conjunction with infrared spectroscopy) has been used to screen a panel of novel psychoactive substances (NPS), which have also been the subject of a study using galvanic replacement of Cu with Ag on coins as cost-effective SERS substrates. Other studies of interest include surface investigations and dose responses of propranolol using molecular imprinted polymers and SERS, as well as the investigation of nanoparticle mediated selection of propranolol enantiomers by SERS, and SERS reading biochips using propranolol as a molecular template.

In this study, we have employed SERS combined with multivariate data analysis in order to develop a portable assay for the detection and quantification of propranolol at relevant concentration ranges found within multiple human biofluids (serum, plasma, and urine). The results achieved in this study clearly demonstrate the potential application of SERS for the detection of propranolol down to 133.1 ng/mL, which is comparable to commonly used techniques such as HPLC, while having the advantage that it is highly portable and could be developed for on-site testing.

[MATERIALS AND METHODS]

Materials. Trisodium citrate, silver nitrate (99.9% purity), sodium chloride, human serum, and human plasma were all purchased from Sigma-Aldrich (Dorset, U.K.). To minimize any batch-to-batch variation, all serum and plasma used originated from the same analytical batch. Amicon Ultra 0.5 mL centrifugal filters (3 kDa) were purchased from Merck Millipore Ltd. (Darmstadt, Germany). Propranolol hydrochloride (99% purity) was purchased from Alfa Aesar (Heysham, U.K.). The chemical structure of propranolol hydrochloride can be seen in Figure 1.

![Figure 1. Chemical structure of propranolol hydrochloride.](image)

Urine Collection. Midstream first morning samples were collected over several weeks in 50 mL Falcon tubes. Samples were briefly kept at ~4 °C immediately following collection, then transported to the laboratory, and stored at ~80 °C within 2 h of collection. Prior to analysis, 50 mL aliquots were allowed to thaw and centrifuged at 5000 g for 10 min at 4 °C, and the supernatant was collected.

Synthesis of Silver Nanoparticles. The silver nanoparticles were prepared following the Lee and Meisel citrate reduction method and also as reported in our previous studies. Briefly, AgNO₃ (90 mg) was dissolved in 500 mL of deionized water; the solution was heated to boiling point, and 10 mL of 1% trisodium citrate was added to a stirring silver nitrate solution drop by drop. The mix was left at boiling temperature for a further 15 min. A green-milky silver colloid was observed, which is stable and usable for several weeks when stored at room temperature. UV–visible spectroscopy and scanning electron microscopy (SEM) were used to assess the colloid and were similar to data collected earlier (data not shown).

Instrumentation. SERS spectra were collected using a DeltaNu Advantage portable Raman spectrometer (Delta Nu, Laramie, WY, USA), equipped with a 785 nm HeNe laser with a power output of ~60 mW on the sample. Daily calibration of the instrument was performed using a polystyrene internal standard as supplied by the instrument manufacturer. All samples were analyzed within the spectral range 400–2000 cm⁻¹, and the laser exposure time was set to 20 s for all samples.

Sample Preparation for SERS Measurements. A stock solution of propranolol at a concentration of 0.5 mM was prepared directly in different biofluids (serum, plasma, and urine). Serial dilution of propranolol with each biofluid of interest was then prepared using these stock solutions with final concentrations ranging from 0 to 120 μM; note no additional water was used at this stage so that the only addition into each
of the three biofluids was the propranolol itself. In order to remove protein residues from the plasma samples (this was not needed for serum), 0.5 mL aliquots were transferred onto Amicon Ultra centrifugal filters and centrifuged at 14000 g for 30 min following manufacturer’s recommended protocol. All samples were then stored at −80 °C until analysis. SERS analysis was performed as follows: 200 μL of silver colloid was added to a glass vial, followed by the addition of 200 μL of the biofluid—propranolol sample, and 50 μL of 0.5 M sodium chloride as the aggregation agent. The vial was vortexed for 2 s and placed into the sample cell attachment. Five biological replicates were prepared for each biological matrix and for each concentration.

Sample Preparation for HPLC-MS Measurements. Propranolol with a final concentration of 200 μM was prepared in water and plasma. The plasma was then filtered to remove the proteins following the standard protocol as explained above for SERS analysis, while the water mixture was used as a control, along with an aliquot of unfiltered plasma.

HPLC-MS Parameters. Samples (200 μL) were added to LC-MS vials for analysis. Analysis was carried out on an Accela UHPLC autosampler system using a Hypersil Gold C18 reversed phase column (100 mm × 2.1 mm × 1.9 μm) coupled to an electrospray LTQ-Orbitrap XL hybrid mass spectrometry system (Thermo Fisher, Bremen, Germany). Xcalibur and TunePlus software were used for instrument operation. Tuning and calibration were carried out as per the manufacturer’s instructions. An aliquot (10 μL) of each sample was injected on to the column, and a methanol/water solvent gradient was used for metabolite separation on the stationary phase (for gradient elution conditions see Supporting Information Table S1). Note that both carrier solvents contained 0.1% formic acid to aid the

Figure 2. Baseline-corrected SERS spectra of propranolol, in the three biofluids: (a) serum, (b) filtered plasma, and (c) urine. In all plots the pure biofluids are shown in red, pure propranolol is shown in green, and 200 μM propranolol spiked in each of the biofluids is in blue. The significant vibrational band of propranolol at 1381 cm⁻¹ was assigned to naphthalene ring and was used for quantification.
ionization within the ESI source of the mass spectrometer. Samples were analyzed in positive ESI mode using the following settings: 1 microscan per 400 ms, 50−2000 m/z range, ESI ion source transfer tube set at 275 °C, tube lens voltage set at 100 V, capillary voltage set at 30 V, sheath gas flow rate set at 40 arbitrary units, auxiliary gas flow set at 5 arbitrary units, and sweep gas at 1 arbitrary unit. Data were collected in centroid mode at a mass resolution of 30,000.

Chemometrics SERS Data Processing. MATLAB software R2013a (The Math Works Inc., Natick, MA, USA) was used to process all SERS spectral data. The propranolol peak was baseline-corrected using asymmetric least-squares (AsLS)34 and the peak areas of its main vibrational band (1381 cm⁻¹, naphthalene ring stretch) in samples at different concentrations were compared using box and whisker plots. All processed spectral data were subjected to the unsupervised method of principal component analysis (PCA).35 The SERS spectral data of urine samples were further assessed by employing the supervised method of principal component−discriminant function analysis (PC-DFA) to improve the separation observed for the different levels of propranolol.36 Partial least-squares regression (PLSR)37 as a multivariate quantitative regression technique generally used for predictive linear modeling, as reported previously by our group,33,38 was also employed to attempt to predict the propranolol concentrations in serum, plasma, and urine using the entire SERS spectra. Three separate PLSR models were generated for serum, plasma, and urine, and each of these used approximately 50% of the data as the training set. Once calibrated these models were then used for the prediction of propranolol concentration in the remaining data (test set) and were checked for validation of the models; that is to say the prediction accuracy and linearity of predictions were assessed (vide infra).

RPLC-MS Deconvolution and Data Processing Pipeline. Initially, RAW data files were converted into netCDF format (this is a common data format encompassing a generic code used for cross-system comparisons via statistical platforms such as Matlab and R) within the software conversion option of Xcalibur and moved forward to deconvolution via the XCMS algorithm. Subsequently, our own in-house peak picking and deconvolution software written in R-code which utilizes the XCMS algorithm (http://masspec.scripps.edu/xcms/xcms.php) was used. The resultant data matrix was retention time versus mass-to-charge ratio (m/z) and peak areas linked to each sample injection. Data were normalized by total ion count and log₁₀ transformed.

RESULTS AND DISCUSSION

Optimization of SERS for Detection of Propranolol in Human Biofluids. There is an increasing need for the accurate assessment of drugs in (human) biological fluids, and in a previous study we reported the optimum conditions for detection of propranolol in water.39 In this present study, propranolol has been spiked into significantly more complex biological matrices and these included human serum, plasma, and urine, which shall be discussed individually below.

Initial studies used our previously optimized conditions for the production of citrate reduced HAuCl₄ using KCl as the aggregating agent solution;39 however, no appreciable SERS signals from propranolol were observed in any of the biofluids. Therefore, the optimization process needed to take into account these complex biological matrices, in particular the
potential interference with other chemical species outcompeting propranolol for some association with the metal surface.

Extensive method development was therefore conducted, in which several colloids (including Ag(I) and Au(III), reduced by citrate and hydroxylamine ions) and aggregation agents (including NaCl and KNO₃) were examined to establish the optimum conditions that provided the highest reproducibility and stability of propranolol spiked into filtered plasma (Figure S1). According to these experiments, the combination of citrate-reduced silver colloid with sodium hydrochloride as the aggregation agent provided the most reproducible and information rich SERS spectra with features that corresponded to propranolol, and thus this selection was consequently employed as the standard method throughout this study. SERS spectra of propranolol and the different biofluids, with and without 200 μM propranolol, are presented in Figure 2, and it is clear that the peak at 1381 cm⁻¹, assigned to the naphthalene ring stretch,²⁹,³⁹ is the main vibrational band of propranolol which is also present in the propranolol spiked serum, filtered plasma, and urine drug mixtures, thus confirming the presence of propranolol.

Previous studies have reported the interference of high abundance proteins (e.g., albumin) present in plasma with the aggregation of the SERS nanoparticles, causing a steric...
repulsion between the nanoparticles which may prevent their successful aggregation with one another. This was also found to be true in our study, as the SERS spectra of the unfiltered plasma did not contain any specific spectral features from either the plasma itself or the target drug propranolol. We therefore decided to reduce the protein content via a filtration process employing ultracentrifugal filters, and we found that this improved the signal and resulted in spectral features that could be assigned to propranolol (Figure S2). This filtration step would seem to allow for the binding and interaction of the silver nanoparticles with other components in the sample. In addition, comparison of the spectral features of plasma samples we collected after filtration with the findings of Premasiri et al. (these authors also used SERS of unfiltered dry plasma using silver citrate) suggested that the protein components of plasma no longer contribute to the SERS spectral features. To check for any loss of propranolol due to this centrifugal-based filtration process, the samples of plasma with and without protein spiked with propranolol (before the filtration step was undertaken) were analyzed by SERS and HPLC-MS. The results of this analysis (Figure 3A,B) show that the filtration process did lower the apparent concentration of propranolol and that the findings of SERS and HPLC-MS show a similar trend. It should be noted that since SERS and HPLC-MS are very different analytical techniques, even though the general trends are similar, the plot of SERS and HPLC-MS predicted concentrations are in good agreement with each other (Figure 3C). Further SERS analyses were undertaken on plasma where

Figure 5. Plots showing PLSR quantitative predictions from training (red triangles) and test data (blue triangles) generated using SERS data sets from propranolol spiked into human biofluids: (a) serum, (b) plasma, and (c) urine.
the propranolol was added to plasma either before (Figure S5) or after (Figure S6) the filtration step, and we calculated the limits of detection to be 147.9 and 73.95 ng/mL, respectively. It was perhaps not surprising that the limit of detection (LOD) was significantly lower when propranolol was added to the plasma after the filtration step as no propranolol would have been removed during the filtration process. It was therefore likely that some of the propranolol had been adsorbed by plasma proteins. Nevertheless, as long as the propranolol is present in the plasma before the filtration step, then this reduction in analyte signal is taken into account during the processing, and the estimates of propranolol concentration will be valid (see below).

Finally, as a benchmark, propranolol was dissolved in water and the area under the naphthalene ring at 1381 cm\(^{-1}\) was used for quantification. The LOD we used was based on the area under the peak at 1381 cm\(^{-1}\) being three times greater than the standard deviation of blank spectra\(^4^3\) and was calculated as 2.4 ng/mL or 8 nM propranolol in water.

**Serum.** Several studies have reported the development and application of SERS for the detection of different diseases such as colorectal cancer,\(^4^4,4^5\) diabetes,\(^4^6\) gastric cancer,\(^4^7\) and nasopharyngeal cancer, using serum samples.\(^4^8\) We therefore used these works to compare our SERS spectra from serum, and we found that the most prominent vibrational bands detected in the serum samples in this series of experiments (Figure 2a) were located at 631, 723, 810, 959, 1096, 1132, 1204, 1330, 1446, 1583, and 1696 cm\(^{-1}\) which are all in agreement with other previously reported studies.\(^4^5,4^9\) The most intense peak was at 723 cm\(^{-1}\) which could be assigned to the C—H bending vibration of adenine. The band at 1132 cm\(^{-1}\) may belong to the C—N stretching vibration of D-mannose, and the bands at 1330 and 1696 cm\(^{-1}\) correspond to amide III and amide I, respectively (Table S2 in the Supporting Information contains assignments for these serum peaks). Whilst our spectra are in agreement with the literature, the assignment of all these features was not possible, given that the focus of the work in terms of detection of drugs in human biofluids was outside the present scope of the study.

As discussed above the naphthalene ring from propranolol (1381 cm\(^{-1}\)) was the most intense vibration that we observed in serum (Figure 2a; from 200 \(\mu\)M propranolol), and therefore a calibration curve from the SERS data (Figure S3a) was constructed using the peak areas at 1381 cm\(^{-1}\) with the varying concentrations of propranolol (0.9—30 \(\mu\)M) (Figure S4a). This plot showed a classic SERS S-shaped response for analyte quantification, where high concentrations of propranolol resulted in a reduction in signal due to the saturation of the nanoparticle with the analyte solution with, consequently, precipitation of the colloidal solution thus reducing the surface area available for colloidal–analyte interaction. From Figure S4a the linear range was observed from 0.9 to 10 \(\mu\)M, and this was used to determine the LOD, which was calculated to be 133.11 ng/mL (0.45 \(\mu\)M).

Rather than just observe a single vibrational “response” in these spectra, the next stage was to perform a multivariate assessment of the whole SERS spectra so that features in addition to the naphthalene ring from propranolol may be considered for the quantification. The PCA scores plot of the SERS spectral data demonstrated a clear concentration-dependent clustering pattern (Figure 4a), with the most significant variation being displayed across the PC1 axis with a total explained variance (TEV) of 96.59%. In PCA the first principal component (PC1) explains the most variance within the data, so this is highly significant. It should be noted that the variance associated with PC2 is relatively low (1.5%) in comparison to TEV of PC1 (96.59%), and hence the drift (across PC2) in the linearity looks exaggerated. According to the PC1 loadings plot (Figure 4d), the propranolol-specific peak (1381 cm\(^{-1}\)) is among the most significant vibrational bands contributing to this separation, as well as many other bands that are specific to serum, presumably because these decline as propranolol interacts more strongly with the silver nanoparticles.

A PLSR model was also constructed for the quantification of propranolol in serum samples using the SERS spectral data from the training set (0, 2, 4, 6, and 8 \(\mu\)M propranolol spiked into serum), while the remaining data (1, 3, 5, 7, and 9 \(\mu\)M propranolol) were used for the validation of the model. The PLSR predictions for the propranolol concentrations are shown in Figure 5a, illustrating acceptable predictive values, with \(R^2 \approx 0.87\) (this shows good linearity of the test set predictions; the closer to 1, the better) and very low prediction error with a root-mean-square error of prediction (RMSEP) of 0.58 in the test set. Table 1 contains a summary of the statistics for the PLS

| Table 1. Comparison of PLSR Models Showing Their Reproducibility and Accuracy of Predicting Propranolol Concentrations Spiked to Biofluids* |
|-----------------|--------|--------|------|------|
| biofluid        | factors | \(R^2\) | \(Q^2\) | RMSEP | RMSECV |
| serum           | 2      | 0.9774 | 0.8740 | 0.5796 | 0.4254 |
| plasma          | 6      | 0.9992 | 0.8328 | 9.6808 | 9.1445 |
| urine           | 5      | 0.9982 | 0.7753 | 1.6919 | 13.4071 |

*Factors specify the number of latent variables used in PLSR; RMSEP is the root-mean-squared error of prediction (from test set); RMSECV is the root-mean-squared error of cross-validation (from training set); \(R^2\) and \(Q^2\) show linearity for the training and test set predictions, respectively.

models of all the biofluids and also details the same statistics for the training set (viz., \(R^2\) and root-mean-square error of cross-validation (RMSECV)).

**Plasma.** With the exception of the ultracentrifugal filtration step used to reduce the proteins in plasma, the SERS conditions used were identical to those for serum, and the same chemometrics process as described for serum was applied for the analyses of the plasma SERS data. Figure 2b illustrates the SERS spectra of plasma with and without 200 \(\mu\)M propranolol. Similar to the serum samples, the peak at 1381 cm\(^{-1}\) was detected in all plasma samples containing propranolol. Although there were many features in the SERS spectra of plasma samples, the most significant peaks that could be assigned to plasma were 634 and 1132 cm\(^{-1}\), which are attributed to tyrosine and D-mannose, respectively.\(^4^8\) (Table S3 summarizes the main band assignments for plasma). According to the calibration curve (Figures S3b and S4b), the LOD of the samples within the linear range (0—80 \(\mu\)M) was found to be 156.77 ng/mL (0.53 \(\mu\)M). All data from the SERS spectra of plasma samples were also investigated further by PCA (Figure 4b). The PCA scores plot for plasma also demonstrated a clear concentration-dependent clustering of the data based on PC1 with TEV of 91.03%, while samples with propranolol concentrations higher than 70 \(\mu\)M were separated on PC2 with TEV of 4.98%. The tight clustering of the replicates again suggests that we have achieved high reproducibility of the SERS
spectra that were generated using our sample preparation method. According to the PC1 loadings plot (Figure 4c) 1381 cm\(^{-1}\) is the main vibrational band contributing toward this propranolol trend observed in PCA. As before, a PLSR model was established for the SERS spectral data using a training set (0, 20, 40, 60, 80, and 100 μM propranolol spiked to plasma) and the model was validated using the test set (10, 30, 50, 70, and 90 μM propranolol). Again, Figure 5b shows that the quantitative modeling was highly acceptable and Table 1 details that the predictive accuracy for the test was high: RMSEP = 9.68, and \(Q^2 = 0.83\).

**Urine.** Finally, a similar approach was also applied for the detection and quantification of propranolol in human urine. Figure 2c illustrates the most prominent SERS peaks observed in human urine, including peaks at 658 and 1133 cm\(^{-1}\) which could be assigned to C–O–N deformation and C–N stretching of uric acid, and also the band located at 1002 cm\(^{-1}\) may be due to C–N stretching vibration, while the peaks at 1218 and 1448 cm\(^{-1}\) are probably characteristic of phenylalanine vibrations. A summary of the main bands detected in the urine samples and their corresponding assignments and vibrational modes are provided in Table S4. Once again, the intense peak of propranolol at 1381 cm\(^{-1}\) was employed for generating the calibration curve (Figures S3c and S4c), which displayed a much wider linear range of 0–120 μM when compared to those results from serum and plasma. Furthermore, the LOD was calculated based on the linear regression curve, which was estimated to be 0.57 μM (168.61 ng/mL). PCA was performed on these data and the scores plot of PC1 versus PC2 revealed very little quantitative information of PC1 versus PC2 revealed very little quantitative information on the level of propranolol spiked into the urine, which could be due to the increased presence of low molecular weight compounds and a much higher salt content in urine that may interact with the metal colloid, and thus generate more complex spectra compared to serum and plasma. Therefore, PC-DF1 was employed and generated a clearer clustering pattern (Figure 4c), once again, illustrated a clear concentration-dependent separation based on the first discriminant function (DF1) axis. Note that the DFA algorithm was programmed with 13 independent groups and that this a priori information was provided categorically and did not reflect the concentration level of propranolol in urine, and therefore the trend seen in DF1 is natural and not artificial. Moreover, according to the DF1 loadings plot, the propranolol peak at 1381 cm\(^{-1}\) contributes significantly toward this clustering pattern (Figure 4f). The PLS model for the training set and test data are shown in Figure 5c, which were processed using the same approach as those for the serum and plasma spectral data. Overall, this demonstrated a RMSEP of 1.69 and a \(Q^2\) of 0.77. The error is higher and linearity lower than that for serum and plasma (Table 1), and this is very likely a reflection of the increased complexity of the sample (as discussed above), as many more chemical species that are naturally found in human urine compete for the surface of the metal colloid. It was also notable that the LOD for propranolol in urine was slightly higher than that for propranolol in blood (0.57 μM in urine compared with 0.45 μM and 0.53 μM for serum and plasma, respectively).

**CONCLUSION**

In this study we report for the first time the detection and quantification of propranolol in multiple human biofluids (viz., serum, plasma, and urine) using SERS on a portable benchtop spectrometer that could easily be deployed remotely for on-site testing in clinical chemistry laboratories. We initially envisaged that the SERS substrate and aggregating agent developed using fractional factorial design and multiobjective evolutionary optimization\(^9\) in water would be an ideal starting point for this study. Unfortunately this was not the case, and we needed to re-design and re-develop the SERS substrate specifically for the analysis of biofluids. We found in the present study that silver nanoparticles prepared by reducing Ag(I) with trisodium citrate, along with NaCl as the aggregating agent, gave the best results for all three biofluids. We compared this method with our previous SERS substrate based on gold nanoparticles,\(^39\) and both gave equivalent LODs in water of 8 nM propranolol. For analysis of propranolol in human samples we found that through adding the colloid directly to serum and urine we were able to detect propranolol down to levels of 0.57 and 0.45 μM, respectively. By contrast for the detection of this drug in plasma the proteins had to first be removed (or at least significantly reduced) by ultracentrifugation, before equivalent detection limits (0.53 μM) were achieved. In addition, the results from SERS and LC-MS for propranolol detection and quantification in plasma were both in agreement and confirmed that the filtration process employed for the removal of plasma proteins contributed to a loss of propranolol which was presumably noncovalently associated with the major proteins found in plasma. It is notable that the detection of propranolol in human biofluids was ca. 6× greater than the LOD for water and this is a consequence of other small molecules (metabolites, organic acids, and ions, etc.) as well as peptides and proteins, competing for the silver surface of the colloid. Comparing the signal of propranolol at the concentration of 10 μM, which is common between all three biofluids, it can be noted that the propranolol response is five times higher in serum and plasma compared to urine at this concentration. This could be attributed to the competitive effect of other analytes in the matrix. Moreover, there is a clear difference in the spectra of all three biofluids; for serum the most intensive peak at 723 cm\(^{-1}\) can be assigned to adenine (C–H bending) and another dominating peak can be assigned to the protein bands (amides I and III). Beside these protein bands, in some cases spectra of serum also display weak bands at 632, 810, 959, 1096, and 1132 cm\(^{-1}\). In plasma the most intensive peak at 634 cm\(^{-1}\) can be assigned to l-tyrosine (C–S), and as for urine the main contribution was from uric acid at 658 and 1133 cm\(^{-1}\). Although the LOD of propranolol using SERS is not as low as other commonly used techniques such as GC (0.05 ng/mL),\(^8\) HPLC (1 μg/L),\(^9\) and CE (0.01 μg/mL),\(^5\) SERS provides the advantage of being a highly portable, rapid, and cost-effective method, which can be used for the detection of various drugs in biofluid samples. In conclusion, our results clearly demonstrate for the first time that the detection and quantification of propranolol in multiple human biofluids using SERS is a promising analytical approach. After further optimization and validation to enhance detection of lower quantities of propranolol, this may provide a rapid, quantitative, portable, and inexpensive tool for future small-molecule diagnostic applications in blood and urine.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b02041.
Details of LC-MS mobile phase conditions (Table S1), vibrational and chemical assignments of SERS peaks from serum (Table S2), plasma (Table S3), and urine (Table S4) along with references used to support these assignments, optimization of SERS for the detection of propranolol including spectra generated with different nanoparticles (Figure S1), SERS spectra of filtered and unfiltered plasma spiked with 0.5 mM propranolol (Figure S2), SERS spectra of propranolol in the three different biofluids (Figure S3), peak areas from 1381 cm⁻¹ versus propranolol concentrations (Figure S4), and effects of pre- and post-filtration of proteins from plasma on SERS (Figures S5 and S6) (PDF)

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**