Technological research article / https://doi.org/10.15446/rcciquifa.v52n3.112481

Development of a surfactant mediated method for direct monitoring of atracurium in exhaled breath condensate

Anali Ali Akbari [1], Zahra Karimzadeh [1,2], Maryam Khoubnasabjafari [3,4], Vahid Jouyban-Gharamaleki [5], Abolghasem Jouyban[1,6], Elaheh Rahimpour [1,7]*

[1] Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

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

[4] Department of Anesthesiology and Intensive Care, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

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

[6] Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7] Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

* Corresponding author: (E. Rahimpour), E-mail address: rahimpour_e@yahoo.com

Received: May 31, 2023
Corrected: September 9, 2023
Accepted: September 16, 2023

Summary

Aim: This study aimed to establish an enhanced fluorometric assay for directly monitoring atracurium in exhaled breath condensate (EBC). The principal of the technique is the rigidity induced medium in the presence of the sodium dodecyl sulfate (SDS) surfactant which eliminates quenching results from collisional and vibrational conversions. This process causes an improvement in the emission intensity of atracurium. **Methods:** Fluorescence recordings were made on a spectrofluorometer at 15 ˚C at 310 nm. The crucial factors for reaching to maximum response

were investigated and optimized. **Results:** The technique was validated and the calibration curve was linear in the range of 0.01-2.0 μg·mL^{-1} of atracurium with a limit of detection of 0.007 μg·mL^{-1}. Finally, the proposed technique was used for the atracurium analysis in EBC of patients receiving the drug. **Conclusion:** The validated technique was found to be accurate and reliable for the quantification of atracurium from the repeatability and reproducibility points of view.

Keywords: Atracurium; Sodium dodecyl sulfate; Fluorescence enhancement, Exhaled breath condensate

Resumen

Desarrollo de un método mediado por surfactante para la monitorización directa de atracurio en el condensado del aliento exhalado

Objetivo: Este estudio tuvo como objetivo establecer un ensayo fluorométrico mejorado para monitorear directamente el atracurio en el condensado del aliento exhalado (EBC). El principio de la técnica es el medio inducido por rigidez en presencia del tensioactivo dodecilsulfato de sodio (SDS), que elimina los resultados de enfriamiento de las conversiones por colisión y vibración. Este proceso provoca una mejora en la intensidad de emisión del atracurio. **Métodos:** Los registros de fluorescencia se realizaron en un espectrofluorómetro a 15 °C a 310 nm. Se investigaron y optimizaron los factores cruciales para alcanzar la máxima respuesta. **Resultados:** La técnica fue validada y la curva de calibración fue lineal en el rango de 0,01-2,0 μg·mL^{-1} de atracurio con un límite de detección de 0,007 μg·mL^{-1}. Finalmente, la técnica propuesta se utilizó para el análisis de atracurio en EBC de pacientes que recibieron el fármaco. **Conclusión:** La técnica validada resultó ser precisa y confiable para la cuantificación de atracurio desde los puntos de vista de repetibilidad y reproducibilidad.

Palabras clave: Atracurio; Dodecil sulfato de sodio; Mejora de la fluorescencia, condensado del aliento exhalado

Resumo

Desenvolvimento de um método mediado por surfactante para monitoramento direto de atracúrio no condensado do ar exalado

Objetivo: Este estudo teve como objetivo estabelecer um ensaio fluorométrico aprimorado para monitorar diretamente o atracúrio no condensado do ar exalado (EBC). O principal da técnica é o meio induzido por rigidez na presença do surfactante dodecil sulfato de sódio (SDS) que elimina os resultados de têmpera de conversões colisionais e vibracionais. Este processo provoca uma melhoria na intensidade de emissão de atracúrio. **Métodos:** Os registros de fluorescência foram feitos em espectrofluorômetro a 15 °C a 310 nm. Os fatores cruciais para atingir a resposta máxima foram investigados e otimizados. **Resultados:** A técnica foi validada e a curva de calibração foi linear na faixa de 0,01-2,0 $\mu g \cdot mL^{-1}$ de atracúrio com limite de detecção de 0,007 $\mu g \cdot mL^{-1.}$ Por fim, a técnica proposta foi utilizada para análise de atracúrio em EBC de pacientes que receberam o medicamento. **Conclusão:** A técnica validada mostrou-se precisa e confiável para a quantificação de atracúrio do ponto de vista da repetibilidade e reprodutibilidade.

Palavras-chave: Atracúrio; Dodecilsulfato de sódio; Aumento de fluorescência, condensação da respiração exalada

Introduction

Atracurium besylate, 2,2'- [1,5-pentanediylbis [oxy(3-oxo-3,1-propanediyl)]] bis[1-[(3,4-dimethoxyphenyl) methyl] -1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolinium] dibenzenesulfonate, is a highly selective and widely used medication in surgical operations. Generally, its action mechanism is competition with acetylcholine receptors of the neuromuscular junction to block the neurons. First of all, the muscles responsible for face fine rapid movements, then muscles of the limbs and torso and finally diaphragm affect by this drug [1]. Disparate surgical anesthesia, the atracurium's pharmacodynamic/pharmacokinetic profile is poorly documented in care unit patients. Its plasma concentration is about 10 $\mu g \cdot mL^{-1}$ with half-life of 2-3.4 min (distribution) and 20 min (terminal). The protein binding of this non-depolarizing skeletal muscle relaxant is 82% and its volume of distribution is approximately 160 mL/kg (range: 120-188 mL/kg). It is metabolized to laudanoside using non-enzymatic cleavage method independent of hepatic/renal function and also ester hydrolysis by nonspecific esterases and Hofmann elimination (retro-Michael addition) [2]. The only dosage form of

this drug is 10 mg·mL^{-1} injectable solution. Overdosage with this drug may escalate the risk of cardiovascular effects and histamine release, especially hypotension. Therefore, the quantification of atracurium is one of the noticeable subjects in clinical and analytical chemistry. Knowing the exact drug amount in the patient's body help the physician to adjust the drug dose more accurately.

Some analytical techniques are revealed in the literature for the determination of atracurium in clinical samples including spectrophotometry [3], high-performance liquid chromatographic (HPLC) with detection system of fluorimetry [4, 5], mass spectroscopy [6, 7], and electrochemiluminescence [8]. Despite the acceptable selectivity reported for the mentioned methods, most of them are time-consuming and need complex apparatus, also have some disadvantages such as difficult running procedures and great and expensive material consumption which is not appropriate for routine clinical applications. It is essential to valid a fast and reliable technique for the analysis of atracurium concentration in biological samples [9]. Usually plasma, blood, or serum are the common biological fluids used for determining drug concentrations. However, exhaled breath condensate (EBC) is an alternative matrix that is accessible, repeatable, easy to collect, and any considerable threat to the patient [10]. For patients under surgical operation and mechanical ventilation, there is an easy way to collect the EBC sampler via expiratory circuit of the ventilator. The literature data demonstrate that many drugs and their metabolites can be exhaled [11] and possibly can be used as diagnostic biomarkers for various diseases [12, 13]. In comparison with more complicated matrices like urine, blood, and other biological samples, EBC samples decrease the number of interfering materials due to its diluted matrix. In the current work, we have tried to improve the inherent atracurium fluorescence employing rigidity-induced substrates and to estimate their performance for direct atracurium analysis in EBC samples. This technique has some benefits over present technique with respect to the facility, analysis range, speed, sensitivity, and less difficulties of operation that has no sample preparation techniques such as separation, extraction, or preconcentration.

Methods And Materials

Reagents and Solutions

The ultrapure deionized water was provided from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). Polyvinylpyrrolidone (PVP, Daana Pharmaceutical Co., Tabriz, Iran), sodium dodecyl sulfate (SDS, Carlo Erba, Milan, Italy), cetyltrimethylammonium bromide (CTAB, Merck, Darmstadt, Germany), β-cyclodextrin (β-CD, Sigma, St Louis MO, USA), and atracurium besylate (Iran Hormone Pharmaceutical Co., Tehran,

Iran) were employed in the curent study. Standard stock (1000 μg·mL^{-1}) solution of atracurium was used and diluted work solutions were prepared with ultrapure deionized water before each experiment.

Apparatus and Instruments

The UV–Vis absorption were determined on a double-beam UV–Vis spectrophotometer model UV-1800 (Shimadzu, Japan) with 1.0 cm quartz cells. Fluorescence spectra were recorded on a FP-750 spectrofluorometer (JASCO Corp., Japan) at 15 °C with a 10 nm band-pass in emission and excitation paths and the "medium" sensitivity. The pH adjustments were done by employing a digital pH-meter model 744 (Metrohm Ltd., Switzerland).

EBC Collection

EBC samples for development and validation of the method were gathered by a homemade cooling trap method [14]. EBC (drug free) samples utilized for the technique optimization and validation were collected from healthy sample donors. Real EBC samples have gotten from the expiratory circuit of the mechanical ventilator [15] of the patients under ventilation after intravenous administration of atracurium for their routine surgical operation without any more intervention. Sample donors signed an agreement form confirmed by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.903).

General Procedure

An appropriate amount of sample or standard solution containing atracurium in the range of 0.01–2.0 μg·mL^{-1} was inserted into a 2 mL vial containing 200 μL of EBC sample, then 10 μL of phosphate buffer with pH: 9 alongside 36 μL of SDS solution (1.0 mmol·L^{-1}) was added to the mixture. The solution was reached to 400 μL volume and mixed well. Then, the fluorescence intensity was documented at 315 nm with excitation at 280 nm.

Results and discussion

Enhancement of Fluorescence Response

The fluorescence spectra and UV–Vis absorption spectra of the atracurium (1.0 μg·mL^{-1}) in the presence and absence of SDS were shown in Fig. 1. From Figs. 1A and 1B, it can be realized that atracurium has an emission peak about 310 nm with an excitation peak of 280 nm in an aqueous solution. There was not any shift in the absorption or emission peak of atracurium by adding SDS. But their intensity has significantly increased.

In the SDS present, the inherent fluorescence intensity of atracurium is enhanced in concentrations higher than a critical micelle concentration value as a rigidity-induced substance which can form a micelle system. It is a fact that the non-radiative transition pathways cause usually dramatic decrease and compete or completely quench the radiation pathway. The quantum yield of the fluorophore is reduced in the presence of such competition [16, 17] which is a crucial subject, particularly in the fluorophore low concentration and decrease the sensitivity of analyses. Beside, other reason reported for the atracurium fluorescence improvement is removal of water molecules surrounding the atracurium and the reduction of the rotational freedom into the SDS micelle that provides a protective medium for atracurium in the excited state. The results in the presence of SDS and other rigidity-induced substance were investigated for benzene [18], indole [19], 8-hydroxyquinoline [20], dapoxy sodium sulphonate [21], warfarin [22], graphitic carbon nitride quantum dots [23] and verapamil [24].

Signal intensity in the analyses is considered a challenge and the methods which amplified response signals have intensely garnered interest. We recognized that emission intensity of atracurium is enhanced in the SDS present which obtains an improvement in the limit of detection of atracurium ~ 2.2 orders of magnitude. In this research, we employed SDS to the amplify inherent emission of atracurium without employing any preparation or pre-concentration technique which decrease the detection limit (LOD) values for direct monitoring of atracurium from 0.028 $\mu g \cdot mL^{-1}$ in the absence of SDS to 0.0077 $\mu g \cdot mL^{-1}$ in the SDS present, respectively. Therefore, the concentration of atracurium could be determined in the EBC of patients by the current technique.

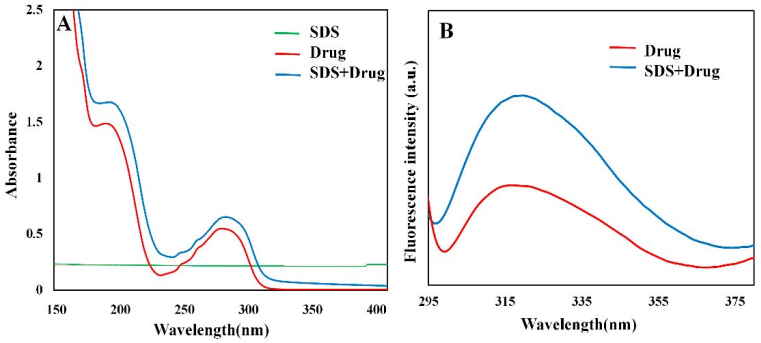

Fig. 1. Absorption (A) and emission (B) spectra of atracurium (1.0 $\mu g \cdot mL^{-1}$), SDS, and SDS+atracurium.

Optimization

To achieve the maximum response, the impact of factors that affect the response such as pH, the type of rigidity-induced substance, concentration of the reagents, incubation time, and temperature were investigated by the one-at-a-time trend. A 1.0 $\mu g \cdot mL^{-1}$ solution of atracurium was used for all records, and each test was replicated three times.

Type of Rigidity-Induced Material

In the current work, several rigidity-induced reagents such as β-CD, SDS, PVP, and CTAB were tested as the protecting materials. As shown in Fig. 2A, SDS shows a good sensitivity in compared to other reagents. It is better to note that, while employing the studied reagents, there was no any change in the wavelength of 310 nm. We studied all reagents in various concentrations including the concentrations higher and lower than the critical micelle concentration for each surfactant. According to the findings and data, SDS with a concentration of 0.09 $mmol \cdot L^{-1}$ was chosen as a suitable rigidity-induced reagent for the next actions.

Effect of pH

The pH impact was examined over a pHs of 3–11, by utilizing sodium hydroxide and hydrochloric acid solutions for pH setting. As seen in Fig. 2B, a maximum response ($\Delta F = F - F_0$, in which F and F_0 are the fluorescence intensity in the presence and absence of SDS) for the atracurium was achieved at pH = 9.0. The pK_a of atracurium with two amino groups is 19.02; hence at pH = 9.0 atracurium is entirely ionized and supplies the positively charged nitrogen required for strong interaction with negatively charged surfactant like SDS.

Effect of Temperature and Incubation Time

The effect of temperature was also examined at 10 to 37 °C. As critical micelle concentration of surfactants can change with the environmental mediums, the temperature impact was also investigated in an SDS concentration of 0.09 $mmol \cdot L^{-1}$. As can be obtained from Fig. 2C, as the temperature increase, the fluorescence response get decrease. So, 15 °C was suggested as a selected temperature. Moreover, the influence of the experiment time on the response was examined. There is no significant difference over time.

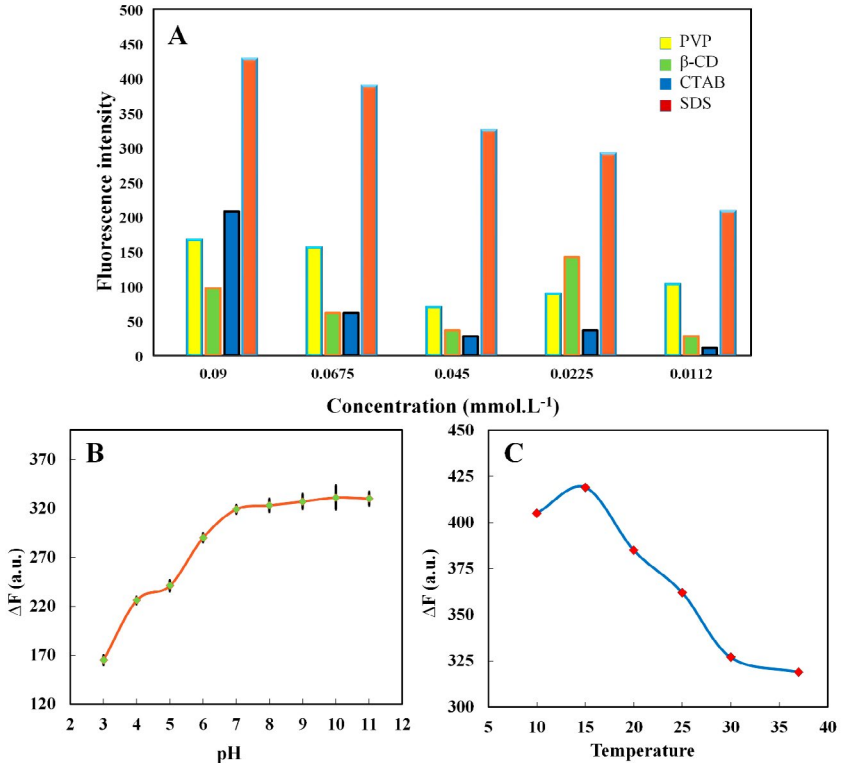

Fig. 2. Effect of the type of rigidity-induced reagent and their concentrations (A), pH (B), and temperature (C) on the fluorescence intensity of atracurium. Condition: 1.0 μg·mL^{-1} of atracurium.

Study of Interferences

With the analytical process set up at optimized condition that mentioned above, the probable interference between atracurium and some other co-administered pharmaceuticals, *i.e.*, acetylsalicylic acid (ASA), losartan, caffeine, nicotinic acid, ibuprofen, ascorbic acid, and diazepam were investigated. Herein, 2.0 μg·mL^{-1} of these species was mixed with the EBC sample spiked with a standard solution of 2.0 μg·mL^{-1} atracurium. The results are shown in Fig. 3. It can be found that ascorbic acid, ibuprofen, nicotinic acid, and losartan had almost no interference in the determination of atracurium. However, among the commonly used drugs investigated for selectivity studies, ASA, diazepam, and caffeine represent significantly decreasing effects demonstrating interference with atracurium determination. It is suggested that this method could be performed for atracurium quantification in the EBC of the patients not receiving ASA, diazepam, and caffeine or an extraction method could be suggested before direct determination.

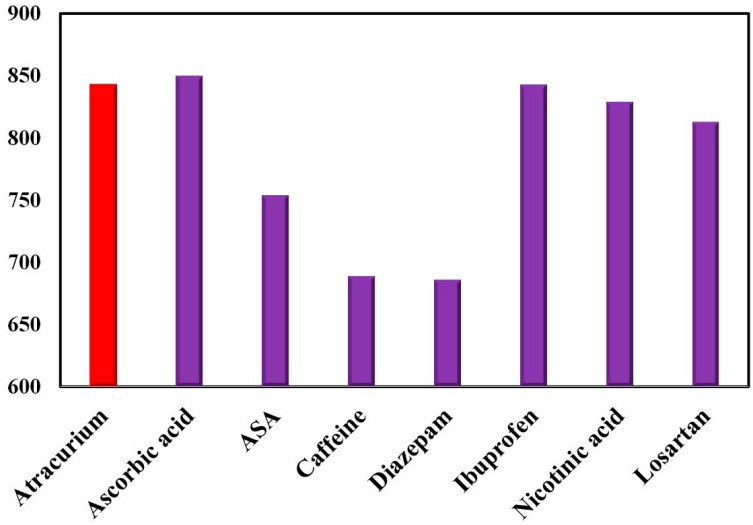

Fig. 3. Selectivity of the probe toward atracurium in the presence of some over-the-counter or some co-administrated drugs in the concentration of 2.0 μg·mL^{-1}

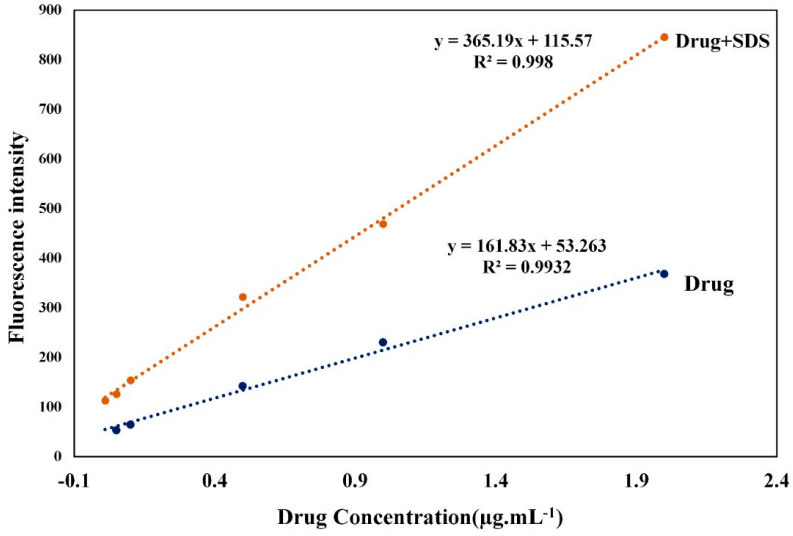

Fig. 4. Calibration curves of atracurium with different concentrations in the absence and presence of SDS

Analytical Figures of Merit

The equation for the regression line of atracurium without SDS adding was $F = 161.83\ C + 53.263$ ($R^2 = 0.9932$), where F is the fluorescence intensity in arbitrary unit, and C is the concentration of atracurium in $\mu g \cdot mL^{-1}$. The calibration chart in direct monitoring of atracurium in the SDS absent was linear at 0.05-2.0 $\mu g \cdot mL^{-1}$ with a LOD and LOQ of 0.028 $\mu g \cdot mL^{-1}$ and 0.093 $\mu g \cdot mL^{-1}$. Whereas, the calibration graph in the SDS present as an enhancer was linear from 0.01 - 2.0 $\mu g \cdot mL^{-1}$ atracurium with the regression equation of $F = 365.19\ C + 115.57$ ($R^2 = 0.998$) and LOD and LOQ of 0.007 $\mu g \cdot mL^{-1}$ and 0.023 $\mu g \cdot mL^{-1}$. With comparing the slope of regression line (Fig. 4), it can find that an enhancement about 2.2 was observed for atracurium florescence intensity in the presence of SDS. This enhancement leads to reach a low LOD for measurements.

Precision

To investigate the method precision, the standard solution having concentration 0.5, 1.0, 2.0 $\mu g \cdot mL^{-1}$ of atracurium were examined during the experiment course on different days and on the same day. For both intra-day and inter-day variations, atracurium solutions were assessed three times and the results are shown in Table 1.

Table 1. Inter-day and intra-day relative standard deviations (%*RSD*) for replicated determinations for different levels of ethanol for redox reaction with dichromate in EBC.

[atracurium] $\mu g \cdot mL^{-1}$	%*RSD*	
	Intra-day	**Inter-day**
0.5	1.87	4.15
1.0	1.28	1.41
2.0	2.53	3.77

Stability

Stability investigations are examined by three concentrations of atracurium. EBC samples containing atracurium are frozen in -20 °C and thawed three cycles with interval of 24 hours (24, 48, 72 hours) and then determined. The variations from initial fluorescence intensity value are summarized in Table 2. According to the results, samples are stable for 2 cycles of freeze and thaw and more than 2 cycles, a relatively high deviation is observed in the results.

Table 2. Stability study for different levels of atracurium.

[atracurium] μg·mL^{-1}	Freeze-thaw stability (%RE)		
	After 24 h	After 48 h	After 72 h
0.5	3.47	7.63	15.03
1.0	6.21	9.48	17.37
2.0	5.16	10.04	20.90

$\%RE = [((I_{Measured}) - (I_{Expected})) / (I_{Expected})] \times 100$.

Robustness

To test the potential variability in the experiment conditions when a technique is done by another analyst or transferred from one laboratory to another, the method robustness was examined and finding are reported in Table 3. As can be achieved, the finding of this examination demonstrate no significant variation on the analytical results representing the method robustness.

Table 3. Robustness study for different levels of atracurium.

Condition	RE% for three level of atracurium (μg·mL^{-1})		
	0.5	1.0	2.0
One laboratory to another	9.23	8.10	2.98
One researcher to another	10.91	9.21	4.97

$\%RE = [((I_{Measured}) - (I_{Expected})) / (I_{Expected})] \times 100$.

Real samples analysis

The method applicability was verified by atracurium analysis in six EBC samples gotten from the patients receiving atracurium. The found concentration varied from 0.10 to 0.53 μg·mL^{-1}. To investigate the model accuracy, the standard addition was performed and recoveries of the spiked concentrations (in two concentration level 0.5 and 1.0 μg·mL^{-1}) were obtained (Table 4). It is better to note that this method is a direct method without any sample preparation technique which can be installed on the mechanical ventilators as a point of care setup. However, as has be reported in the interferences studies, some interferents have negative or positive effect on the analytical response and should be considered in the monitoring procedure. The high variations in the recovery values of sample 2 and 3 could be assigned into such interferents.

Table 4. Determination of atracurium in patient EBC samples.

No.	Added ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)[a]
1	- 0.5 1.0	0.25 0.54 1.10	- 108.0 110.0
2	- 0.5 1.0	0.53 0.91 1.41	- 76.0 88.0
3	- 0.5 1.0	0.36 0.79 1.46	- 86.0 110.0
4	- 0.5 1.0	0.10 0.61 1.15	- 102.0 105.0
5	- 0.5 1.0	0.10 0.57 1.14	- 94.0 104.0
6	- 0.5 1.0	<LOD 0.48 1.16	- 96.0 116.0

[a] Recovery (%) = [atracurium concentration in samples (after spiking – before spiking)/Added] × 100.

Conclusions

In the current study, a reliable fluorescence-enhancement method was confirmed for the direct monitoring of atracurium in EBC of the patients receiving atracurium. The benefits of the technique are developing a simple fluorometric method including the least time needed for sample analysis without any pre-concentration or sample preparation which makes it a valuable technique for routine clinical utilizations. The procedure is based on an improvement in the emission of atracurium in the SDS present as an enhancer. We ascribe the detected fluorescence improvement of atracurium to the viscosity increasing of the microenvironment into the SDS micelle which limits molecular movements of atracurium and results in a fluorescence improvement. This technique is fast, reproducible, sensitive, simple, and easy to use in routine utilizations. The proposed method could be further developed online monitoring system installed on the mechanical ventilators for providing real time analysis of atracurium.

Acknowledgments

We thank Iran Hormone Pharmaceutical Company for gifting atracurium besylate solution. Grant number 70227.

Conflict of interests

M. Khoubnasabjafari, V. Jouyban-Gharamaleki and A. Jouyban patented the EBC collection setup in the Iranian Patent Office. The other authors claim that there is no conflict of interest.

References

1. T.A. Torda, The 'new'relaxants: A review of the clinical pharmacology of atracurium and vecuronium, *Anaesth. Intensive Care Med.*, **15**, 72-82 (1987).

2. V. Fodale, L.B. Santamaria, Laudanosine, an atracurium and cisatracurium metabolite, *Eur. J. Anaesthesiol.*, **19**(7), 466-473 (2002).

3. P. Lakshmi, C. Rambabu, Simple and selective spectrophotometric determination of atracurium besylate in pharmaceutical formulations using acidic dyes, *Anal. Chem.*, *An Indian J.*, **15**, 466-473 (2015).

4. C. Farenc, M. Audran, J.Y. Lefrant, I. Mazerm, F. Bressolle, High-performance liquid chromatographic method for the determination of atracurium and laudanosine in human plasma. Application to pharmacokinetics, *J. Chromatogr. B*, *Biomed. Sci. Appl.*, **724**, 117–126 (1999).

5. M.M. Ayad, F. Belal, M.M. Hosney, H. Elmansi, N. Elsayed, Simultaneous HPLC determination of cisatracurium and propofol in human plasma via fluorometric detection, *J. Chromatogr. Sci.*, **56**, 524–530 (2018).

6. A. Błazewicz, Z. Fijałek, M. Warowna-Grześkiewicz, M. Jadach, Determination of atracurium, cisatracurium and mivacurium with their impurities in pharmaceutical preparations by liquid chromatography with charged aerosol detection, *J. Chromatogr. A*, **1217**, 1266–1272 (2010).

7. S.D .Ferrara, A. Nalesso, F. Castagna, M. Montisci, S. Vogliardi, D. Favretto, Simultaneous determination of atracurium and its metabolite laudanosine in

post-mortem fluids by liquid chromatography/multiple-stage mass spectrometry on an ion trap, *Rapid Commun. Mass Spectrom.*, **21**, 2944–2950 (2007).

8. M. Zuo, J. Gao, X. Zhang, Y. Cui, Z. Fan, M. Ding, Capillary electrophoresis with electrochemiluminescence detection for the simultaneous determination of cisatracurium besylate and its degradation products in pharmaceutical preparations, *J. Sep. Sci.*, **38**, 2332–2339 (2015).

9. M.E. El Sharkasy, M. Walash, F. Belal, MM. Salim, Conventional and first derivative synchronous spectrofluorimetric methods for the simultaneous determination of cisatracurium and nalbuphine in biological fluids, *Spectrochim. Acta A, Mol. Biomol. Spectrosc.*, **228**, 117841 (2020).

10. M. Khoubnasabjafari, E. Rahimpour, A. Jouyban, Exhaled breath condensate as an alternative sample for drug monitoring, *Bioanalysis*, **10**, 61–64 (2018).

11. N. Hashemzadeh, E. Rahimpour, A. Jouyban, Applications of exhaled breath condensate analysis for drug monitoring and bioequivalence study of inhaled drugs, *J. Pharm. Pharm. Sci.*, **25**, 391-401 (2022).

12. C. Berchtold, M. Bosilkovska, Y. Daali, B. Walder, R. Zenobi, Real-time monitoring of exhaled drugs by mass spectrometry, *Mass Spectrom. Rev.,* **33**, 394–413 (2014).

13. F. Di Francesco, R. Fuoco, M. Trivella, A. Ceccarini, Breath analysis: trends in techniques and clinical applications, *Microchem. J.*, **79**, 405–410 (2005).

14. A. Jouyban, M. Khoubnasabjafari, K. Ansarin, V. JouybanGharamaleki, *Breath sampling setup*, Iranian patent. 81363, 2013.

15. M. Khoubnasabjafari, H. Pourfathi, A. Mahmoodpoor, A. Jouyban, Waste of mechanical ventilators as a biological sample for follow up of biomarkers and drugs, *Pharm. Sci.*, **26**, 343 (2020).

16. Chapter 2: Instrumentation for Fluorescence Spectroscopy, in: J.R. Lakowicz (editor), *Principles of Fluorescence Spectroscopy*, Springer, Boston, MA, 2006. p. 27–61. https://doi.org/10.1007/978-0-387-46312-4_2

17. Y.C. Tsang, R. Pop, P. Gordon, J. Hems, M. Spino, High variability in drug pharmacokinetics complicates determination of bioequivalence: Experience with verapamil, *Pharm. Res.,* **13**, 846–850 (1996).

18. M. Hoshino, M. Imamura, K. Ikehara, Y. Hama, Fluorescence enhancement of benzene derivatives by forming inclusion complexes with .beta.-cyclodextrin in aqueous solutions, *J. Phys. Chem.*, **85**, 1820–1823 (1981).

19. A. Orstan, J.B.A. Ross, Investigation of the .beta.-cyclodextrin-indole inclusion complex by absorption and fluorescence spectroscopies, *J. Phys. Chem.*, **91**, 2739–2745 (1987).

20. H. Abdolmohammad-Zadeh, E. Rahimpour, $CoFe_2O_4$ nano-particles functionalized with 8-hydroxyquinoline for dispersive solid-phase micro-extraction and direct fluorometric monitoring of aluminum in human serum and water samples, *Anal. Chim. Acta,* **881**, 54–64 (2015).

21. K. Pal, S. Mallick, A.L. Koner, Complexation induced fluorescence and acid–base properties of dapoxyl dye with γ-cyclodextrin: A drug-binding application using displacement assays, *Phys. Chem. Chem. Phys.*, **17**, 16015–16022 (2015).

22. J.M. Vasquez, A. Vu, J.S. Schultz, V.I. Vullev, Fluorescence enhancement of warfarin induced by interaction with beta-cyclodextrin, *Biotechnol. Prog.*, **25**, 906–914 (2009).

23. H. Abdolmohammad-Zadeh, E. Rahimpour, A novel chemosensor based on graphitic carbon nitride quantum dots and potassium ferricyanide chemiluminescence system for Hg(II) ion detection, *Sens. Actuators B Chem.*, **225**, 258–266 (2016).

24. F. Pourkarim, E. Rahimpour, M. Khoubnasabjafari, V. Jouyban-Gharamaleki, A. Jouyban, Direct monitoring of verapamil level in exhaled breath condensate samples, *Pharm. Sci.*, **25**, 50–56 (2019).

How to cite this article

A.A. Akbari, Z. Karimzadeh, M. Khoubnasabjafari, V. Jouyban-Gharamaleki, A. Jouyban, E. Rahimpour, Development of a surfactant mediated method for direct monitoring of atracurium in exhaled breath condensate, *Rev. Colomb. Cienc. Quim. Farm.*, **52**(3), 1247-1261 (2023). https://doi.org/10.15446/rcciquifa.v52n3.112481