UDC 615.065:54.061/.062:547.712.22:001.8

ANALYTICAL DIAGNOSING OF MILNACIPRAN POISONINGS

S.V.Baiurka, S.A.Karpushyna, V.P.Moroz

National University of Pharmacy

Key words: chemico-toxicological analysis; milnacipran; isolation from the biological material; TLC-screening; high pressure liquid chromatography

The method of milnacipran isolation with chloroform from the dehydrated biological material with subsequent extraction purification in the n-hexane-acetonitrile solvent system has been developed. The method developed has allowed to isolate 47±5% of the antidepressant. The TLC-screening method of a number of antidepressants has been developed using four mobile phases with a low correlation and the sequential scheme of visualization by a set of chromogenic reagents. It has allowed to separate milnacipran, venlafaxine, amitriptyline, fluoxetine and sertraline. As differentiating reagents the Liebermann's reagent and the Mandelin's reagent in modification consisting in sequential treatment of the sample by the Mandelin's reagent and formaldehyde vapours have been suggested. The methods of identification and quantitative determination of milnacipran in the biological material after the TLC purification using HPLC with multiwave UV-spectrophotometric detection have been developed. The calibration curve of the dependence of the peak area on the concentration was described by the following equation: $Y=5.14 \cdot 10^{-5}X$; linearity was within the concentration range of 24.2-500 μg/ml; LOD and LOQ were 8.0 and 24.2 μg/ml (at 262 nm), respectively. The results obtained can be used in forensic toxicology for diagnosing milnacipran poisonings.

Milnacipran – (1R,2S)-rel-2-(Aminomethyl)-N,N-diethyl-1-phenylcyclopropanecarboxamide is a novel third-generation thymoleptic. Its pharmacological effect is due to the dual action, it is a selective serotonin and norepinephrine reuptake inhibitor (SNRI). Milnacipran is used for the treatment of moderate and severe endogenous depression [3]. Fatal intoxications associated with milnacipran overdoses and co-administration of fluoxetine and sertraline in therapeutic doses [8], as well as ethanol [10] have been reported, in these cases peripheral blood concentrations of milnacipran are 21.5 mg/l and 3.15 μg/ml, respectively.

Bioanalytical methods for analysis of milnacipran in the blood and plasma using high pressure liquid chromatography with UV spectrophotometric [7], diode array [12] and MS- [5] detection have been developed. Methods of analysing the biological material for the presence of milnacipran have not been developed. The general isolation methods appeared to be ineffective for a number of antidepressants, in particular for amitriptyline, fluoxetine and sertraline [1, 2], due to the lipophilic properties of these substances [6]. In this regard, the practical interest is the study of the efficiency of milnacipran isolation (Vd=5.5 l/kg [6]) from the biological material with chloroform as a lipophilic solvent with subsequent extraction purification in the *n*-hexane-acetonitrile solvent system [4]. Therefore, the aim of this study was to determine the optimal conditions for milnacipran isolation from the biological material, develop the TLC-screening scheme for the group of antidepressants, which can be co-administrated with milnacipran, as well as the methods of identification and quantitative determination for milnacipran in the biological material by HPLC with multiwave UV-spectrophotometric detection.

Materials and Methods

The method of milnacipran isolation with chloroform with subsequent extraction purification in the n-hexane-acetonitrile solvent system. Add 1 ml of the aqueous solution containing 574 mg of milnacipran hydrochloride (corresponding to 500 mg of the milnacipran base) to the powdered biological sample (5 g of the liver tissue) and allow to stand for 24 h. Simultaneously perform the blank experiment.

Isolation of milnacipran from the liver with chloroform was carried by the method given in the work [7].

The method of Thin Layer Chromatography. Two types of chromatographic plates – Merk (Silica gel 60 F254, 10x20 cm in size) and Sorbfil (PTLC-P-A) (10x10 cm in size) were used for thin-layer chromatographic studies.

Evaporate 10-30 ml aliquots of the final chloroform extract obtained from the tissue spiked with milnacipran and extracts from the blank tissue (drug-free) to the minimum volume (~ 0.05 ml) and spot as a band onto the start line. Next to it spot 10 ml of the standard solution of milnacipran in methanol (1 mg/ml). At first develop the chromatograms in chloroform to separate the drug from endogenous impurities, then use four mobile phases (listed below).

Thin-layer chromatographic studies were performed as described in the work [1, 2, 4]. The antidepressant was detected on the plate with the chromogenic reagents pre-

S.V.Baiurka – Candidate of Pharmacy, associate professor of the Department of Toxicological Chemistry of the National University of Pharmacy (Kharkiv)

Table 1

The results of antidepressant visualization in TLC-screening

Antidepressant	Reagent (colour and sensitivity, μg per a sample)				
	Dragendorff's reagent by Munier	acidified iodoplatinate	Liebermann's reagent	Mandelin's reagent	Mandelin's reagent + formaldehyde
Milnacipran	orange (5.0)	blue (1.0)	yellow → orange (5.0)	–	–
Venlafaxine	orange (3.0)	blue-violet (2.0)	brown → cherry → red (5.0)	brown → cherry → blue → green (4.0)	light blue (5.0)
Amitriptyline	orange (2.0)	blue-violet (0.2)	yellowish-brown (3.0)	brown (0.5)	brown (0.5)
Fluoxetine	orange (1.0)	blue-violet (0.5)	brown (3.0)	blue (3.0)	green (3.0)
Sertraline	orange (1.0)	violet (1.0)	violet → brown → discoloration (2.0)	–	–

Table 2

Chromogenic reagents for the final stage of the antidepressant visualization in TLC-screening

Antidepressant	Reagent (colour and sensitivity, μg per a sample)		
Milnacipran	ninhydrin (*pink-violet*, 4.0)	mercury sulphate saturated solution (*blue*, 6.0)	Van Urk's (*yellow*, 3.0)
Venlafaxine	Froehde (*greenish → violet (on the edge)*, 8.0)		
Amitriptyline	concentrated sulphuric acid (*orange*, 0.2)	Froehde (*brick-red → green*, 5.0)	
Fluoxetine	ninhydrin (*orange*, 5.0)	Froehde (*blue*, 4.0)	
Sertraline	potassium permanganate solution (*blue-violet*, 5.0)	Van Urk's (*yellow*, 5.0)	Froehde (*brownish-green*, 5.0)

sented in Tables 1, 2. Elute milnacipran from the chromatogram band untreated by the location reagents with methanol. Evaporate the eluate, and reconstitute the residue 1 ml of methanol.

The method of HPLC. The HPLC study was performed using a "MiLiChrome A-02" microcolumn high pressure liquid chromatograph with a multiwave UV-spectrophometric detector by the method presented in the work [4]. The injected volume was 10 μl. Quantitative determination of milnacipran in eluates from chromatograms was carried out at the wavelength of 262 nm.

Results and Discussion

According to the epidemiological studies [9] the most poisonings with novel antidepressants are combined. The scheme of milnacipran detection by thin layer chromatography screening method in the presence of a number of antidepressants from different groups such as venlafaxine (SNRI), amitriptyline (TCA), sertraline and fluoxetine (SSRI) has been developed.

The simultaneous use of four mobile phases (MP) with a high distributive power in relation to drugs under study and the lowest correlation between them was proposed. They are ethyl acetate – methanol – 25% ammonium hydroxide solution (85:10:5) (MP 1), methanol – 25% ammonium hydroxide solution (100:1.5) (MP 2), cyclohexane – toluene – diethylamine (75:15:10) (MP 3), and toluene – acetone – ethanol – 25% ammonium hydroxide solution (45:45:7.5:2.5) (MP 4). The Rf values of antidepressants in the mobile phases selected on two types of the chromatographic plates (Merk and Sorbfil) are shown in Table 3. The Dragendorff's reagent modified by Munier and acidified iodoplatinate solution were the most sensitive common reagents for detection of the substances studied. As differentiating reagents the Liebermann's reagent and the Mandelin's reagent in modification consisting in sequential treatment of the sample by the Mandelin's reagent and formaldehyde vapours were suggested (Table 1). Using a particular set of additional chromogenic reagents (Table 2) it was proposed to use four reagents, and it was sufficient for reliable identification of toxic substances according to the TIAFT recommendations [7] for the final stage of visualization. The degree of milnacipran elution with methanol from

Table 3

The Rf values of antidepressants in TLC-screening systems

Antidepressant	Mobile phase (N_o)							
	1		2		3		4	
	Merk	Sorbfil	Merk	Sorbfil	Merk	Sorbfil	Merk	Sorbfil
Milnacipran	0.31	0.53	0.28	0.37	0.05	0.09	0.42	0.47
Venlafaxine	0.84	0.90	0.65	0.66	0.57	0.70	0.71	0.92
Amitriptyline	0.91	0.92	0.51	0.61	0.88	0.85	0.67	0.92
Fluoxetine	0.90	0.95	0.78	0.72	0.30	0.28	0.40	0.92
Sertraline	0.54	0.90	0.67	0.68	0.64	0.88	0.75	0.71

Table 4

The results of HPLC determination of milnacipran isolated from the liver with chloroform followed by purification in the *n*-hexane-acetonitrile system

Amount of milnacipran added to 5 g of the liver, μg	Amount of milnacipran extracted ($\bar{X}$, %)	Metrological characteristics			
		S	$S_{\bar{x}}$	$\Delta\bar{X}$ (P=0.95%, ν=4)	ε, %
500	47	3.8	1.7	5	10

the chromatographic plates was 97.8±1.0%.

Identification of milnacipran in the eluates by HPLC was carried out by the retention time and the absorbance ratios ($R=S_\lambda/S_{210}$), they were 17.00±0.06 min (n=5, RSD=0.15%, ε=0.37%) and 0.811±±0.007; 0.291±0.004; 0.030±0.002; 0.012±0.002; 0.014±0.002; 0.0021±±0.0002; 0.0014±0.0003, respectively. Quantitative determination was performed using the calibration curve of the dependence of the peak area on the concentration at the wavelength of 262 nm. After verifying the significance of the intercept in the linear regression equation it was concluded about the possibility of transition to the equation in the form of: $Y=5.14\cdot10^{-5}X$ (Table 4). The method showed linearity in the range of 24.2-500 mg/mL. The LOD and LOQ values were calculated based on the parameters of the calibration curve; they were 8.0 μg/ml and 24.2 μg/ml, respectively. Accuracy and precision of the method developed were 101.8 % (RSD= =1.4%) at the low concentration level, 100.8% and 100.5% (RSD= =1.0%) at the middle and high concentration levels, respectively. Therefore, they satisfy the requirements for the methods used in forensic toxicology [11].

The method of milnacipran isolation with chloroform was quite efficient and allowed isolating 47±5% of the antidepressant studied (Table 4). The results obtained can be used in forensic toxicology for diagnosing milnacipran poisonings.

CONCLUSIONS

1. The method of milnacipran isolation with chloroform from the biological material dehydrated by triturating with anhydrous sodium sulphate with subsequent extraction purification in the *n*-hexane-acetonitrile solvent system has been developed. The method developed has allowed to isolate 47±5% of the medicine.

2. The TLC-screening method of a number of antidepressants has been developed using four mobile phases with low correlation and the sequential scheme of visualization by chromogenic reagents. It has allowed to separate milnacipran, venlafaxine, amitriptyline, fluoxetine and sertraline.

3. The sensitive and specific methods of identification and quantitative determination of milnacipran in the biological material by HPLC with multiwave UV-spectrophotometric detection have been developed.

REFERENCES

1. *Баюрка С.В., Бондар В.С., Карпушина С.А. // Вісник фармації. – 2009. – №3 (59). – С. 23-26.*
2. *Баюрка С.В., Карпушина С.А., Мороз В. П. // Фармация. – 2015. – №2. – С. 7-9.*
3. *Фурсов Б.Б. // Психические расстройства в общей медицине. – 2009. – №2. – С. 63-65.*
4. *Baiurka S., Karpushina S. // J. Chem. Pharm. Res. – 2013. – Vol. 5 (12). – P. 1110-1120.*
5. *Castaing N., Titier K., Receveur-Daurel M. et al. // J. Anal. Toxicol. – 2007. – Vol. 31 (6). – P. 334-341.*

6. *Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material: 4-th ed. / A.C.Moffat, M.D.Osselton, B.Widdop et al. – London, Chicago: Pharmaceutical Press, 2011. – 2736 p.*
7. *Dallet P., Labat L., Richard M. et al. // J. Liq. Chromatogr. Relat. Technol. – 2002. – Vol. 25 (1). – P. 101-111.*
8. *Fanton L., Bévalot F., Grait H. et al. // J. Forensic Leg. Med. – 2008. – Vol. 15 (6). – P. 388-390.*
9. *Rop P.P., Sournac M.H., Burle J. et al. // J. Anal. Toxicol. – 2002. – Vol. 26 (2). – P. 123-126.*
10. *Flanagan R.J. // Hum. Psychopharmacol. – 2008. – Vol. 23 (Suppl. 1). – P. 43-51.*
11. *SOFT / AAFS Forensic Laboratory Guidelines. – 2006. – 1-24 p.*
12. *Titier K., Castaing N., Scotto-Gomez E. et al. // Ther. Drug Monit. – 2003. – Vol. 25 (5). – P. 581-587.*

АНАЛІТИЧНА ДІАГНОСТИКА ОТРУЄНЬ МІЛНАЦИПРАНОМ

С.В.Баюрка, С.А.Карпушина, В.П.Мороз

Національний фармацевтичний університет

Ключові слова: хіміко-токсикологічний аналіз; мілнаципран; ізолювання з біологічного матеріалу; ТШХ-скринінг; високоефективна рідинна хроматографія

Розроблено методику ізолювання мілнаципрану хлороформом із зневодненого біологічного матеріалу з наступною екстракційною очисткою за допомогою системи розчинників н-гексан – ацетонітрил, яка дозволила виділити 47±5% антидепресанта. Розроблено методику ТШХ-скринінгу ряду антидепресантів з використанням чотирьох рухомих фаз з низькою кореляцією та послідовною схемою візуалізації за допомогою набору хромогенних реактивів, яка дозволяла розділити мілнаципран, венлафаксин, амітриптилін, флуоксетин та сертралін. Як диференціюючі реагенти нами запропоновано використання реактиву Лібермана та реактиву Манделіна у модифікації, яка полягала у послідовній обробці проби реактивом Манделіна та парою формальдегіду. Розроблені методики ідентифікації та кількісного визначення мілнаципрану в біологічному матеріалі після ТШХ-очистки методом ВЕРХ з мультихвильовим УФ-спектрофотометричним детектуванням. Калібрувальний графік залежності площі піку від концентрації описувався рівнянням: $Y=5,14\cdot10^{-5}X$; діапазон лінійності – 24,2-500 мкг/мл; LOD та LOQ становили, відповідно, 8,0 та 24,2 мкг/мл (при 262 нм). Отримані результати можуть бути використані в судовій токсикології для аналітичної діагностики отруєнь мілнаципраном.

АНАЛИТИЧЕСКАЯ ДИАГНОСТИКА ОТРАВЛЕНИЙ МИЛНАЦИПРАНОМ

С.В.Баюрка, С.А.Карпушина, В.П.Мороз

Национальный фармацевтический университет

Ключевые слова: химико-токсикологический анализ; милнаципран; изолирование из биологического материала; ТСХ-скрининг; высокоэффективная жидкостная хроматография

Разработана методика изолирования милнаципрана хлороформом из обезвоженного биологического материала с последующей экстракционной очисткой с помощью системы растворителей н-гексан-ацетонитрил, которая позволила выделить 47±5% антидепрессанта. Разработана методика ТСХ-скрининга ряда антидепрессантов с использованием четырех низкокоррелирующих подвижных фаз и последовательной схемы визуализации с помощью набора хромогенных реактивов, которая позволила разделить милнаципран, венлафаксин, амитриптилин, флуоксетин и сертралин. В качестве дифференцирующих реагентов нами предложено использование реактива Либермана и реактива Манделина в модификации, которая заключалась в последовательной обработке пробы реактивом Манделина и парами формальдегида. Разработаны методики идентификации и количественного определения милнаципрана в биологическом материале после ТСХ-очистки методом ВЭЖХ с мультиволновым УФ-спектрофотометрическим детектированием. Калибровочный график зависимости площади пика от концентрации описывался уравнением $Y=5,14\cdot10^{-5}X$; диапазон линейности – 24,2-500 мкг/мл; LOD и LOQ составляли, соответственно, 8,0 и 24,2 мкг/мл (при 262 нм). Полученные результаты могут быть использованы в судебной токсикологии для аналитической диагностики отравлений милнаципраном.

Address for correspondence:
4, Valentynivska str., Kharkiv, 61168, Ukraine.
Tel. (572) 67-91-92. E-mail: svitkrp@gmail.com.
National University of Pharmacy

Received in 04.03.2016