Azhar Int J Pharm Med Sci 2024; Vol 4 (2): 79-87
(Research Article.)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

New eco-friendly spectrofluorometric approaches for the analysis of three antiepileptic drugs based on quenching of Acriflavine and silver nanoparticles

Fawzia Ibrahim [1], Rasha M. El-Sharawy [2, *] and Sawsan Abd El-Razeq [2]

[1] Department of analytical chemistry, Faculty of pharmacy, Mansoura University, Mansoura, Egypt.
[2] Department of analytical chemistry, Faculty of pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
***** Correspondence: rasha.elsharawy@yahoo.com

Article history: Received 2023-07-29 Revised 2023-10-18 Accepted 2024-03-16

Abstract: Two new spectrofluorometric methods have been developed for the quantitation of three antiepileptic drugs. The first method relied on monitoring the quenching effect of lamotrigine and carbamazepine on native fluorescence of acriflavine dye at 503nm (λex260 nm) in Britton Robinson buffer [pH 4]. The second one included the measuring of the quenching effect of carbamazepine and oxcarbazepine on the fluorescence intensity of silver nanoparticles at 531 nm (λex265 nm). Good linearity was obtained in method I over concentration range of 0.5 – 12 µg/mL (r2 = 0.9999),with LOD of 0.16 and 0.157 and LOQ of 0.485 and 0.476 for lamotrigine and carbamazepine respectively. While in method II the linearity was in the range 1.0 -16 µg/mL (r2 = 0.9998), with LOD of 0.167 and 0.259 and LOQ of 0.507 and 0.785 for carbamazepine and oxcarbazepine, respectively . The greenness of the methods was checked by three approaches; national environmental method index [NEMI], the analytical eco-scale and green analytical procedure index [GAPI].

Keywords: spectrofluorometry; quenching; lamotrigine; carbamazepine; oxcarbazepine; eco-friendly.

1. INTRODUCTION

Lamotrigine (LMT) fig. 1(A) is a drug used to treat epilepsy, belongs to the class of phenyltriazine and it stabilizes the mood in bipolar disorder [1]. Lamotrigine was approved to use in USA in 1994 and it is considered the first-line treatment for primary seizures. Carbamazepine (CBZ) fig. 1 (B) is an anticonvulsant drug, described to cure bipolar disorder [2]; it works by reducing the electrical activity in the brain. Oxcarbazepine (OXC) fig. 1 (C) is a drug used in treating partial epilepsy as it is described for focal or generalized seizures [3,4] and it is a derivative of carbamazepine has been introduced to the market in 2000.

Different analytical techniques have been developed to determine the three cited drugs as spectrophotometry [5-11], spectrofluorometriy [12-14], chemiluminescence [15], electrochemistry [16-18], HPTLC [19-21], HPLC [22-26], and UPLC [27-30].

In our survey in literature only two fluorometric methods have been reported for the determination of lamotrigine [12, 13] one through reaction with o-phthalaldehyde in the presence of 2-mercaptoethanol and the other by quenching the fluorescence of N-doped graphene quantum dots after its solid-phase extraction using magnetic graphene oxide and only one method has been reported for the determination of carbamazepine through reaction with NBD-Cl [14].

The purpose of this work is to develop a sensitive, fast and simple fluorometric method for the quantitation of lamotrigine and carbamazepine in their tablet formulations through quenching of native florescence of acriflavine dye. Again carbamazepine and oxcarbazepine have been determined by

Cite this article: Ibrahim F., El-Sharawy RM., Abd El-Monem S. New eco-friendly spectrofluorometric approaches for the analysis of three antiepileptic drugs based on quenching of Acriflavine and silver nanoparticles**.** Azhar International Journal of Pharmaceutical and Medical Sciences, 2024; 4(2): 79-87. doi: 10.21608/AIJPMS.2024.225813.1228

DOI: 10.21608/AIJPMS.2024.225813.1228

quenching of the native fluorescence of silver nanoparticles because of their unique properties.

2. METHODS

2.1. Instruments

- Pure samples of Lamotrigine (99%) as stated by the supplier were obtained from GlaxoSmithKline pharmaceutical, Heliopolis, Cairo, Egypt and checked by TLC [19].
- Pure samples of Carbamazepine (99.7%) and Oxcarbazepine (99%) as stated by the supplier were obtained from Novartis Pharma, Zaytoun Al Qebleya, Cairo, Egypt and checked by TLC [20, 21].
- Lamictal® 50 mg supplied by (GlaxoSmithKline pharmaceutical S.A., Poland), with batch no.≠ WL2Y.
- Lamictal® 100 mg supplied by (GlaxoSmithKline pharmaceutical S.A., Poland), with batch no.≠ W52D.
- Tegretol® 200 mg product of (Novartis Pharma), with batch no.≠ Y0632.
- Tegretol CR® 400 mg product of (Novartis Pharma), with batch no.≠ Y0382.
- Trileptal ® 300 mg product of (Novartis Pharma), with batch no.≠ 112521A.
- Acriflavine aqueous solution (Sigma Aldrich, USA) 5×10^{-6} M was prepared in distilled water.
- Britton Robinson buffer solution was made by mixing up (0.04 M) acetic acid, (0.04 M) boric acid and (0.04 M) phosphoric acid [34].
- Aqueous 0.2 M NaOH (Merck, Germany).
- Methanol [HPLC grade] was supplied by Sigma Aldrich, USA.
- Sodium borohydride (98%) were purchased from Fisher Chemical (UK) ;(2×10^{-3} M) freshly prepared in water by dissolving 0.0189g in 250 mL water.
- Silver nitrate (99.8%) was supplied by Sigma-Aldrich Co.; (1×10^{-3} M) in water.
- Distilled water was used during this work.

2.2. Preparation of Ag nanoparticles

The Ag nanoparticles were synthesized following a previously described procedure [35].30 mL of freshly prepared aqueous $NaBH_4$ (2×10^{-3} M) was transferred to 250 mL flask in ice bath on a magnetic stirrer. Add 10 mL of $AgNO_3$ solution (1×10^{-3} M) to the stirred $NaBH_4$ solution approximately 1drop per second to obtain a (2.7×10^{-4} M) Ag nanoparticles. This solution was stable for one month [36].

2.3. Stock drug solutions

Stock solutions of LMT, CBZ or OXC had been prepared as 0.1mg/mL in methanol separately.

2.4. General procedure

- **Method** I_ In two separate groups of 10 mL volumetric flasks 1.5 ml acriflavine solution (5×10^{-6} M), 1.5 ml Britton–Robinson buffer [pH 4], and 0.05 – 1.2 mL standard solutions (0.1 mg/mL) of LMT or CBZ were transferred and mixed well. The mixtures were kept for 15min at ambient temperature, and then completed with water to the mark. The fluorescence intensity has been measured for a blank against each experiment at 503 nm (λ_{ex} = 260 nm). The calibration curves were plotted by drawing ΔF versus the concentration of the drug and regression analysis was performed.

- **Method** II_ In two separate groups of 10 mL volumetric flasks, aliquots (0.1-1.6 mL) of standard solution of CBZ or OXC and 0.4 mL of (2.7×10^{-4} M) Ag nanoparticles solution were mixed well. Then diluted to 10 mL with distilled water and leave for 5 min then completed with water to volume.

The blank fluorescence intensity had been measured against each experiment at 531 nm (λ_{ex} 265 nm). ΔF was plotted at 531 nm against drug concentration in µg/mL to draw calibration curves and regression analysis was performed.

2.5. Application to pharmaceutical preparation

Ten tablets of each of Lamictal® or Tegretol® or Trileptal® were weighed precisely, finely ground, and mixed well.

A precisely weighed amount of each fine powder amounting to 10 mg of the drug was relocated into a small conical flask and extracted with 3 × 30 mL of methanol. Then the extracts and washings were filtered into a 100 mL volumetric flask and completed to the mark with methanol to get a solution labeled to contain 0.1 mg/mL. The later solutions of each drug were analyzed by quenching spectrofluorometriy as detailed under general procedure.

3. RESULTS and DISCUSSION

3.1. Methods I and II

Acriflavine is a member of the acredine derivative and used as a reagent with high native fluorescence intensity at 503 nm (λ_{ex} 260 nm). It was found to be quenched by adding many pharmaceutical compounds [31-33]. Thus, the

quenching effect of LMT or CBZ onto acriflavine solution was studied in method I; fig. 2 (A, B).
This quenching effect was attributed to formation of new fluorescent ion-associated complexes composed of acriflavine and each of the studied drugs. The different parameters which affect sensitivity of the proposed methods were studied.

In method II nanotechnology was used which is a new and remarkable field of recent research deals with design, synthesis and manipulation of particles structure which their size ranged from 1 to 100 nm. Recently, usage of metal nanoparticles in luminescence measurements enhances and increases their luminescence [34-37].

Because of the unique properties of silver nanoparticles, they are in area of interest, so it used for determination of CBZ and OXC; Fig. 3(A,B). It noteworthy to mention that lamotrigine gave unreliable results with silver nanoparticles.

3.2. Optimization of experimental conditions

Buffer medium_ Britton–Robinson buffer and Acetate buffer were tried as a medium for the reaction of each drug with acriflavine. Britton–Robinson buffer solution pH 4 ± 0.1 gave better quenching of fluorescence intensity, so it was the buffer of choice; Fig. 4 A. Using different volumes of it (0.5- 2.5 mL) to the reaction mixture, illustrated an optimum volume of 1.5± 0.2; Fig. 4 B.

Concentration and volume of Acriflavine_ different concentrations of acriflavine reagent (0.4 - 2.0 × 10^{-5} M) were studied on the quenched signal where maximum ΔF was obtained with 5 × 10^{-6} M acriflavine; therefore this concentration was selected throughout the study. It also found that ΔF increased by increasing volume of acriflavine, hence 1.5 ± 0.1 mL of 5 × 10^{-6} M acriflavine solution was found to be optimum.

Effect of the Silver Nanoparticles concentration _ The fluorescence intensity of silver NPs was increased and reached maximum value, when the nanoparticles' concentration ranged from 2.0 × 10^{-4} to 3.0 × 10^{-4} M. Further increase of concentration resulted in the decrease of the fluorescence intensity. Thus (2.7 × 10^{-4} M) was considered optimum concentration of silver NPs.

Reaction time_ it was observed that maximum quenching effect of each drug was obtained after 15± 2 min of mixing with the reagent, after which ΔF wasn't affected; therefore, reaction was allowed for this time. Fig. 5A
Again, it was observed that maximum quenching effect of carbamazepine or oxcarbazepine on silver NPs was obtained after 5 min of mixing with the reagent, after which ΔF wasn't affected till one hour; therefore, reaction was allowed to be measured after 5 min. Fig. 5B

Effect of diluting solvent_ numerous diluents for the reaction mixture of the two methods were examined. Distilled water gave the greatest ΔF for the studied drugs which adds to the benefits of the method.

3.3. Stoichiometry of the fluorometric reaction

Limiting logarithmic method has been used to determine the stoichiometric ratio of the fluorescence reaction between LMT or CBZ and acriflavine. Fig. 6 (A) displays plots of log concentration of [Lamotrigine] versus log ΔF at a fixed concentration of acriflavine and log [acriflavine] versus log ΔF at a fixed concentration of Lamotrigine. Also, Fig. 6 (B) displays plots of log concentration of [Carbamazepine] versus log ΔF at a fixed concentration of acriflavine and log [acriflavine] versus log ΔF at a fixed concentration of CBZ.

Both graphs gave straight lines with the slopes' values 1.43 and 1.4 for LMT and 0.972 and 0.958 for CBZ, respectively and from the slopes, the ratio of the reaction of the LMT or CBZ: complex of acriflavine can be considered as 1 : 1. The formation of an ion-associated complex was the proposed mechanism for the reaction quenching reaction [38]. (Scheme 1)

3.4. TEM images and characteristics of Silver nanoparticles UV spectrum

Transmission electron microscope (TEM) image Fig. 7 (A) showed the dark spherical shaped, mono-dispersed silver nanoparticles (Ag NPs) with size about 50 nm. Fig. 7 (B) showed the narrow absorption peak of Ag NPs near 400 nm which confirms the high level of dispersion of the Ag NPs.

3.5. The mechanism of fluorescence quenching in the proposed methods

The fluorescence quenching takes place through various mechanisms, such as inner filter effect [IFE], dynamic quenching or static quenching.

It was found that the spectra of excitation of acriflavine or Ag NPs and UV absorbance of three drugs showed some overlap, suggesting that the quenching might be related to IFE. So IFE could be evaluated for the three drugs by the following equation [39] :

$$\mathbf{F_{(corrected)} = F_{(observed)} \times antilog\ \{(Aex\text{-}Aem)/2\}}$$

In which $F_{(corrected)}$ is the fluorescence intensity

after subtracting IFE, $F_{(observed)}$ was the measured fluorescence, A_{ex} and A_{em} were the absorbance of the three drugs at the excitation and emission wavelengths of fluorophore. Then (%E) the suppressed efficiency was obtained for the observed and corrected fluorescence from this equation:

$$\% E = \left[1 - \frac{F}{F^0}\right] \times 100$$

From drawing % E of both $F_{(corrected)}$ and $F_{(observed)}$ of the three drugs versus their concentrations (Fig. 8), It was found that %E decreased. This decrease indicate that IFE of the three drugs was the main cause of quenching by about 31% and 23% for LAM and CBZ respectively in method I and by 21% and 13% for CBZ and OXC respectively in method II.

Stern–Volmer equation was also applied [40].In static quenching, the quencher substance complexes with the fluorescent material (in ground state). However, in dynamic quenching the quencher substances collide with the fluorescent materials (in their excited state). Stern–Volmer equation was:

$$F^0/F = 1 + D_{SV} \; C_{que}$$

In which F and F^0 are the fluorescence intensities of fluorophore (Acriflavine or Silver NPs) with or without the quencher; C_{que} is the quencher's concentration and D_{SV} is the Stern–Volmer quenching constant.

Stern–Volmer constant was calculated at different temperatures. When there is an inverse relationship between Stern–Volmer quenching constant D_{SV} and temperature, the mechanism of quenching is static quenching. However positive relationship between Stern–Volmer quenching constant D_{SV} and temperature indicated dynamic quenching.

The three temperatures (293^0F, 303^0F and 313^0F) were examined and Stern–Volmer graphs of F^0/F vs. C_{que} are presented in Fig. 9 (A-D). F^0/F and C_{que} had a linear relationship at the three temperatures where with increased temperature D_{SV} value decreased, so the mechanism of quenching was static quenching.

3.6. Method validation

The methods were validated relied on ICH Q2R1 guidelines [41]

Linearity and range

A good linear regression was obtained by drawing the ΔF between blank and experiment versus the final drug concentration ranged between 0.5– 12 µg/mL for LMT and CBZ in method I and 1.0– 16 µg/mL for CBZ and OXC in method II under the optimum experimental conditions. These equations were obtained from data analysis of the linear relationship:

- method I

Δ F = 7.593 + 30.55 C (r^2 = 0.9999) for LMT

Δ F= 65.214 + 36.912 C (r^2 = 0.9999)for CBZ

- method II

Δ F = 76.514 + 47.29 C (r^2 = 0.9998) for CBZ

Δ F = 37.99 + 51.65 C (r^2 = 0.9998)for OXC

Where: ΔF is the difference in fluorescence intensity between blank and experiment; r^2 is the correlation coefficient and C is the drug concentration in µg/mL.

The data was analysed statistically [42] showed small values of SD of residuals ($S_{y/x}$); 1.86 for LMT and 2.2 for CBZ in method I and are 3.01 for CBZ and 5.744 for OXC in method II with a good value for the correlation coefficient (r^2) of the regression equations, which indicate small scattering of the points around the calibration plots. LOQ and LOD were calculated according to ICH Q2R1 recommendation [41]:

LOQ =10 S_a/b

LOD = 3.3 S_a/b

LOQ was between 0.476 and 0.785 µg/mL while LOD ranged between 0.157 and 0.256 µg/mL, Table 1.

Accuracy and Precision

Pure samples of LMT, CBZ and OXC over the working concentration range were examined by the developed methods. The obtained results were in a good agreement with the results of comparison methods with concentration range 0.5 - 12 µg for LMT or CBZ and 1.0 – 16 µg for CBZ or OXC.

The intraday and interday precisions were assayed by analyzing three concentrations of each LMT, CBZ and OXC in the pure form three sequential times in a day and on three sequential days, where intraday %RSD had range between 0.14 and 1.63 and interday% RSD were between 0.31 and 1.87. Table 2.

Table 1. Analytical performance data for the developed florescence quenching methods.

Parameters	Lamotrigine (method I)	Carbamazepine		Oxcarbazepine (method II)
		method I	method II	
Linearity range (µg mL^{-1})	0.5 – 12	0.5 – 12	1-16	1-16
Intercept (a) ± SD	7.593 ± 1.4	65.214 ± 1.7	76.514 ± 2.39	37.99 ± 4.05
Slope (b) ± SD	30.55 ± 0.19	36.912 ± 0.22	47.29 ± 0.25	51.65 ±0.44
SD of residual	1.864	2.2096	3.01	5.744
Correlation coefficient	0.9999	0.9999	0.9998	0.9998
LOD (µg mL^{-1})	0.16	0.157	0.167	0.259
LOQ (µg mL $^{-1}$)	0.485	0.476	0.507	0.785
Accuracy	99.87±0.78	99.98±0.62	99.91±0.67	100.22± 1.15
% Error	0.296	0.234	0.253	0.434

Robustness

It was determined by estimating the impact of small variation of experimental variables:

- In Method I: Volume of Britton Robinson buffer (1.5 mL ± 0.2) and its pH (4 ± 0.2), acriflavine (1.5 mL ± 0.2).
- In Method II: Volume of Ag NPs was found to be (0.4 mL ± 0.05).

The results had not been affected significantly by little changes in the variables; so, the developed quenching fluorimetric methods were found to be robust; Table 3.

Application to the analysis of pharmaceutical preparation

Both developed and reported methods were used to determine LMT, CBZ and OXC in Lamictal®, Tegretol® and Trileptal® and then the results of the developed methods were statistically compared with those of the reported methods [6,9,14].

Tables 4, 5 showed values of Student t- test and variance F ratio and revealed that there was no considerable difference between reported and developed methods regarding accuracy and precision. However, the developed quenching fluorometric methods were much more selective for determination of the three cited drugs regarding the usage of two wavelengths (λ_{ex} and λ_{em}).

Selectivity

The methods' selectivity had been checked by monitoring any confusion from the widespread tablet additives like starch, talc and lactose which did not have any effect on the results of the developed fluorometric method.

3.7. Estimation of the developed methods Greenness

Nowadays it is very significant to estimate any analytical method greenness, so three approaches were used to evaluate greenness of the developed fluorometric methods. The first one is [NEMI] the national environmental method index [43]; Fig. 10 (A), the developed methods meet the standards of the NEMI approach.

The second one is the analytical eco-scale [42], which relies on counting the penalty point; Table 6 summarized the obtained PPs for the proposed methods which are a score of 83 for method I and 77 for method II. Although the reported methods were found to be green according to eco-scale [44] table 6, the proposed methods were much more selective and sensitive than the reported methods.

The score obtained approved that the developed methods are acceptable and excellent green ones. The third approach is [GAPI] the green analytical procedure index [45] which is one of the new tools to estimate the greenness of the methods and gathers the both advantages of eco-scale and NEMI tools as

it estimate the greenness starting from the sample collection to the final determination. Fig. 11 and table 7 clarify the GAPI assessment of the proposed methods.

Table 2. Precision data for the developed fluorometric methods for the analysis of pure lamotrigine, carbamazepine and oxcarbazepine

.

Drug			Taken Concentration (µg/mL)	Found Concentration* (µg/mL)	% RSD	% Error
Lamotrigine	Method I	Intraday	1	1.001	1.11	0.64
			8	7.856	0.14	0.08
			10	10.102	0.31	0.18
		Interday	1	0.989	1.07	0.62
			8	8.005	0.31	0.18
			10	9.986	0.73	0.42
Carbamazepine	Method I	Intraday	1	1.012	0.68	0.39
			5	4.92	0.35	0.20
			10	10.04	1.44	0.83
		Interday	1	0.989	1.50	0.87
			5	4.96	0.92	0.53
			10	10.13	0.44	0.26
	Method II	Intraday	1	1.008	0.67	0.39
			8	7.902	0.25	0.15
			14	13.9	1.33	0.77
		Interday	1	0.980	1.54	0.89
			8	7.934	0.64	0.37
			14	14.26	1.73	1.00
Oxcarbazepine	Method II	Intraday	1	1.018	0.92	0.71
			7	6.919	1.08	0.63
			15	15.150	1.63	0.94
		Interday	1	1.010	1.87	1.03
			7	6.867	1.68	0.97
			15	15.168	0.65	0.37

* Average of three determinations

5. CONCLUSIONS

Accurate and green spectrofluorometric methods through quenching of acriflavine dye or silver nanoparticles were used for the quantitation of Lamotrigine, Carbamazepine and Oxcarbazepine in pure forms and in their pharmaceutical preparations.

LOD and LOQ of the developed methods were 0.16 and 0.485 µg/mL for Lamotrigine, 0.157 and 0.476 µg/mL for Carbamazepine in method I, 0.167 and 0.507 µg/mL for Carbamazepine in method II and 0.259, 0.785 µg/mL for Oxcarbazepine in method II respectively. The two methods proved to be robust and successfully applied to the pharmaceutical preparations of the cited drugs with high sensitivity. Hence, they can be used in quality control laboratories and can be used for application in biological fluids.

Funding: This research doesn't receive any funding from any organization.

Conflicts of Interest: No conflict of interests for all the authors.

Author Contribution: All authors contributed in this work and have read and approved the final submitted manuscript.

REFERENCES

1. Prabhavalkar KS, Poovanpallil NB, Bhatt LK: Management of bipolar depression with lamotrigine: an antiepileptic mood stabilizer. Front. Pharmacol. 2015 ;23;6:242.

2. The American Society of Health-System Pharmacists. Archived from the original 2015.

3. "Oxcarbazepine Monograph for Professionals". Drugs.com. American Society of Health- System Pharmacists; 2019.

4. British National Formulary: BNF 76 (76 ed.). Pharmaceutical Press. 2018. pp. 319–320.

5. Alizadeh, N., Khakinahad, R., Jabbari, A.; Spectrophotometric determination of lamotrigine in pharmaceutical preparations and urine by charge-transfer complexation; Die Pharmazie, Nov 2008; 63(11): 791-795.

6. Rajendraprasad N, Basavaiah K, Vinay KB and Ramesh PJ; Simple and Sensitive Spectrophotmetric Determination of Lamotrigine in Pure Form and in Dosage Forms; Pharmaceutica Anaytica Acta 2012,3:9.

7. Frag EYZ, Zayed MA, Omar MM, Elashery SEA, Mohamed GG.; Spectrophotometric determination of carbamazepine and mosapride citrate in pure and pharmaceutical preparations; Arabian Journal of chemistry (2012) 5 , 375-382.

8. Basavaiah K, Rajendraprasad N, Cijo M X, Vinay KB, Ramesh PJ.; Development and validation of stability indicating spectrophotometric methods for the determination of oxcarbazepine in pharmaceuticals; Journal of Scientific & Industrial Research, 2011; 70:346–351.

9. Ramaa CS, Chothe PP, Naik AA and Kadam VJ.; Spectrophotometric method for the estimation of oxcarbazepine in tablets; Indian Journal of Pharmaceutical Sciences 2006; 68:265–266. DOI: 10.4103/0250-474X.25734

10. Satish MA and Nagendrappa G.; Spectrophotometric determination of oxcarbazepine in pharmaceutical formulation; International Journal of Pharmacy and pharmaceutical Sciences 2010; 2: 93–98.

11. Krishna Ch. M, Rao S.V. V, Rao N.V.S. M, Rambabu C.; A Simple Spectrophotometric determination of oxcarbazepine by condensation reactions using 2-chlorophenylhydrazine and anthranilic acid; Journal of Pharmacy Research 2011; 4(10):3317–3319.

12. El Enany N., El Sherbiny D., Abdelal A., Belal F.; Validated Spectrofluorimetric Method for the Determination of Lamotrigine in Tablets and Human Plasma Through Derivatization with o-phthalaldehyde; Journal of Fluorescence 2009; 20(2):463-72.

13. Bazrafshan E., Dadfarnia Sh., Shabani A., Afsharipour R., Determination of lamotrigine by fluorescence quenching of N-doped graphene quantum dots after its solid-phase extraction using magnetic graphene oxide; Spectrochimica Acta Part A 2022; 267(1).

14. Walash M. I., El Enany N., Askar H.; Validated spectrofluorimetric method for the determination of carbamazepine in pharmaceutical dosage forms after reaction with 4-chloro-7--nitrobenzo-2-oxa-1,3-diazole (NBD-Cl); Luminescence 2015; 30(7): 1119-1124.

15. Hai tao, X.; Determination of carbamazepine by chemiluminescence analysis with Ce(IV)-Na2SO3 system; Chinese Journal of Pharmaceutical analysis 2012; 32(10):1866-1869.

16. Burgoa M. E., Dominguez R. O., Martinez M. J.;Determination of lamotrigine by adsorptive stripping voltammetry using silver nanoparticle-modified carbon screen-printed electrodes; Talanta 2007; 74(1): 59-64.

17. Pan M. L., Lin W. Y., Wang H. Y., Tsai S. C., Hsieh P. F., Su Y. L. O., Huang P. W.; Determination of carbamazepine: A comparison of the differential pulse

voltammetry (DPV) method and the immunoassay method in a clinical trial; Journal of Analytical Chemistry 2014; 69(1): 57-61.

18. Calvo MEB, Renedo OD, Martínez MJA.; Determination of oxcarbazepine by square wave adsorptive stripping voltammetry in pharmaceutical preparations; Journal of Pharmaceutical and Biomedical Analysis 2007; 43(3):1156–60.

19. Mennickent S., Fierro R., Vega M., de Diego M., Godoy C. G.; Quantification of lamotrigine in human serum by high-performance thin-layer chromatography; Journal of Planar Chromatography-ModernTLC 2011; 24(3): 222-226.

20. Naguib A., Nesma A., Fadwa A., El Ghobashy M. ,Fatma F.; Validation and eco-scale assessment of stability-indicating HPTLC method for quantitative analysis of carbamazepine and its degradation product, iminostilbene, in pure forms, pharmaceutical preparations, and spiked human plasma UV detection at 230 nm; Journal of Planar Chromatography-Modern TLC 2020; 33(3): 219–229.

21. Bhoite SD, Dhole NS, Bhoir S, Sangole P, Thorat S.; Development and validation of stability indicating HPTLC method for determination of oxcarbazepine in bulk and pharmaceutical formulation; International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(3):127–132.

22. Asadi M., Dadfarnia S., Shabani A. M. H., Abbasi B.; Simultaneous extraction and quantification of lamotrigine, phenobarbital, and phenytoin in human plasma and urine samples using solidified floating organic drop microextraction and high-performance liquid chromatography; Journal of Separation Science 2015; 38(14): 2510-2516.

23. Serralheiro A., Alves G., Fortuna A., Rocha M., Falcao A.; First HPLC-UV method for rapid and simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma; Journal of Chromatography B: 2013; 925(1): 1-9.

24. Contin M., Mohamed S., Candela C., Albani F., Riva R., Baruzzi A. ;Simultaneous HPLC-UV analysis of rufinamide, zonisamide, lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in deproteinized plasma of patients with epilepsy; Journal of Chromatography B: 2010; 878(3-4): 461-465.

25. Heideloff C.,Bunch D.R.,Wang S.; A novel HPLC method for quantification of 10 antiepileptic drugs or metabolites in serum/plasma using a monolithic column; Therapeutic Drug Monitoring 2010; 32(1): 102-106.

26. Ibrahim F.A , El-Yazbi A. , A Barary M., Wagih M.M.; Sensitive inexpensive HPLC determination of four antiepileptic drugs in human plasma: application to PK studies; Bioanalysis, 2016 ;8(21):2219-2234.

27. Karinen R., Vindenes V., Hasvold I., Olsen K. M., Christophersen A. S., Oiestad E.; Determination of a selection of anti-epileptic drugs and two active metabolites in whole blood by reversed phase UPLC-MS/MS and some examples of application of the method in forensic toxicology cases; Drug Testing and Analysis 2014; 7(7): 634-644. Doi: 10.1002/dta.1733.

28. Kuhn J.,Knabbe C.; Fully validated method for rapid and simultaneous measurement of six antiepileptic drugs in serum and plasma using ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry; Talanta 2013; 110(1): 71-80.

29. Shibata M., Hashi S., Nakanishi H., Masuda S., Katsura T., Yano I.; Detection of 22 antiepileptic drugs by ultra-performance liquid chromatography coupled with tandem mass spectrometry applicable to routine therapeutic drug monitoring; Biomedical Chromatography 2012; 26(12): 1519-1528.

30. Beig A., Dahan A.; Quantification of carbamazepine and its 10,11-epoxide metabolite in rat plasma by UPLC-UV and application to pharmacokinetic study;

Biomedical Chromatography 2014; 28(7): 934-938.

31. Fathy M.,Aly F.,El Enany N.,Tolba M.,Abdel Aziz H.; Green and sensitive spectrofluorimetric method for the determination of two cephalosporins in dosage forms; Royal Society Open Science 2021;8(8):210329.

32. Ali L., Qader A., Salih M., Aboul Enein H.; Sensitive spectrofluorometric method for the determination of ascorbic acid in pharmaceutical nutritional supplements using acriflavine as a fluorescence reagent; Luminescence 2019; 43(2) : 168-174.

33. Qader A., Fakhre N.; Spectrofluorometric determination of furosemide in some pharmaceutical product using acriflavine as a reagent; AIP Conference Proceedings 2017; 1888(1):020042.

34. Kamruzzaman M, Alam AM, Lee SH, Suh YS, Kim YH, Kim GM.; Method for determination of fluoroquinolones based on the plasmonic interaction between their fluorescent terbium complexes and silver nanoparticles; Michrochimica Acta 2011;174:353–60.

35. Hassan Korbekandi and Siavash Iravani ; Silver Nanoparticles; Research Gate. May 2012 ; Chapter: 1.

36. Alothman Z., Bukhari N., Haider , S., Wabaidur S. and Alwarthan A; Spectrofluorimetric determination of fexofenadine hydrochloride in pharmaceutical preparation using silver nanoparticles; Arabian Journal of Chemistry 2010;3, 251-255.

37. S.D. Solomon, M. Bahadory, A.V. Jeyarajasingam, S.A. Rutkowsky, C. Boritz, and L. Mulfinger; Synthesis and study of silver nanoparticles; Journal of Chemical Education, (2007) 84, 322-325 .

38. I. L. Finar; Organic chemistry; vol.1 sixth edition, publisher ELBS with Longman, U.K., 1994.

39. Weitner T., Friganović T., and Šakić D.; Inner Filter Effect Correction for Fluorescence Measurements in Microplates Using Variable Vertical Axis Focus ,Analytical chemistry; 2022,94(19). 7107-7114.

40. Marcelo H Gehlen; The centenary of the Stern-Volmer equation of fluorescence quenching: From the single line plot to the SV quenching map, Journal of photochemistry and Photobiology C; 2020, 42, 100338.

41. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Current Step 4 Version, Parent Guidelines on Methodology Dated November 6 1996, incorporated in November 2005, http://www.ich.org/LOB/media/MEDIA41.pdf, accessed 15 February 2008.

42. Miller J. C. and Miller J. N., Statistics and Chemometrics for Analytical Chemistry, Pearson Education Limited, Harlow, England, 5th edn, 2005, pp. 39–73,107-149, 256.

43. Tobiszewski, M. ; Metrics for green analytical chemistry; Analytical Methods (2016), 8, 2993–2999.

44. Van Aken K., Strekowski L., Patiny L. ; EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters; Beilstein Journal of Organic Chemistry (2006); 2(1):3, 1–7.

45. Płotka-Wasylka, J.; A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta 2018, 181, 204–209. DOI:10.1016/j.talanta.2018.01.013