The validated method for the analysis of a mixture of psychotropic medicines olanzapine, clorazepate and escitalopram using a high-performance liquid chromatography method

Mamdouh Alasabashi,

Augusta Ževžikovienė*,

Mindaugas Marksa,

Laura Rimkienė,

Andrejus Ževžikovas

Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, 13 Sukilėliai Avenue, 50161 Kaunas, Lithuania

It was established that self-poisoning with antipsychotics, anti-anxiety drugs and antidepressants is caused by consumption of the drug in higher doses than prescribed. So it is very important to determine fast the materials causing poisoning and use the method which would allow one to identify several substances during only one analysis. Reference and test solutions were prepared in ethanol. A qualitative and quantitative analysis was carried out on a Waters Alliance 2695 liquid chromatograph equipped with a Waters 996 PDA detector and an ACE C18 (250 mm × 4.6 mm × 5 µm) column. The mobile phase consisted of solvent trifluoroacetic acid (0.1 %) and acetonitrile. The linear gradient elution profile was used. For the validation process the following parameters were chosen: specificity, repeatability, precision and limits (LOD and LOQ). According to the study results, it can be concluded that the validated method for the qualitative and quantitative evaluation analysis of olanzapine, clorazepate and escitalopram was developed.

Keywords: olanzapine, clorazepate, escitalopram, high-performance liquid chromatography, qualitative and quantitative determination

INTRODUCTION

According to the State Medicines Control Agency of Lithuania, the last four-year consumption of 2017, 2018, 2019 and 2020 showed that the general usage of antipsychotic drugs had a tendency of augmentation by DDD/1,000/day. According to the data, Lithuania (LT) had the highest consumption of antipsychotics among the three countries [1]. In LT, the olanzapine consumption (DDD 10 mg) increased during the years 2016–2020 as follows: 2.674 (2016), 2.942 (2017), 3.301 (2018), 3.331 (2019) and 3.646 (2020) [1, 2]. The LT consumption of clorazepate (DDD 20 mg) decreased in 2016–2020 as follows: 0.704 (2016), 0.667 (2017), 0.600 (2018), 0.572 (2019) and 0.529 (2020) [1, 2]. LT has the highest consumption of escitalopram in comparison to Estonia and Latvia. The consumption of escitalopram (DDD 10 mg) still increased during the years 2016–2017 as follows: 7.916 (2016) and 9.670 (2017); it decreased to 8.616 (2018) and increased again: 9.586 (2019) and 9.958 (2020) [1, 2].

Antipsychotics, anti-anxiety drugs and antidepressants are the classes, which are commonly prescribed. They have shown self-poisoning due

* Corresponding author. Email: augusta.zevzikoviene@lsmuni.lt

to an inappropriately high drug dosage. Poisoning of antipsychotics, anti-anxiety drugs and antidepressants, particularly olanzapine, clorazepate and escitalopram, is usually caused by consumption of the drug in higher doses than prescribed; however, it is very unusual to cause death, unless the medicine is used simultaneously with alcohol or other drugs (barbiturates, opioids or tricyclic antidepressant). It is very rare to result in death after hospital admission [3–8].

In every case, it is important to determine fast the materials causing poisoning, for which selective and effective methods of analysis are requested. To make the toxicological laboratory work more efficient and practical, methods which would allow one to identify several substances during only one analysis are very important. The analysis of the literature data showed that olanzapine, clorazepate and escitalopram were analysed as separate compounds, but not as components of the mixture. So, it was decided to use the literature data as a base and to develop the HPLC methods, which would be suitable for the analysis of all three substances and their mixture.

The aim of the experiment was to optimise HPLC methodics, suitable for the qualitative and quantitative evaluation of olanzapine, clorazepate and escitalopram.

EXPERIMENTAL

Solvents and reagents
HPLC-grade and analytical-grade reagents were used: trifluoroacetic acid, acetonitrile (Sigma Aldrich, Steinheim, Germany); standards of olanzapine, clorazepate and escitalopram (Sigma Aldrich, Steinheim, Germany); ethanol (96%) (Vilniaus Degtinė, Vilnius, Lithuania); tested medicines Olanzapine Actavis 5 mg tablets (Actavis Group PTC ehf.); Tranxene 5 mg hard capsules (Sanofi-Aventis Lietuva); Cipralex 20 mg film-coated tablets (H. Lundbeck A/S). Water used for the sample preparation was produced using a Super Purity Water System (Millipore, USA).

Reference and test solutions
Reference solutions of the tested substances were prepared by dissolution of the standard in ethanol: olanzapine RSO, clorazepate RSC and escitalopram RSE. A reference mixture solution was prepared

consequently (RST OCE) by mixing 1 ml of each standard solution to be prepared.

Test solutions for the analysis were prepared from the medicinal products obtained at pharmacy stores in Lithuania. The test solutions of a concentration of 0.1 mg/ml were prepared, respectively: olanzapine TSO, clorazepate (TSC) and escitalopram (TSE). The tablets were crushed and grinded well in a mortar. The capsule content was used directly without grinding. Powders obtained were transferred to a volumetric flask and dissoluted with ethanol. Further the obtained suspensions were mixed well for 10 min with the help of an ultrasound bath. The obtained precipitate was separated by a centrifuge. The solutions were filtrated using a PVDF filter with a pore size of 0.45 mm. The resulting solutions were transferred to cork closed flasks and used for upcoming investigations. A mixture solution was prepared consequently (TS OCE) by using 1 ml of each solution of the test substances.

HPLC-PDA CONDITIONS

The qualitative and quantitative analysis was carried out on a Waters Alliance 2695 liquid chromatograph equipped with a Waters 996 photodiode array detector (PDA) and an ACE C18 (250 mm × 4.6 mm × 5 μm) column (Advanced Cromatography Technologies, Aberdeen, Scotland). The mobile phase consisted of solvent A (trifluoroacetic acid (0.1%)) and B (acetonitrile). The linear gradient elution profile was as follows: 98% A/2% B at 0 min, 98% A/2% B at 1 min, 2% A/98% B at 20 min, 2% A/98% B at 23 min and 98% A/2% B at 24 min. The flow rate was 1 mL/min and the injection volume was 10 μL. The absorption was measured in the range from 200 to 400 nm. The detection was performed using the standards of tested substances.

Validation
For the validation process the following parameters were chosen: specificity, repeatability, precision and limits (LOD and LOQ).

RESULTS AND DISCUSSION

HPLC techniques are applied for the separation, qualitative and quantitative analysis of many

compounds and mixtures, including benzodiazepines and SSRI [9–11]. In the European Pharmacopoeia (Eur. Ph. 9.0 and 9.7), the HPLC methods for the qualitative determination of all three investigated medicines and the methods for the quantitative analysis of olanzapine have been found [12–14]. Clark's analysis [15] describes different methods for the HPLC analysis of olanzapine and clorazepate, but there are no much information about the analysis of escitalopram. In UNODC (United Nations Office on Drugs and Crime), all three substances were identified, but not quantificated [16]. In other publications, data about the analysis of separate compounds was found [17–21]. Moreover, retention times in these systems are very close, so the analysis of the mixture using described methods is not possible. Because of the listed reasons, it was necessary to obtain the methods, which can be applied for all three substances.

Firstly, substances should be separated and for this several different chromatographical columns, solvent system, gradients and flow rates were tested. After the experiment with Sunfire C_{18} (150 mm × 3 mm × 3.5 μm), Supelco LC_{18} (150 mm × 4.6 mm × 5 μm) and ACE C_{18} (250 mm × 4.6 mm × 5 μm), the best separation was achieved using the ACE C_{18} chromatography column (250 mm × 4.6 mm × 5 μm). The solvent system was evaluated by the retention time of the analytes, the symmetry of the peaks and baseline stability. The separation of olanzapine, clorazepate and escitalopram mixture was achieved by elution with a solvent system consist-

ing of 0.1% trifluoroacetic acid (TFA) aqueous solution (A) and acetonitrile (ACN) (B). The examination of the mixture separation was performed when the mobile phase flow rate was from 0.1 to 1 ml/min. The optimal separation was observed when the flow rate of the eluent was 1.0 ml/min. The duration of general chromatographic analysis was 24 min.

Secondly, the analysis method for qualification and quantification should be validated. This was done according to the specificity, precision, linearity, the limit of detections and the limit of determination.

The analyte specificity is demonstrated by comparison of the standard and analyte retention time and spectral overlaps (Figs. 1, 2). The retention times for the analytes were the following: olanzapine – 9.29 min, clorazepate – 12.17 min and escitalopram – 13.4 min. The validation data are presented in the Table.

Once the optimum conditions were set at which olanzapine, clorazepate and escitalopram can be identified in the mixture, the methodology was applied in the test solutions TS O, TS C and TS E prepared from the medicinal products obtained at pharmacy stores located in Lithuania. The examination of medicinal products was identified by the following criteria: retention time and UV-light absorption spectrum. For all medicinal products retention times were close to that of the reference solution and UV-absorption spectra were identical to those of the literature data.

In order to assess the suitability of selected HPLC methods for the quantitative determination
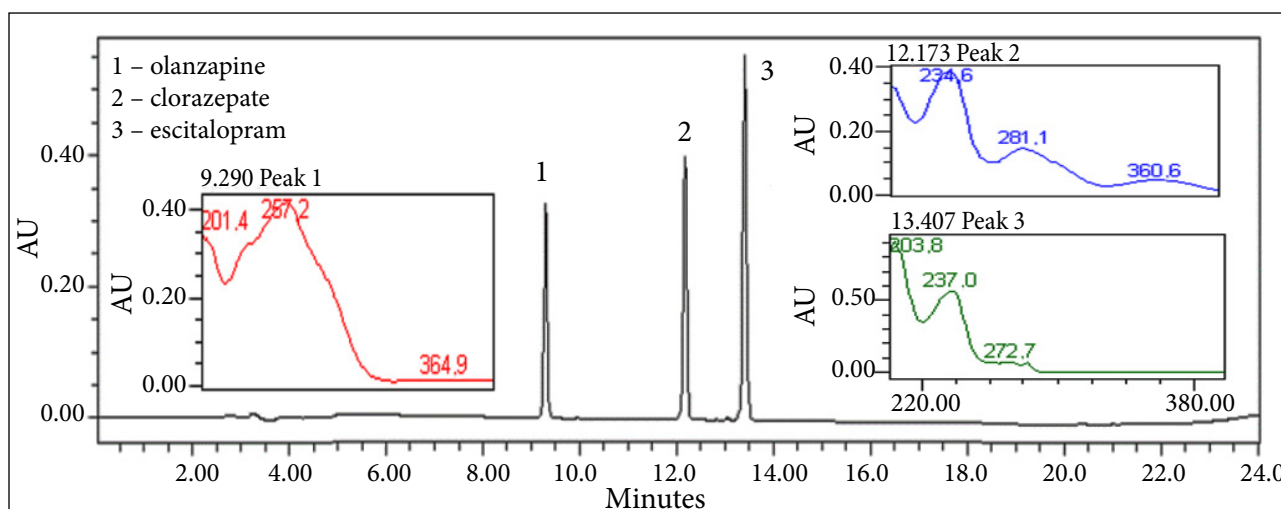

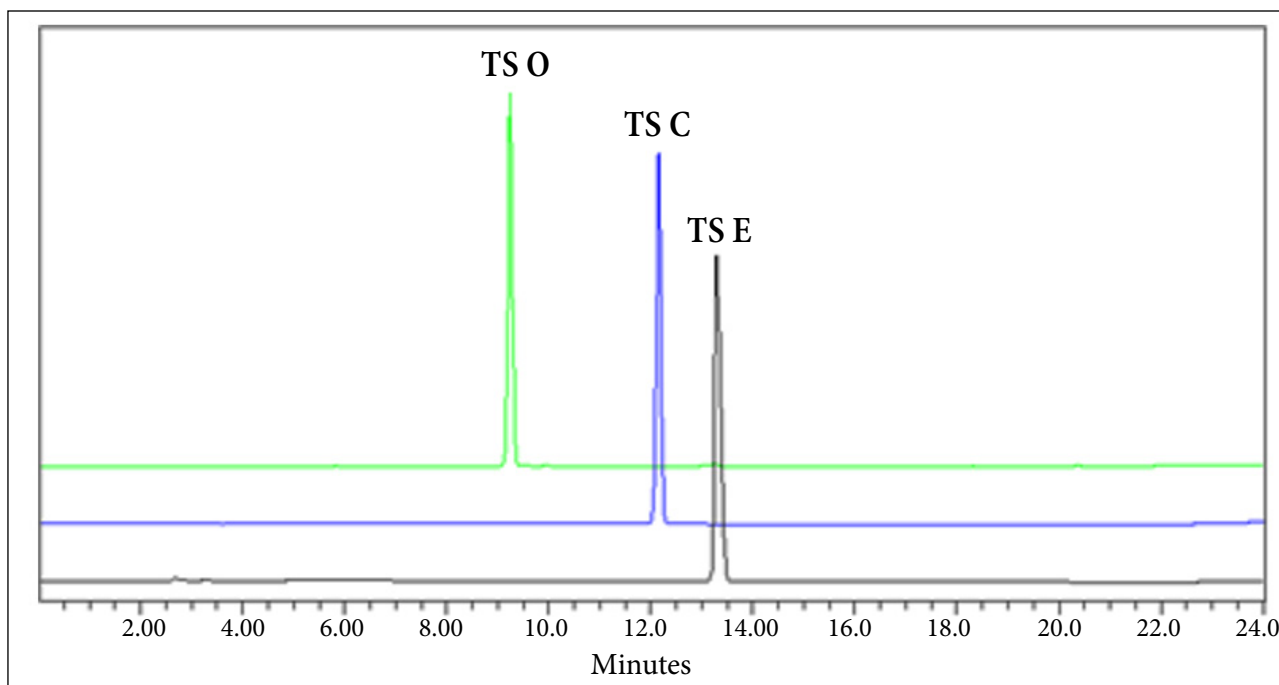**Fig. 1.** A chromatogram of the mixture of reference solutions

Fig. 2. A chromatogram of the mixture of tested solutions

T a b l e . **Validation data**

Tested solution	t_R, min	Intraday RSD, %		Interday RSD, %		R^2	Calibration curve equation	LOD, µg/ml	LOQ, µg/ml
		a	b	a	b				
Olanzapine	9.29	0.6	0.12	0.7	0.16	0.999917	$f(x) = 3.66 \cdot 10^4 \times + 7.34 \cdot 10^3$	0.195	0.75
Clorazepate	12.17	0.9	0.06	0.9	0.08	0.999890	$f(x) = 2.95 \cdot 10^4 \times + 8.64 \cdot 10^3$	0.39	1.46
Escitalopram	13.40	0.7	0.04	0.8	0.04	0.999923	$f(x) = 4.15 \cdot 10^4 \times + 1.15 \cdot 10^4$	0.39	1.49

Note: t_R, retention time; RSD, relative standard deviation by (a) peak area and (b) retention time; R^2, correlation coefficient; LOD, limit of detection; LOQ, limit of quantification.

of test materials calibration curves of light absorption peak height dependence on substance concentration were prepared. The higher the tonnage the higher the peak. The prepared standard solutions were prepared at different concentrations to create calibration curve solutions by diluting standards in halves. The calibration curve for clorazepate was made from 6 points and the one for olanzapine and escitalopram from 7 points. The linearity limits (mcg/ml) were the following: for olanzapine – 50–0.78, for clorazepate – 50–1.56 and for escitalopram – 100–1.56.

With reference to the study results, it can be concluded that the validated method for the qualitative and quantitative evaluation analysis of olanzapine, clorazepate and escitalopram was developed.

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Mamdouh Alasabashi, Augusta Ževžikovienė, Mindaugas Marksa, Laura Rimkienė, Andrejus Ževžikovas

PSICHOTROPINIŲ VAISTŲ MIŠINIO, SUDARYTO IŠ OLANZAPINO, KLORAZEPATO IR ESCITALOPRAMO, ANALIZĖ VALIDUOTU EFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODU

S a n t r a u k a

Nustatyta, kad vartojant didesnes nei paskirta antipsichotikų, vaistų nuo nerimo ir antidepresantų dozes apsinuodijama, todėl labai svarbu kuo greičiau nustatyti medžiagas, sukėlusias apsinuodijimą ir naudoti metodą, leidžiantį identifikuoti kelias medžiagas vienos analizės metu. Analizei ruošti etanoliniai etaloniniai ir tiriamieji tirpalai. Kokybinė ir kiekybinė analizė atlikta „Waters Alliance 2695" skysčių chromatografu su „Waters 996 PDA" detektoriumi ir „ACE C18" (250 mm × 4,6 mm × 5 µm) kolonėle. Mobiliąją fazę sudarė trifluoracto rūgštis (0,1 %) ir acetonitrilas, naudotas linijinis gradientinis eliuavimas. Validacijai pasirinkta įvertinti specifiškumą, pakartojamumą, tikslumą, ribas (LOD ir LOQ). Remiantis tyrimo rezultatais, galima daryti išvadą, kad buvo sukurtas validuotas olanzapino, klorazepato ir escitalopramo kokybinio ir kiekybinio įvertinimo metodas.