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# SIMULTANEOUS DETERMINATION OF LAMOTRIGINE AND OXCARBAZEPINE IN PLASMA USING -HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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**ABSTRACT**
A simple, fast and efficient method based on ultrasound-assisted emulsification microextraction (USAEME) followed by HPLC has been developed for simultaneous determination of oxcarbazepine (OXZ and Lamotrigine (LTG) in biological samples. The influence of different parameters affecting the USAEME procedure was evaluated to optimize the efficiency of the process. At these conditions, validation of the proposed method was performed based on European medicines agency guidelines. The proposed method showed good linearity in plasma samples ranging from 0.25 to 6 µg/mL and 0.13 to 6 µg/mL ($R^2 \geq 0.99$) for OXZ and LTG, respectively. The precision of the proposed method was evaluated for repeatability which was ≤12% ($n$ = 5). The detection limits of 0.06 and 0.05 µg/mL were obtained for OXZ and LTG, respectively. The proposed method can be applied as an effective technique for therapeutic drug monitoring of OXZ and LTG in plasma of epileptic patients.
**KEYWORDS:** *ultrasound-assisted emulsification microextraction, oxcarbazepine Lamotrigine, HPLC, plasma analysis*

oxcarbazepine and Lamotrigine are anticonvul-sant drugs generally used to prevent or reduce the severity of epileptic fits and other convulsions [1]. Antiepileptic drugs are of those drugs that require therapeutic drug monitoring (TDM) in order to main- tain relatively constant concentration of drug in bloodstream to optimize patients' clinical outcome [2, 3]. Hence, there is a need for routine monitoring of antiepileptic drug concentrations in blood samples especially of OXZ and LTG with narrow therapeutic ranges to prevent the unwanted effects and to improve clinical management of patients with epilepsy. The recommended therapeutic concentrations of OXZ are between 4–12 µg/mL in serum and those of LTG are between 1–4 µg/mL in plasma [3].

The most common method for TDM of antiepilep- tic drugs in biological fluids and drug products is based on HPLC [4–6]. The advantages of HPLC for antiepileptic drugs determination are its adaptability and simplicity of sample preparation as well as a broad linearity of detectors. In addition, gas chromatography has been described in the literature for determination of LTG and OXZ in biological f luids and drug prod- ucts [7, 8].

Determination of drug concentrations in blood samples requires an excellent sample preparation

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pro- cedure which influences the purity of the sample introduced into the chromatographic system. The most common ways to extract these antiepileptic drugs are liquid–liquid extraction (LLE) [9, 10] and solid- phase extraction (SPE) [11, 12]. However, in spite of their general co-administration by epileptic patients, very few methods have been proposed for simultane-ous determination of LTG and OXZ [7, 9, 13].

Application of traditional LLE and SPE methods was limited due to such disadvantages as solvent losses, large secondary wastes, tedious procedure and complex equipment. The microextraction techniques effectively overcome these problems through eliminat- ing or reducing the amount of organic solvent. Addi-tionally, extraction and preconcentration are done in one step. Solid-phase microextraction [7, 14] and dis-persive liquid–liquid microextraction [15, 16] have been applied for some antiepileptic drugs. Ultra-sound–assisted emulsification microextraction with applying both low and heavy density organic solvents is one of the alternative dispersion techniques in which the need of disperser solvent was eliminated through using the ultrasonic radiation [17–19].

In this work, USAEME based on emulsification of micro volumes of low-density organic solvents in aqueous samples coupled with HPLC was successfully developed for simultaneous determination of OXZ and LTG in human plasma.

## EXPERIMENTAL

**Materials.** oxcarbazepine and Lamotrigine were purchased from Aldrich. The stock solutions (1000 μg/mL) of them were prepared in methanol and were stored at −4°C far from the light. The working standard solutions of OXZ and LTG were prepared by diluting the above stock solutions with double distilled water.

All materials and solvents, such as toluene, cyclo- hexanol, *n*-dodecan, 1-octanol and 2-octanon, of analytical grade were purchased from Merck Com- pany (Darmstadt, Germany). Methanol and acetonitrile (HPLC grade) were purchased from Caledon (Georgetown, Canada) for HPLC mobile phase.

**Instrumentation.** Separation and determination of analytes were performed on a PerkinElmer HPLC system Series 200 equipped with a manual injector and a UV-Vis detector. All chromatographic separations were carried out on an Eclipse plus C18 column (100 × 4.6 mm, 3.5 μm particle size) from Agilent. A mixture of 10 mM potassium dihydrogen phosphate buffer at pH 6 and acetonitrile (70 : 30, v/v) at a flow rate of 1.0 mL/min was used as the mobile phase in isocratic elution mode. The detection was performed at the wavelength of 220 nm.

An ElmaSonic ultrasonic bath (frequency of 37 kHz and power of 0.138 kW) was used for emulsification of extraction organic solvent. All pH measure- ments were done at 25 ± 0.1°C using a pH meter (713, Metrohm, Herisau Switzerland) with a standard uncertainty of 0.1 mV.

**Ultrasound-assisted emulsification microextraction procedure.** All extractions were performed in a special centrifuge tube with a capillary tube at the top of the centrifuge vial which is suitable to collect the floated low-density organic extraction solvents [20]. The tube was placed into an ultrasonic water bath. Then, 60 μL of 1-octanol was gently injected into 10 mL of sample solution (pH 10) containing OXZ and LTG and 20% (w/v) NaCl. A cloudy solution was observed after son-ication for 30 s due to the formation of emulsified fine droplets of organic solvent in the aqueous solution. The solution was then centrifuged at 5000 rpm for 8 min leading to disruption of emulsified 1- octanol. Finally, 20 μL of floated organic solvent was injected into the HPLC-UV instrument for analysis.

**Sample preparation.** Drug-free plasma samples obtained from six healthy volunteers (taking no medication) spiked with OXZ and LTG were used to pre- pare calibration curves and to conduct validation tests. All plasma samples were stored at –18°C prior to anal- ysis. The blood samples were taken from four patients with epilepsy in therapy with Tegretol® 200 mg, Lam- ictal® 50 mg and Lamogin 100 mg twelve hours after taking the previous dose (before the morning dose), transferred to plasma separator tubes containing EDTA-Na and centrifuged at 3000 rpm for 15 min to obtain plasma. Working plasma samples were prepared by diluting 2 mL of plasma with 8 mL of with phosphate buffer solution (pH 10) to bring the concentrations of OXZ and LTG in the working range.

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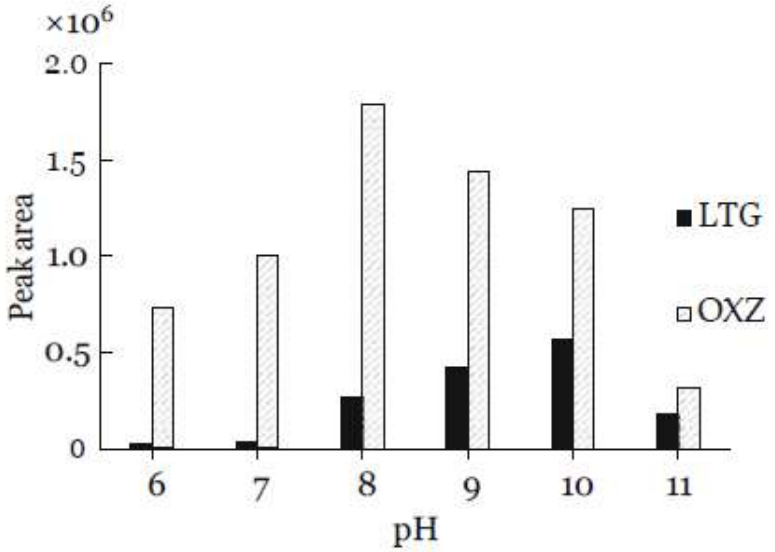



**Fig. 1. Effect of pH on the extraction efficiency of oxcarbazepine and Lamotrigine. Extraction conditions: sample solution—10 mL of 2 µg/mL oxcarbazepine and Lamo-trigine in phosphate buffer (pH 10), extraction solvent—50 µL of toluene, salt concentration – 20%, emulsification time—30 s, centrifugation time—8 min.**

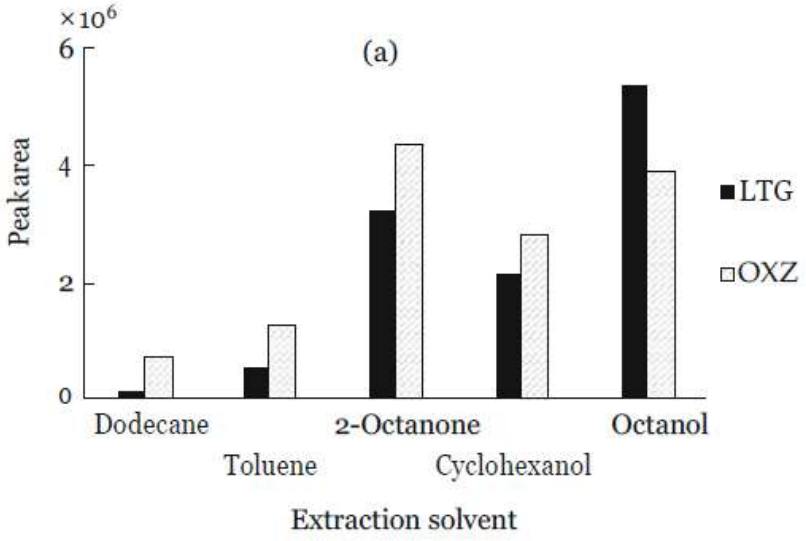
## RESULTS AND DISCUSSION

In the present study, simultaneous measurement of two drugs, Lamotrigine and oxcarbazepine was car- ried out in human plasma using the USAEME provid- ing enhanced extraction efficiency and reduced extraction time with small consumption of organic solvent.

In order to obtain high-enrichment factor, the effects of different parameters were optimized. These parameters were the type and volume of the extraction solvent, pH, extraction time and ionic strength. Finally, these optimal conditions were applied to determine OXZ and LTG in plasma samples.

Effect of pH on the extraction efficiency. pH opti- mization is one of the crucial steps for basic and acidic analytes. Adjusting the sample pH to the value where the compound is in the neutral form with more hydro- phobic property drastically enhances the extraction efficiency. As the studied drugs contain basic func- tional groups, the pH effect was investigated within range of 6–11 shown in Fig. 1. The maximum extraction efficiency was obtained at pH 8 and 10 for OXZ and LTG, respectively. The adjustment of solu- tion to pH greater than 8 resulted in OXZ and LTG existing mainly in non-ionic forms. Therefore, pH 10 in which both drugs had acceptable extraction effi- ciency was chosen as the optimum pH in all subse- quent experiments.

Influence of extraction solvent and its volume on the extraction efficiency. In the USAEME method like other extraction methods, solvent should have low



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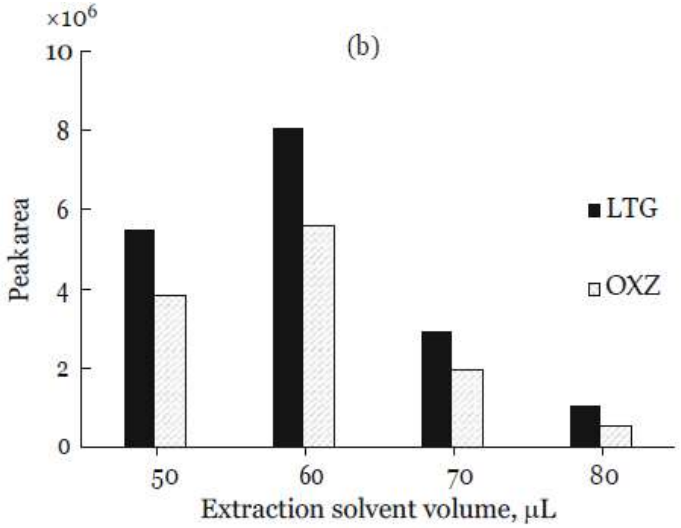


**Fig. 2. Effect of different extraction solvents (50 µL) (a) and extracting solvent volume (b) on the extraction efficiency of Carba-mazepine and Lamotrigine. Extraction conditions: sample solution—10 mL of 2 µg/mL oxcarbazepine and Lamotrigine in phosphate buffer (pH 10), salt concentration—20%, emulsification time—30 s, centrifuge time—8 min.**

water solubility, high extraction capability of target analytes and it should be compatible with HPLC. Organic solvents with lower density than water includ- ing toluene, cyclohexanol, *n*-dodecan, 1-octanol and 2-octanon were examined. The results shown in Fig. 2a revealed that 1-octanol offered the highest peak area among the solvents. Different extraction efficiencies of these solvents are attributed to their dif- fering polarities, viscosities and emulsification pow- ers. Based on observed results, 1-octanol was chosen as the optimum extraction solvent for subsequent studies.

In order to optimize the extraction solvent volume, the volume of 1-octanol was studied in the range of 30–100 µL. As shown in Fig. 2b, the highest peak area was obtained at 60 µL. When 30 and 40 µL of 1-octanol were used the volume of collected extraction sol- vent was very small to inject into the HPLC system. At higher volumes (>60 µL), the volume of sedimented phase was increased while the peak area was decreased. This observation might be attributed to the decreased enrichment factor in larger extraction solvent volume. So, the volume of 60 µL was selected as the optimal extraction solvent volume for further studies.

**Effect of ionic strength.** Adding a salt to increase the ionic strength usually improves the extraction efficiency due to decrease in the solubility of analytes in the aqueous phase (the salting out effect). The most common salt to investigate the ionic strength is sodium chloride. Different concentrations of NaCl in the range of 0–20% (w/v) were examined. The obtained results are shown in Fig. 3. The results indi- cated that the extraction efficiency increased with increasing NaCl concentration. Thus, 20% salt was used for preparing sample solutions in further experi- ments.

**Method evaluation.** Linearity behavior of the USAEME-HPLC method for extraction of OXZ and LTG was tested under optimum conditions. The calibration graphs in plasma were linear in the ranges of 0.25–6 µg/mL and 0.13–6 µg/mL for OXZ and LTG, respectively. The reported detection limit (LOD) values were obtained based on practical experiments. For this purpose, the concentrations of analytes were reduced until the obtained HPLC-UV responses for the drugs created detectable signals-to-noise ratios of 3 at the retention times related to target drugs. The limit of quantitation (LOQ) values were considered as the signal-to-noise ratios of 10. The obtained LODs and LOQs for OXZ and LTG were in the ranges of 0.03 to 0.04 and 0.10 to 0.13 µg/mL in water and 0.05 to 0.06 and 0.17 to 0.20 µg/mL in plasma samples, respectively. The results are summarized in Table 1. The preconcentration factor (PF) was defined as the ratio of the final analyte concentration in the acceptor phase (*c*f, a) to the initial concentration of analyte in the sample solution (*c*i, S), where *c*f, a was calculated from a calibration graph obtained from the direct injection of the standard solution. The obtained PF values for OXZ and LTG were in the ranges of 85 to 117 and 56 to 65 in water and plasma samples, respectively. To evaluate the repeatability of the proposed method, peak areas of five

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replicate measurements at the con- centration of 3 µg/mL (50% of the calibration curve range) of OXZ and LTG were used and expressed as the relative standard deviation (RSD, %).

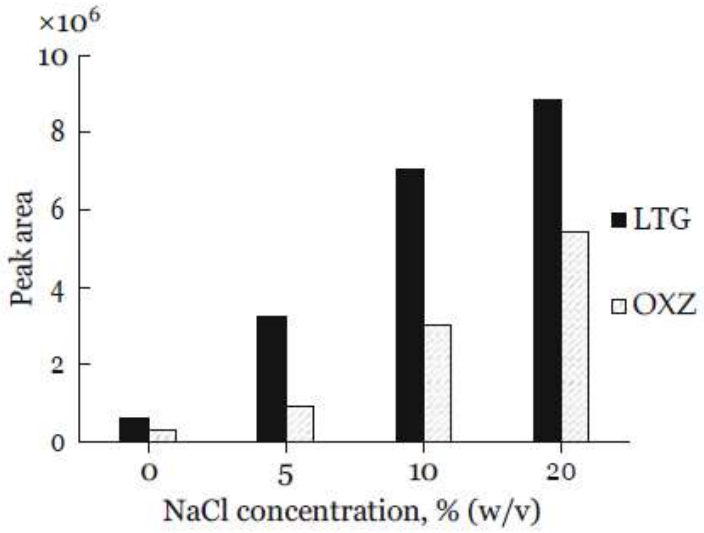


**Fig. 3. Effect of salt concentration on the extraction effi- ciency of oxcarbazepine and Lamotrigine. Extraction conditions: sample solution—10 mL of 2 µg/mL oxcarbazepine and Lamotrigine in phosphate buffer (pH 10), extraction solvent—1-octanol, extraction solvent vol- ume—60 µL, emulsification time—30 s, centrifuge time— 8 min.**

**Table 1. Figures of merit of the ultrasound-assisted emulsification microextraction–HPLC method for oxcarbazepine and Lamotrigine determination in water and plasma samples**

| Drug | Matrix | DLR[a], µg/mL | LOD, µg/mL | LOQ, µg/mL | RSD, % ($n$ = 5) | EF[b] | $R^2$ |
|---|---|---|---|---|---|---|---|
| OXZ | Water | 0.05–6 | 0.04 | 0.13 | 5.3 | 117 | 0.999 |
| | Plasma | 0.25–6 | 0.06 | 0.20 | 8.9 | 65 | 0.998 |
| LTG | Water | 0.05–6 | 0.03 | 0.10 | 4.2 | 85 | 0.999 |
| | Plasma | 0.13–6 | 0.05 | 0.17 | 7.7 | 56 | 0.997 |

[a] DLR—dynamic linear range, [b] EF—enrichment factor.

**Table 2. Method validation data for target drugs determination in spiked healthy human plasma using the ultrasound- assisted emulsification microextraction–HPLC procedure**

| Drug | Spiked concentration, µg/mL | Found ± SD[a], µg/mL | Accuracy[b], % | RR[c], % | Precision[d], % | |
|---|---|---|---|---|---|---|
| | | | | | intra-day[e] | inter-day[f] |
| OXZ | 0.25 | 0.23 ± 0.00 | −8.0 | 92 | 10.4 | 11.1 |
| | 0.75 | 0.68 ± 0.07 | −9.3 | 91 | 9.4 | 10.6 |
| | 3.3 | 3.3 ± 0.3 | +0.3 | 100 | 8.9 | 8.3 |
| | 4.1 | 4.0 ± 0.3 | −3.6 | 96 | 6.5 | 7.2 |
| LTG | 0.13 | 0.14 ± 0.02 | +7.7 | 108 | 11.6 | 11.9 |
| | 0.39 | 0.35 ± 0.04 | −10.3 | 90 | 10.2 | 11.5 |
| | 2.8 | 2.6 ± 0.2 | −6.8 | 93 | 7.7 | 8.4 |
| | 4.1 | 4.3 ± 0.3 | +4.4 | 104 | 7.2 | 9.5 |

[a] SD—standard deviation; [b] expressed as relative error, %; [c] RR—relative recovery; [d] expressed as RSD, %; [e] intra-day precision was cal- culated by analysis of five replicates samples within one day ($n$ = 5); [f] inter-day precision was calculated by analysis of five replicates over a period of 5 days ($n$ = 5).

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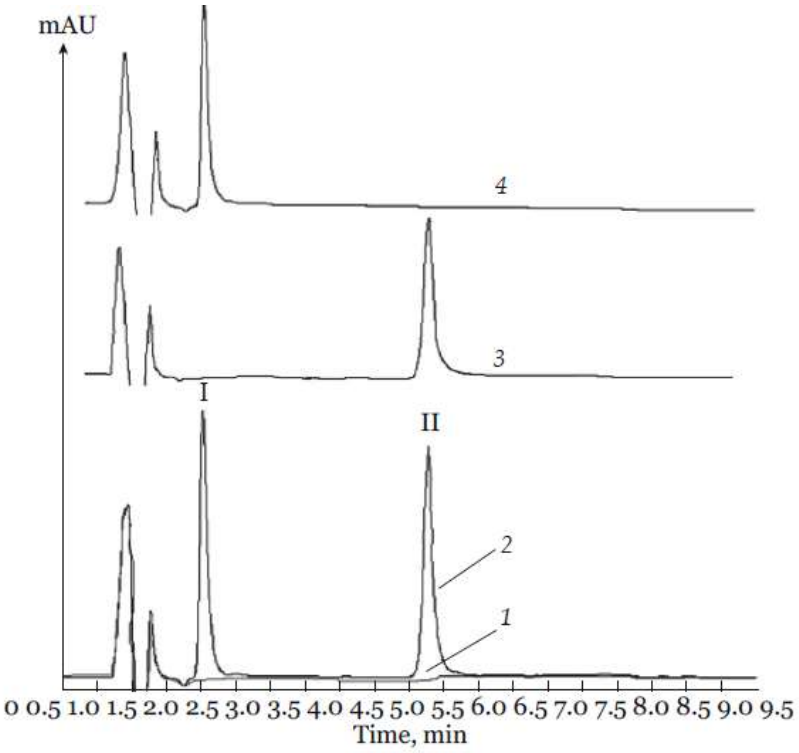


**Fig. 4. Chromatograms obtained by the USAEME–HPLC method from non-spiked healthy human plasma (blank plasma) (*1*), spiked healthy human plasma with 5 µg/mL Lamotrigine (I) and oxcarbazepine (II) (2), non-spiked plasma of epileptic patient in therapy with Tegretol® daily 400 mg (subject 2) (*3*) and non-spiked plasma of epileptic patient in therapy with Lamogin® daily 100 mg (subject 3) (*4*).**

As the quality control (QC) sample was not avail- able, the spiking method was used to validate the pro- posed method. For this purpose, the intra-day preci- sion (repeatability) and inter-day precision (reproduc- ibility) values were calculated by analyzing five replicates of spiked healthy plasma within one day and

**Table 3.** oxcarbazepine and Lamotrigine determination in plasma of epileptic patients using ultrasound-assisted emul-sification microextraction–HPLC

| Subject[a] | Drug | Found ± SD, µg/mL | Precision (RSD, %, $n$ = 3) |
|---|---|---|---|
| 1[b] | OXZ | 5.3 ± 0.4 | 7.9 |
| 2[c] | OXZ | 3.0 ± 0.3 | 8.6 |
| 3[d] | LTG | 3.7 ± 0.3 | 8.4 |
| 4[e] | LTG | 0.53 ± 0.05 | 10.2 |

[a] All blood samples were taken 10 h after taking the previous dose, [b] epileptic patient in therapy with Tegretol® 200 mg (400 mg/day), [c] epileptic patient in therapy with Tegretol® 200 mg (200 mg/day), [d] epileptic patient in therapy with Lamogin® 100 mg (100 mg/day), [e] epileptic patient in therapy with Lamictal® 50 mg (50 mg/day).

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during five consecutive days, respectively. The results are summarized in Table 2. The precisions were calculated at four different concentration levels of LTG and OXZ according to European medicines agency recommendations [21]: the lower limit of quantification (LLOQ, the lowest calibration standard), within three times the LLOQ (low QC), around 50% of the calibra- tion curve range (medium QC) and at 75% of the upper calibration curve range (high QC). The intra- and inter-day precisions were expressed as the percent RSD. The obtained results represented favorable inter- and intra-day precisions (less than 12%) for OXZ and LTG determination in plasma samples. The accuracy (relative error) values of the method were in the range of –10.2 to +7.7%. In addition, relative recovery (RR, %) of the proposed method was calculated by the following equation [22]:

$$RR(\%) = (c_{found} - c_{real})/c_{added\ or\ spiked} \times 100. \quad (1)$$

Finlay, the USAEME-HPLC method was used for therapeutic drug monitoring of under OXZ and LTG therapy. Figure 4 shows the HPLC chromatograms of plasma samples taken from a healthy and two patients with epilepsy receiving LTG and OXZ treatment. Peak shapes in patient chromato-

**Table 4. Comparison of some figures of merit reported in literature for oxcarbazepine and Lamotrigine determination in plasma**

| Method | Drug | Linearity range, µg/mL | LOQ, µg/mL | Extraction time, min | Reference |
|---|---|---|---|---|---|
| SPME[a]-LC | OXZ | 0.05–10 | 0.2 | 30 | [23] |
| | LTG | 0.2–20 | 0.5 | | |
| SBSE[b]/HPLC-UV | OXZ | 0.08–40 | 0.08 | 50 | [6] |
| SPE-LC-DAD[d] | OXZ | 2–40 | 0.08 | 60 | [24] |
| LLE-HPLC-UV | OXZ | 0.1–5 | 0.173 | 60 | [25] |
| USAEME-HPLC-UV | OXZ | 0.25–6 | 0.13 | 0.5 | Present work |
| | LTG | 0.13–6 | 0.13 | | |

[a] SPME—solid-phase microextraction, [b] SBSE—stir bar-sorptive extraction, [c] DAD—diode array detector.

grams were similar to those obtained using spiked blank plasma and no interferences form matrix were observed. No interfering peaks appeared at the reten- tion times of the drugs. The results indicated good per- formance of the presented method for LTG and OXZ determination in plasma samples. Drug concentra- tions found in these subjects are shown in Table 3.

Table 4 shows a comparison between the character- istics of the proposed method and those of some methods described in the literature for determination of OXZ and LTG in plasma. The proposed method presents LODs comparable with those reported by other methods. In most reported techniques, the extraction step requires much time comparing to the USAEME (less than 1 min). The application of ultra- sonic radiation accelerates the mass-transfer process between two immiscible phases. Due to the large con- tact area between two phases, extraction efficiency increases in a short time. Moreover, the use of just a few microliters of organic solvent for extraction makes the proposed method environmentally friendly.

## CONCLUSIONS

In the present study, the applicability of the USAEME-HPLC-UV method was evaluated for OXZ and LTG determination in plasma samples. The obtained results demonstrate that the proposed method exhibites simplicity and high preconcentra- tion factor in a very short time and, therefore, can be successfully applied for determination OXZ and LTG in different biological samples. Application of the USAEME as an effective preconcentration microex- traction technique provided LODs and precisions acceptable for TDM of OXZ and LTG in plasma sam- ples.

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## REFERENCES

1. *Battino, D., Estienne, M., and Avanzini, G., Clin. Pharmacokinet., 1995, vol. 29, p. 341.*
2. *Gross, A.S., Br. J. Clin. Pharmacol., 1998, vol. 46, p. 95.*
3. *Farhadi, Kh., Hatami, M., and Matin, A.A., Biomed. Chromatogr., 2012, vol. 26, p. 972.*
4. *Zhu, Y., Chiang, H., Wulster-Radcliffe, M., Hilt, R., Wong, P., Kissinger, C.B., and Kissinger, P.T., J. Pharm. Biomed. Anal., 2005, vol. 38, p. 119.*
5. *Van Rooyen, G.F., Badenhorst, D., Swart, K.J., Hun- dt, H.K., Scanes, T., and Hundt, A.F., J. Chromatogr. B: Anal. Technol. Biomed. Life Sci., 2002, vol. 769, p. 1.*
6. *Cost, R.H., Queiroz, R.H.C., Bertucci, C., Mal- fará, W.R., Dreossi, S.A.C., Chaves, A.R., Valério, D.A.R., and Queiroz, M.E.C., J. Pharm. Biomed. Anal., 2008, vol. 48, p. 428.*
7. *Queiroz, M.E.C., Silva, S.M., Carvalho, D., and Lanças, F.M., J. Chromatogr. Sci., 2002, vol. 40, p. 219.*
8. *Roger, J.C., Rodgers, G., and Soo, A., Clin. Chem., 1973, vol. 19, p. 590.*
9. *Queiroz, M.E.C., Carrilho, E., Carvalho, D., and Lanças, F.M., Chromatographia, 2001, vol. 53, p. 485.*
10. *Barbosa, N.R. and Midio, A.F., J. Chromatogr. B: Biomed. Sci. Appl., 2000, vol. 741, p. 289.*
11. *Liu, H., Delgado, M., Forman, L.J., Eggers, C.M., and Montoya, J.L., J. Chromatogr. B: Biomed. Sci. Appl., 1993, vol. 616, p. 105.*
12. *Bompadre, S., Tagliabracci, A., Battino, M., and Gior- getti, R., J. Chromatogr. B: Anal. Technol. Biomed. Life Sci., 2008, vol. 863, p. 177.*
13. *Ramu B, Chittela KB. High Performance Thin Layer Chromatography and Its Role Pharmaceutical Industry [Review]. Open Sci. J. Biosci. Bioeng. 2018;5(3):29–34.*
14. *Vuckovic, D., Shirey, R., Chen, Y., Sidisky, L., Au- rand, C., Stenerson, K., and Pawliszyn, J, Anal. Chim. Acta, 2009, vol. 638, p. 175.*
15. *Mashayekhi, H.A., Abroomand-Azar, P., Saber-Teh- rani, M., and Husain, S.W., Chromatographia, 2010, vol. 71, p. 517.*
16. *B. Ramu, Chandrul KK, Pandiyan PS, Bio-Analytical Method Development of Repaglinide Drug Delivery Systems, Journal of Drug Delivery and Therapeutics. 2019; 9(6):140-142 http://dx.doi.org/10.22270/jddt.v9i6.3718*
17. *Aghamohammadi, M., Shahdousti, P., and Harooni, B., Microchem. J., 2016, vol. 124, p. 188.*
18. *Shahdousti, P., Ghamohammadi, M., Seidi, Sh., Ha- rooni, B., and Kalhor, H., J. Iran. Chem. Soc., 2015, vol. 12, p. 1757.*
19. *Aghamohammadi, M., Faraji, M., Shahdousti, P., Kalhor, H., and Saleh, A., Phytochem. Anal., 2015, vol. 26, p. 209.*
20. *Saleh, A., Yamini, Y., Faraji, M., Rezaee, M., and Ghambarian, M., J. Chromatogr. A, 2009, vol. 1216, p. 6673.*
21. *Science Medicines Health: Guideline on Bioanalytical Method Validation, European Medicines Agency, 2011. http://www.ema.europa.eu/docs/en_GB/document_ library/Scientific_guideline/2011/08/ WC500109686. Accessed January 20, 2015.*
22. *Rezaee, M., Assadi, Y., Milani Hosseini, M.R., Aghaee, E., Ahmadi, F., and Berijani, S., J. Chromatogr. A, 2006, vol. 1116, p. 1.*
23. *Queiroz, M.E.C., Silva, S.M., Carvalho, D., and Lanças, F.M., J. Sep. Sci., 2002, vol. 25, p. 91.*
24. *Bugamelli, F., Sabbioni, C., Mandrioli, R., Kenndler, E., Albani, F., and Raggi, M.A., Anal. Chim. Acta, 2002, vol. 472, p. 1.*
25. *Dordević, S., Kilibarda, V., and Stojanović, T., Vo- jnosanit. Pregl., 2009, vol. 66, p. 347.*