



Research Article

Direct Monitoring of Verapamil Level in Exhaled Breath Condensate Samples

Fariba Pourkarim¹, Elaheh Rahimpour², Maryam Khoubnasabjafari³, Vahid Jouyban-Gharamaleki⁴, Abolghasem Jouyban^{2,5*}

¹Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

²Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

³Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

⁴Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

⁵Kimia Idea Pardaz Azarbayjan (KIPA) Science Based Company, Tabriz University of Medical Sciences, Tabriz, Iran.

Article Info

Article History:

Received: 25 June 2018

Revised: 31 August 2018

Accepted: 2 September 2018

ePublished: 18 March 2019

Keywords:

-Verapamil

-Fluorescence enhancement

-SDS

-Biosensing

ABSTRACT

Background: In this research, an enhanced fluorimetric assay was developed for the direct monitoring of verapamil in exhaled breath condensate (EBC). The method is based on a binding-induced rigidity inside the sodium dodecyl sulfate (SDS) micelle which eliminate collisional quenching and vibrational modes responsible for non-radiative decay. This process produces an enhancement in the emission intensity of verapamil.

Methods: Fluorescence intensity measurements were made at 15 °C on a FP-750 spectrofluorometer with maximum excitation and emission wavelengths of 280 nm and 310 nm, respectively. The important parameters influencing the analytical signal in experimental steps were investigated and optimized. The method was validated with considering of the linearity, recovery and limit of detection.

Results: Under the optimized experimental conditions, the calibration graph was linear in the range of 0.02 – 12.0 µg.mL⁻¹ of verapamil with a detection limit of 0.008 µg.mL⁻¹.

Conclusion: The proposed method was found to be suitable and accurate for the determination of verapamil and the validated method was successfully used for analysis of verapamil in EBC of patients receiving verapamil with the satisfactory results.

Introduction

Verapamil, 5[(3,4-dimethoxyphenylethyl)methyl-amino]-2-(3,4-dimethoxyphenyl)-2-isopropyl-valeronitrile, as a L-type calcium channel blocker is widely used in the treatment of angina, arrhythmia, essential hypertension, certain cardiomyopathies, cluster headaches and modifying agent in tumors.^{1,2} Overdoses of this drug cause cardiac output decreasing, hypotension, and shock.³ It is mostly applied in two types of oral dosage of verapamil (40–180 mg and 120–140 mg in the case of commonly used tablets and slow releasing tablets, respectively).⁴ The therapeutic plasma concentration of verapamil has been reported in variable ranges from 20 to 500 ng.mL⁻¹ depending on drug form used.⁵ Optimal dosage of drugs is different for each person due to some parameters such as age, genetics, diet and medications taken concurrently. This shows that patients need frequent adjustments of individual dosage of drug due to individual variance in verapamil pharmacodynamics and/or individual variance in verapamil pharmacokinetics. Because of all these properties, determination of verapamil is one of considerable issues in analytical chemistry and clinical viewpoints.

Several analytical methods are reported in the literature for quantification of verapamil in plasma, urine, drugs and some other clinical samples. Some of these reports including fluorimetry,⁶ spectrophotometry,^{7,8} gas chromatography,⁹⁻¹² ion-chromatography,¹³ HPLC,¹⁴⁻¹⁸ HPLC/MS-MS,^{19,20} LC-ESI-MS,²¹ SPME-LC-ESI-MS,²² MIP-LC-MS,²³ and electrochemical methods.²⁴ In spite of higher selectivity presented by above mentioned methods, most of them require sophisticated equipment and are costly, time consuming and also suffer from some disadvantages such as intricate and tedious operational procedure, and high material consumption which is quite inconvenient for routine clinical applications. It is necessary for future individualized patient treatment to develop a simple, rapid and highly sensitive method for the monitoring of verapamil doses in the biological fluids. Knowing the drug concentration in body, make it possible for the physician to adjust the required drug dose before any unfortunate incident for the patient. Usually, plasma, serum or blood are used for the measuring drug concentrations. However, in compared with blood or other biological sample analysis, there are several advantages of exhaled breath condensate (EBC) tests.

*Corresponding Author: Abolghasem Jouyban, E-mail: ajouyban@hotmail.com

©2019 The Authors. This is an open access article and applies the Creative Commons Attribution (CC BY), which permits unrestricted use, distribution and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

EBC sampling, as a fairly accessible matrix, is easy to collect, repeatable, unassociated with side effects and any appreciable discomfort or risk to the subject.²⁵ It also reduces the number of interfering compounds in compared with more intricate matrices such as blood, urine and other biological fluids. The studies show that many drugs or metabolites are exhaled and can potentially be applied as diagnostic biomarkers.^{26,27} So, EBC analysis may provide instantaneous information about a pharmacokinetics of drugs or subject's health status. To our knowledge, there are no reports of direct verapamil determination in the literature. To continue our earlier work on analysis of verapamil in EBC using a combination of dispersive liquid-liquid microextraction and spectrophotometric measurement of verapamil at 200 nm, in this study, we have attempted to enhance the inherent fluorescence of verapamil with rigidity-induced materials and to evaluate their performance for direct monitoring of verapamil in EBC samples. This method has outstanding advantages over existing methods with respect to sensitivity, determination range, facility, speed, and ease operation without any sample preparation methods such as extraction, separation and/or pre-concentration methods.

Methods and Materials

Reagents and solutions

All reagents were of analytical-reagent grade and ultrapure deionized water obtained from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran) was employed throughout the study. Sodium dodecyl sulfate (SDS, Carlo Erba, Milan, Italy), β -cyclodextrin (β -CD, Sigma, St Louis MO, USA), polyvinylpyrrolidone (PVP, Daana Pharmaceutical Company, Tabriz, Iran), cetyl trimethylammonium bromide (CTAB, Merck, Darmstadt, Germany) and verapamil hydrochloride (Sobhan Darou Pharmaceutical Company, Rasht, Iran) were used in this work. Standard stock ($1000 \mu\text{g}\cdot\text{mL}^{-1}$) solution of verapamil was prepared by dissolving a proper amount of verapamil hydrochloride in methanol. Verapamil working solutions were prepared by dilution of the stock solution with ultrapure deionized water before each experiment.

Apparatus and instruments

Fluorescence intensity measurements were performed at 15°C on a FP-750 spectrofluorometer (JASCO Corp., Japan) with 10 nm band-pass in both of the excitation and emission paths and the sensitivity of medium (except for verapamil concentration range of $0.5\text{--}12 \mu\text{g}\cdot\text{mL}^{-1}$ which it was adjusted in low sensitivity). The instrument is equipped with a 150 W xenon lamp, 1.0 cm quartz cell, dual monochromators, Peltier thermostated single cell holder model ETC-272 (JASCO Corp., Japan), and supported with PC-based Windows® Spectra Manager TM software for JASCO Corporation. The UV-Vis absorption spectra were recorded on a double-beam UV-vis spectrophotometer model UV-1800 (Shimadzu, Japan) with 1.0 cm quartz cells.

The pH adjustments of the used solutions were performed

using a digital pH-meter model 744 (Metrohm Ltd., Switzerland) equipped with a glass-combined electrode. An electronic analytical balance model AB204-S (Mettler Toledo, Switzerland) was employed for weighing the materials.

Exhaled breath condensate (EBC) collection

EBC samples were collected by using a lab-made cooling trap system.²⁸ EBC samples used for method validation were a pool of samples collecting from healthy subjects. Patient EBC samples were collected from three patients after oral administration of verapamil. Sample donors signed a written consent form approved by the Ethics Committee of Tabriz University of Medical Sciences.

General procedure

A proper aliquot of standard or sample solution containing verapamil in the range of $0.02\text{--}12 \mu\text{g}\cdot\text{mL}^{-1}$ was placed into a 2 mL vial, then $80 \mu\text{L}$ of SDS solution ($100 \text{mmol}\cdot\text{L}^{-1}$) was added. The solution was diluted with deionized water to 0.5 mL and mixed well. After 15 min the fluorescence intensity was recorded at 310 nm with excitation at 280 nm.

Results and Discussion

Enhancement of fluorescence response

The UV/Vis absorption spectra and fluorescence spectra of the verapamil ($0.2 \mu\text{g}\cdot\text{mL}^{-1}$) in the absence and presence of SDS are shown in Figure 1. From the figures, it can be seen that verapamil in aqueous solution has a fluorescence with 280 nm excitation peak and about 310 nm emission peak. It is not observed any shift in the absorption peak of verapamil by adding SDS. However, it shows an increase in the absorbance intensity. The inherent fluorescence intensity of verapamil can be enhanced in the present of SDS as a rigidity-induced material due to micelle formation in the concentrations at above a critical micelle concentration (CMC) value ($3.34 \text{mmol}\cdot\text{L}^{-1}$). It is well known that the non-radiative transition pathways always compete with the optical emission relaxation and cause usually dramatically decrease or, in some cases, e.g. low concentration of fluorophore, elimination of the emission. In the presence of such competition, the quantum yield of the fluorophore is reduced^{29,30} which can be considered an important issue especially in the low concentration of fluorophore and increase the detection limit.

In the other words, it may be said that the observed fluorescence enhancement of verapamil result from elimination of water molecules surrounding the verapamil and the decrease in their rotational freedom inside the SDS micelle which provide a protective environment for verapamil in excited state. This consequence with SDS and other rigidity-induced materials previously well assayed for fluorescent compound such as dapoxyl sodium sulphonate,³¹ graphitic carbon nitride quantum dots,³² 8-hydroxyquinoline,³³ indole,³⁴ benzene,³⁵ and warfarin.³⁶

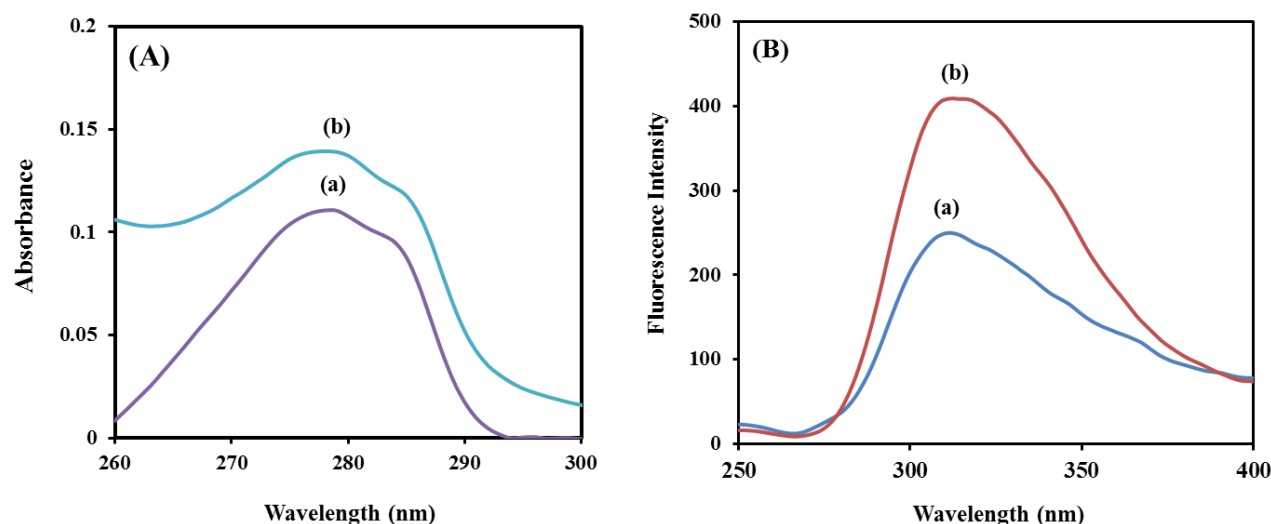


Figure 1. Absorption (A) and fluorescence (B) spectrum of verapamil ($0.2 \mu\text{g}\cdot\text{mL}^{-1}$) in the absence (a) and presence (b) of SDS.

Signal intensity in analytical methods is frequently a challenge and hence the interest in techniques that produce amplified response signals has been intensely increased. We found that the fluorescence intensity of verapamil in the presence of SDS can be enhanced 1.7 orders which it provides an improvement in the detection limit of verapamil determination at least 5 orders of magnitude. Our previous study³⁷ showed that the concentration range of verapamil in EBC of the patients receiving verapamil is about $0.07\text{--}0.1 \mu\text{g}\cdot\text{mL}^{-1}$ which practically is not detectable without a preparation and /or pre-concentration method before the analysis. In that work, we used a dispersive liquid-liquid micro extraction technique coupled with HPLC-UV method for determination of verapamil in the patient's EBC samples. Here, we used SDS for the enhancement of inherent fluorescence intensity of verapamil and without using any preparation and /or pre-concentration method which reduced the limit of quantitation (LOQ) and limit of detection (LOD) values for direct determination of verapamil from $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ and $0.04 \mu\text{g}\cdot\text{mL}^{-1}$ in the absence of SDS to $0.02 \mu\text{g}\cdot\text{mL}^{-1}$ and $0.008 \mu\text{g}\cdot\text{mL}^{-1}$ in the presence of SDS, respectively. Consequently, the concentration of verapamil could be monitored in the EBC of patient individuals by this developed method.

Optimization of reaction condition

In order to obtain the maximum fluorescence intensity, the effect of parameters which influence the analytical signal including type of rigidity induced material, pH, reagents concentration, temperature and incubation time were studied by one-at-a-time method. A $0.2 \mu\text{g}\cdot\text{mL}^{-1}$ solution of verapamil was employed for all measurements and each experiment was repeated three times and mean values were reported.

Type of rigidity-induced materials

In this study various rigidity-induced materials including SDS, β -CD, CTAB, and PVP were examined as the protecting agents. As seen from Figure 2A, the sensitivity

was much better in the presence of SDS than in its absence or in the presence of other protecting agents. It should be mentioned that, upon using the investigated agents, there is no changes observed in the position of the fluorescence maxima. All the used agents were studied in different concentrations including the concentrations of close and higher than the CMC for surfactants. Based on the obtained results, SDS with the concentration of $16 \text{mmol}\cdot\text{L}^{-1}$ was selected as an appropriate rigidity-induced material for next experiments.

Effect of pH

The effect of pH was studied over a pH range of 5–12, using hydrochloric acid and sodium hydroxide solutions for pH adjustment. As shown in Figure 2B, a maximum fluorescence intensity for the analyte was obtained in the $\text{pH} < 9$. The pK_a of verapamil is 9.04,³⁸ hence at $\text{pH} < 9$, verapamil is completely ionized and provides the positively charged nitrogen needed for strongly interaction with a negatively charged surfactants such as SDS. As the pH of EBC is within the range of 7–8, typical of that for human blood,³⁹ the next experiments were performed without pH adjustment.

Effect of temperature and incubation time

The influence of determination temperature was also investigated at 15 to 35 °C. As CMC of surfactants can vary by changing the environmental conditions,⁴⁰ the effect of temperature was studied in the concentration ranges of $4\text{--}32 \text{mmol}\cdot\text{L}^{-1}$ for SDS. As can be seen from Figure 2C, decrease in temperature led to a considerable increase in the fluorescence intensity. However, in the case of temperature lower than 15 °C, it was observed a deformation in the spectra shape. So, 15 °C was recommended as an optimum temperature. Furthermore, the effect of the reaction time on the fluorescence response was investigated. As shown in Figure 2D, the fluorescence intensity increased and reached a constant value after 15 min.

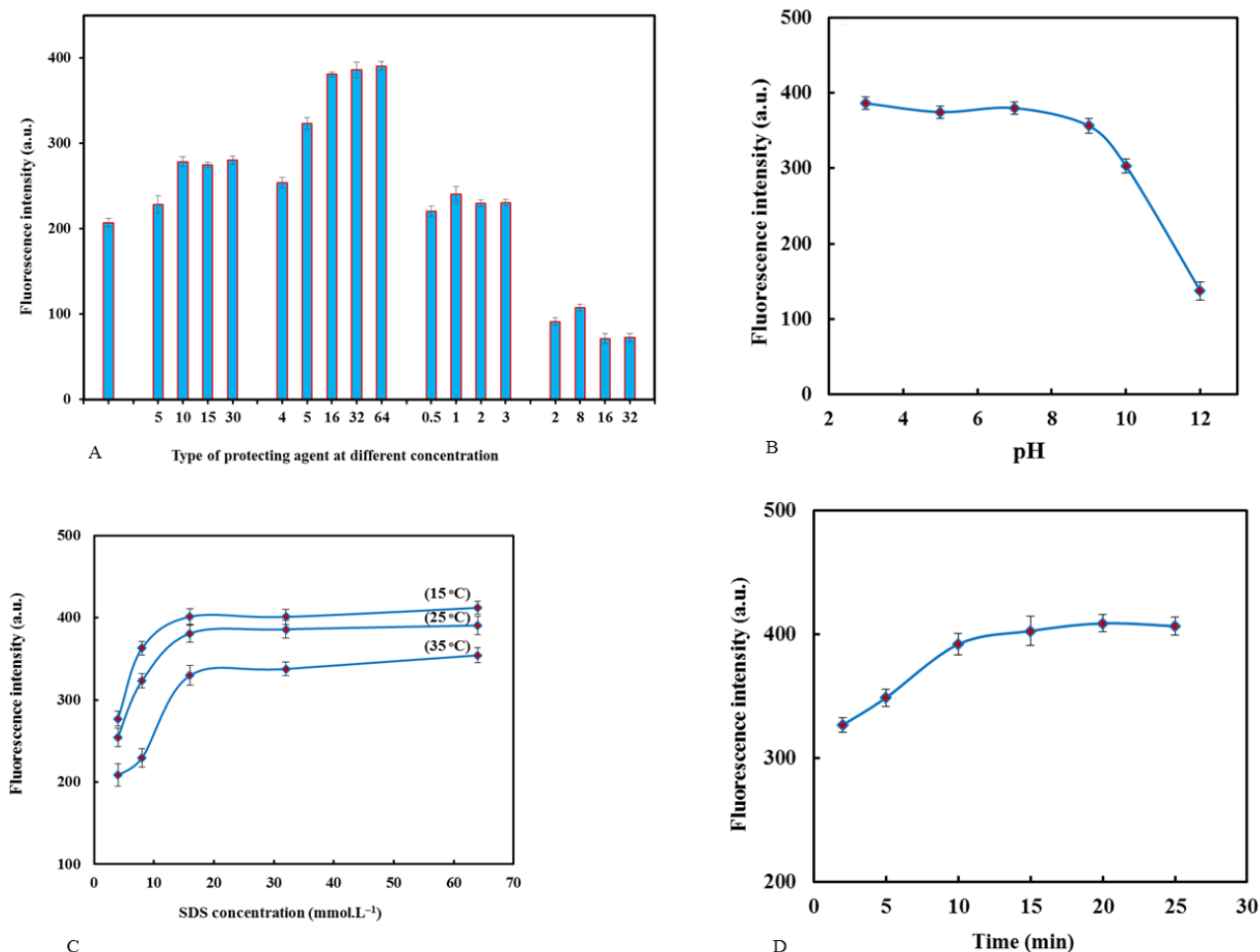


Figure 2. Effect of (A) type of protecting agent and its concentration, (B) pH, (C) temperature and (D) time on the fluorescence intensity of verapamil. Concentration units: PVP: $\times 10^{-2}$ w/v%, β -CD, SDS and CTAB: mmol.L⁻¹.

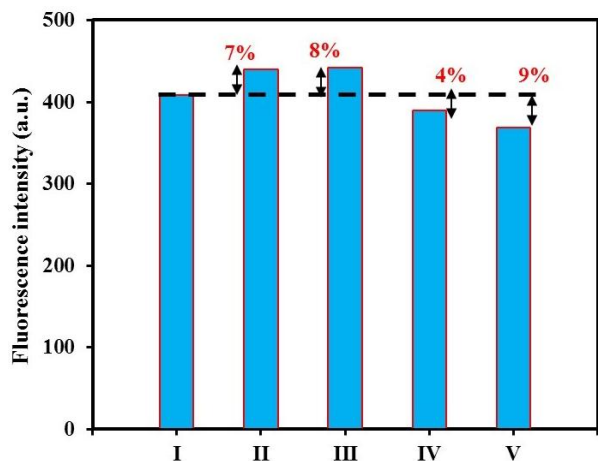


Figure 3. Effect of some co-administered drugs on the fluorescence intensity of verapamil. The concentrations of all drugs are $0.2 \mu\text{g.mL}^{-1}$. (I) Verapamil, (II) verapamil + losartan, (III) verapamil + caffeine, (IV) verapamil + pantoprazole, and (V) verapamil + carvedilol.

Study of interferences

With the analytical procedure established at above optimized condition, the possible interference between

verapamil and other co-administered drugs, several drugs (i.e. losartan, caffeine, pantoprazole, and carvedilol) were studied. For this purpose, $0.2 \mu\text{g.mL}^{-1}$ of these species were added into the EBC sample spiked with standard solution of $0.2 \mu\text{g.mL}^{-1}$ verapamil. The results are presented in Figure 3. As can be seen, all the drugs tested had no interference on the determination of verapamil ($>10\%$ is considered tolerated). These results showed that the method is selective for the analysis of verapamil in EBC samples.

Analytical figures of merit

The calibration graph in direct determination of verapamil in the absence of SDS was linear at $0.1 - 1 \mu\text{g.mL}^{-1}$ with a detection limit of $0.04 \mu\text{g.mL}^{-1}$. The equation for regression line was $I_F = 558.28C + 129.93$, where I_F is the fluorescence intensity in arbitrary unit, and C is the concentration of verapamil in $\mu\text{g.mL}^{-1}$. The calibration curve in the presence of SDS as a fluorescence enhancer was linear from $0.02 - 0.2 \mu\text{g.mL}^{-1}$ verapamil with the equation $I_F = 950.12C + 201.99$ and from $0.5 - 12.0 \mu\text{g.mL}^{-1}$ verapamil with the equation $I_F = 59.267C + 24.905$. Detection limits for each concentration range were 0.008 and $0.18 \mu\text{g.mL}^{-1}$, respectively.

Table 1. Details of the real samples and found concentration of verapamil in patients' EBC samples

No.	Gender	Age (year)	Daily dosage (mg)	Co-administered drugs	Concentration ($\mu\text{g.mL}^{-1}$)
1	Male	72	40	Warfarin, Digoxin, Pirastam	<LOD
2	Female	44	40 BID ^a	–	0.067
3	Male	68	40 BID ^a	Sodium valproate, Nitroglycerin, Ginkgo	0.059

^aTwo times a day

As can be seen, dynamic linear range of verapamil concentration increase from $0.1 - 1 \mu\text{g.mL}^{-1}$ in the absence of SDS to $0.02 - 12.0 \mu\text{g.mL}^{-1}$ in the presence of SDS. It is also observed an improvement in detection limit from $0.04 \mu\text{g.mL}^{-1}$ to $0.008 \mu\text{g.mL}^{-1}$. The precision of the method was investigated by repeated analysis of verapamil on the same day and on the different days. The intra-day and inter-day relative standard deviations for four replicate determinations in two determined level were 0.72% and 1.6% for $0.2 \mu\text{g.mL}^{-1}$ verapamil and 2.1% and 3.2% for $4 \mu\text{g.mL}^{-1}$ verapamil, respectively.

Real samples analysis

The applicability of the validated method was tested by verapamil determination in three EBC samples taken from the patients receiving verapamil. The results are given in Table 1. As can be seen, the concentration of verapamil in sample number 1 is below of LOD value of validated method. In order to study of the accuracy of the reported values and providing an evidence for no interference of other co-administered drugs in determination of verapamil by this method, the sample number 1 was spiked with different levels of the known amount of verapamil and analyzed according to the general procedure. The obtained results are given in Table 2. The recoveries were 96% and 98% for two added amount of verapamil (i.e. 0.05 and $0.1 \mu\text{g.mL}^{-1}$), indicating the good accuracy of the procedure and its independence from the matrix effects.

Table 2. Results of recoveries of spiked on sample No. 1 with different amounts of verapamil to investigate the matrix effect.

Added amount ($\mu\text{g.mL}^{-1}$)	Found amount ($\mu\text{g.mL}^{-1}$)	Recovery (%)
0.05	0.048	96.0
0.1	0.098	98.0

^aDetails of sample donor of number 1 are given in Table 1.

Conclusions

In this work, a simple fluorescence based assay was validated for the direct determination of trace levels of verapamil in EBC of the patients receiving verapamil. Advantages of this method is utilizing a simple fluorimetry detection include the minimal time required to analyze samples without any sample preparation or pre-concentration steps which make it useful method for routine clinical applications. The method is based on an enhancement in the emission intensity of verapamil in the presence of SDS. We attribute the observed fluorescence enhancement of verapamil to the increased effective viscosity of the microenvironment inside the SDS micelle which constrains molecular motions of verapamil and causes a fluorescence enhancement. This method is

simple, rapid, sensitive, reproducible and easy to use in routine applications.

Acknowledgments

We thank Sobhan Darou Pharmaceutical Company for gifting verapamil hydrochloride powder.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Thomas H, Coley HM. Overcoming multidrug resistance in cancer: An update on the clinical strategy of inhibiting P-glycoprotein. *Cancer Control*. 2003;10(2):159-65. doi:10.1177/107327480301000207
2. Beck E, Sieber WJ, Trejo R. Management of cluster headache. *Am Fam Physician*. 2005;71(4):717-24.
3. Patel NP, Pugh ME, Goldberg S, Eiger G. Hyperinsulinemic euglycemia therapy for verapamil poisoning: Case report. *Am J Crit Care*. 2007;16(5):518-9.
4. Ivanova V, Zendelovska D, Stefova M, Stafilov T. HPLC method for determination of verapamil in human plasma after solid-phase extraction. *J Biochem Biophys Methods*. 2008;70(6):1297-303. doi:10.1016/j.jbbm.2007.09.009
5. Buzinkaiova T, Skacani I, Netroiova J. Quantitative assay of verapamil in drugs and serum by capillary isotachopheresis. *Pharmazie*. 1995;50(12):799-805.
6. McAllister RG, Howell S. Fluorometric assay of verapamil in biological fluids and tissues. *J Pharm Sci*. 1976;65(3):431-2. doi:10.1002/jps.2600650330
7. Rahman N, Hoda M. Spectrophotometric determination of verapamil hydrochloride in drug formulations with chloramine-T as oxidant. *Anal Bioanal Chem*. 2002;374(3):484-9. doi:10.1007/s00216-002-1521-6
8. Long Y, Feng JZ, Tong SY. Spectrophotometry of verapamil tablets with charge transfer reaction. *Chin J Pharm*. 1993;24:267-70.
9. Spiegelhalter B, Eichelbaum M. Determination of verapamil in human plasma by mass fragmentography using stable isotope labelled verapamil as internal standard. *Arzneimittelforschung*. 1977;27(1):94-7.
10. Hege HG. Gas chromatographic determination of verapamil in plasma and urine. *Arzneimittelforschung*. 1979;29(11):1681-4.
11. McAllister RG, Tan TG, Bourne DWA. GLC assay of verapamil in plasma: Identification of fluorescent metabolites after oral drug administration. *J Pharm Sci*. 1979;68(5):574-7. doi:10.1002/jps.2600680515

12. Shin HS, Oh-Shin YS, Kim HJ, Kang YK. Sensitive assay for verapamil in plasma using gas-liquid chromatography with nitrogen-phosphorus detection. *J Chromatogr B Biomed Sci Appl.* 1996;677(2):369-73. doi:10.1016/0378-4347(95)00513-7
13. Piotrovskii VK, Rumiantsev DO, Metelitsa VI. Ion-exchange high-performance liquid chromatography in drug assay in biological fluids. II. Verapamil. *J Chromatogr B Biomed Sci Appl.* 1983;275:195-200. doi:10.1016/s0378-4347(00)84361-3
14. Ceccato A, Chiap P, Hubert P, Toussaint B, Crommen J. Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorometric detection. *J Chromatogr A.* 1996;750(1-2):351-60. doi:10.1016/0021-9673(96)00471-2
15. Tsang YC, Pop R, Gordon P, Hems J, Spino M. High variability in drug pharmacokinetics complicates determination of bioequivalence: Experience with verapamil. *Pharm Res.* 1996;13(6):846-50. doi:10.1023/A:1016040825844
16. Harapat SR, Kates RE. Rapid high-pressure liquid chromatographic analysis of verapamil in blood and plasma. *J Chromatogr A.* 1979;170(2):385-90. doi:10.1016/S0021-9673(00)95464-5
17. Cole SCJ, Flanagan RJ, Johnston A, Holt DW. Rapid high-performance liquid chromatographic method for the measurement of verapamil and norverapamil in blood plasma or serum. *J Chromatogr A.* 1981;218:621-9. doi:10.1016/S0021-9673(00)82087-7
18. Kuwada M, Tateyama T, Tsutsumi J. Simultaneous determination of verapamil and its seven metabolites by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl.* 1981;222(3):507-11.
19. Borges NCD, Mendes GD, Barrientos-Astigarraga RE, Galvinas P, Oliveira CH, De Nucci G. Verapamil quantification in human plasma by liquid chromatography coupled to tandem mass spectrometry: An application for bioequivalence study. *J Chromatogr B.* 2005;827(2):165-72. doi:10.1016/j.jchromb.2005.07.012
20. Alebic-Kolbah T, Zavitsanos AP. Chiral bioanalysis by normal phase high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry. *J Chromatogr A.* 1997;759(1-2):65-77. doi:10.1016/S0021-9673(96)00756-X
21. Dethy JM, Ackermann BL, Delatour C, Henion JD, Schultz GA. Demonstration of direct bioanalysis of drugs in plasma using nano-electrospray infusion from a silicon chip coupled with tandem mass spectrometry. *Anal Chem.* 2003;75(4):805-11. doi:10.1021/ac026069z
22. Walles M, Mullett WM, Levsen K, Borlak J, Wunsch G, Pawliszyn J. Verapamil drug metabolism studies by automated in-tube solid phase microextraction. *J Pharm Biomed Anal.* 2002;30(2):307-19. doi:10.1016/s0731-7085(02)00267-4
23. Mullett WM, Walles M, Levsen K, Borlak J, Pawliszyn J. Multidimensional on-line sample preparation of verapamil and its metabolites by a molecularly imprinted polymer coupled to liquid chromatography-mass spectrometry. *J Chromatogr B.* 2004;801(2):297-306. doi:10.1016/j.jchromb.2003.11.041
24. Chamjangali MA, Goudarzi N, Bagherian G, Reskety AA. Development of a new electrochemical sensor for verapamil based on multi-walled carbon nanotube immobilized on glassy carbon electrode. *Measurement.* 2015;71:23-30. doi:10.1016/j.measurement.2015.04.012
25. Chow S, Yates DH, Thomas PS. Reproducibility of exhaled breath condensate markers. *Eur Respir J.* 2008;32(4):1124-6. doi:10.1183/09031936.00085408
26. Berchtold C, Bosilkovska M, Daali Y, Walder B, Zenobi R. Real-time monitoring of exhaled drugs by mass spectrometry. *Mass Spectrom Rev.* 2014;33(5):394-413. doi:10.1002/mas.21393
27. Di Francesco F, Fuoco R, Trivella MG, Ceccarini A. Breath analysis: Trends in techniques and clinical applications. *Microchem J.* 2005;79(1-2):405-10. doi:10.1016/j.microc.2004.10.008
28. Jouyban A, Khoubnasabjafari M, Ansarin K, Jouyban-Gharamaleki V. Breath sampling setup, Iranian patent. 81363;2013.
29. Lakowicz JR. Principles of fluorescence spectroscopy. New York: Plenum Press;1983.
30. Guilbault GG. Practical fluorescence. 2nd ed. New York: Marcel Dekker;1990.
31. Pal K, Mallick S, Koner AL. Complexation induced fluorescence and acid-base properties of dapoxyl dye with γ -cyclodextrin: a drug-binding application using displacement assays. *Phys Chem Chem Phys.* 2015;17(24):16015-22. doi:10.1039/C5CP01696G
32. Abdolmohammad-Zadeh H, Rahimpour E. A novel chemosensor based on graphitic carbon nitride quantum dots and potassium ferricyanide chemiluminescence system for Hg (II) ion detection. *Sens Actuators B Chem.* 2016;225:258-66. doi:10.1016/j.snb.2015.11.052
33. Abdolmohammad-Zadeh H, Rahimpour E. CoFe₂O₄ nano-particles functionalized with 8-hydroxyquinoline for dispersive solid-phase microextraction and direct fluorometric monitoring of aluminum in human serum and water samples. *Anal Chim Acta.* 2015;881:54-64. doi:10.1016/j.aca.2015.04.035
34. Orstan A, Ross JA. Investigation of the β -cyclodextrin-indole inclusion complex by absorption and fluorescence spectroscopies. *J Phys Chem.* 1987;91(11):2739-45. doi:10.1021/j100295a019
35. Hoshino M, Imamura M, Ikehara K, Hama Y. Fluorescence enhancement of benzene derivatives by forming inclusion complexes with β -cyclodextrin in aqueous solutions. *J Phys Chem.* 1981;85(13):1820-3. doi:10.1021/j150613a012

36. Vasquez JM, Vu A, Schultz JS, Vullev VI. Fluorescence enhancement of warfarin induced by interaction with β - cyclodextrin. *Biotechnol Prog.* 2009;25(4):906-14. doi:10.1002/btpr.188
37. Pourkarim F, Shayanfar A, Khoubnasabjafari M, Akbarzadeh F, Sajedi-Amin S, Jouyban-Gharamaleki V, et al. Determination of verapamil in exhaled breath condensate by using a microextraction and liquid chromatography. *Curr Pharm Anal.* 2019; In-press. doi:10.2174/1573412914666180717125434
38. Tatavarti AS, Mehta KA, Augsburg LL, Hoag SW. Influence of methacrylic and acrylic acid polymers on the release performance of weakly basic drugs from sustained release hydrophilic matrices. *J Pharm Sci.* 2004;93(9):2319-31. doi:10.1002/jps.20129
39. Kang B, Wang H, Ren F, Gila B, Abernathy C, Pearton S, et al. Exhaled-breath detection using AlGaN/ GaN high electron mobility transistors integrated with a peltier element. *Electrochemical and Solid-State Letters.* 2008;11(3):J19-21. doi:10.1149/1.2824500
40. Mohajeri E, Dehghan Noudeh G. Effect of temperature on the critical micelle concentration and micellization thermodynamic of nonionic surfactants: polyoxyethylene sorbitan fatty acid esters. *J Chem.* 2012;9(4):2268-74. doi:10.1155/2012/914974