ФАРМАЦИЯ
PHARMACIA

Том/Volume LVIII **2011** **Книжка/Number 1-4**

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С Ъ Д Ъ Р Ж А Н И Е

Оригинални статии

C O N T E N T S

Original articles

ФАРМАЦИЯ 1-4/2011

ISSN 0428-0296 УДК 615

Организационен секретар *Св. Цветанова*
Стилова редакция *Св. Цветанова и д-р Б. Станчева (на англ. ез.)*
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Форматиране *О. Маркова*

Подписана за печат на 09.01.2011 г.
Печатни коли 15, формат 60 x 90/8

Централна медицинска библиотека
1431 София, ул. „Св. Г. Софийски" № 1, тел. 952-16-45, Fax: 851 82 65
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HPLC STUDY OF BINARY AND TRIPLE MIXTURES CONTAINING VENLAFAXINE, CITALOPRAM AND SIBUTRAMINE

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Summary. Analytical study for quality control of antidepressants from SSRIs and SNRIs groups was performed using HPLC methods. The obtained data from analysis of venlafaxine HCl, citalopram and sibutramine were compared on the base of ICH and European Pharmacopoeia criteria and on the developing the chromatographic and analytical parameters. The substances were examined as pure substances, model binary and triple mixtures and in drug formulations. The method was developed for the purposes of the analytical and the toxicological practices. The HPLC method was made in the following conditions: isocratic and gradient mode of Acetonitrile/Methanol in different ratios, UV – detection at 230 nm analytical wavelength, different flow rates and solutions, prepared at varied pH.

Key words: Venlafaxine, Citalopram, Sibutramine, Validation, System suitability test, HPLC

ИЗСЛЕДВАНЕ С ВИСОКОЕФЕКТИВНА ТЕЧНА ХРОМАТОГРАФИЯ НА ДВОЙНИ И ТРОЙНИ СМЕСИ, СЪДЪРЖАЩИ VENLAFAXINE, CITALOPRAM, SIBUTRAMINE

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Резюме. Бе извършено аналитично проучване за контрол на качеството на лекарства антидепресанти от групите SSRIs и SNRIs с помощта на високоефективна течна хроматография. Получените данни от анализа и резултатите от хроматографските и аналитичните параметри за Venlafaxine HCl, Citalopram и Sibutramine бяха сравнени на базата на изисквания и критерии на ICH и Европейската фармакопея. Веществата са изследвани като чисти субстанции, двойни и тройни модели смеси, както и смеси от хранителни добавки. Методите са разработени за целите на аналитичната и токсикологичната практика. Процедурата съдържа следните хроматографски условия: изократичен и линеен градиентен режим, подвижни фази ацетонитрил/метанол в различни съотношения, дължина на вълната 230 nm, различни скорости на потока и различно pH на разтворите.

Key words: Venlafaxine, Citalopram, Sibutramine, Validation, System suitability test, HPLC

Introduction

The pharmaceutical companies could not benefit by the pharmaceuticals' patent protection during the whole period of patent validity and aim to increase the patent life cycle of their products [18, 28].

Studies of the patent protection of antidepressants are relatively limited and focus on the juridical case for patent violation, information about the status of the active patents and territory of protection [5], barriers in front the generic entrance on the market [5, 6] requirements and practice of patent maintaining [26, 24], influence of the patent protection on the market as well as on the legislation [8]. Serious concerns have been raised that the monopoly higher prices of patented products could negatively impact the access to medicines and thus the mental public health [12]. Manufacturers working on the performulation of the active substances invest more in promotion and thus additionally hamper the access [16].

Citalopram is an antidepressant drug of the selective serotonin reuptake inhibitor (SSRI) class.

Its chemical name is ((RS) – 1 - [3-(dimethylamino) propyl] – 1 - (4 – fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) (Fig. 1) and it is defined as hydrobromide salt.

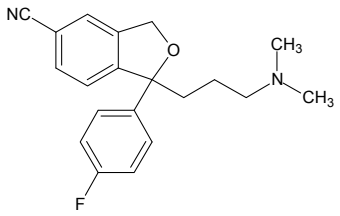

Fig. 1. *Structure of citalopram*

Venlafaxine is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class [1, 7, 23]. Its chemical name is (RS)-1-[2-dimethylamino-1-(4-methoxyphenyl)-ethyl]cyclohexanol (Fig. 2).

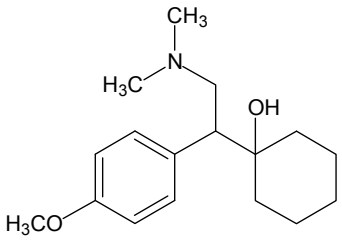

Fig. 2. *Structure of venlafaxine*

Sibutramine is a neurotransmitter reuptake inhibitor that reduces the reuptake of serotonin (by 53%), norepinephrine (by 54%), and dopamine (by 16%), thereby increases the levels of these substances in synaptic clefts and helps enhance satiety; the serotonergic action, in particular, is thought to influence appetite. Its chemical name is (dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-N,N,3-trimethylbutan-1-amine), usually available as sibutramine hydrochloride monohydrate and it is structurally related to amphetamines [21, 30]. Its mechanism of action is distinct [3, 13]. The product is manufactured also under brand names Reductil, Meridia and Sibutrex [4, 15]. Recently, the usage of Sibutramine is under suspension by European regulations because of an intensive increasing of unlegislated distribution as supplement with unknown contamination (Fig. 3).

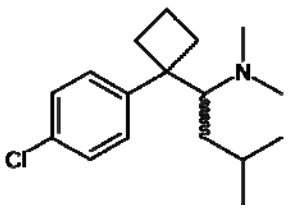

Fig. 3*. Structure of sibutramine*

There are several methods of analysis of venlafaxine, citalopram and sibutramine for each substance individually, but there is no method for identification and for quantitation of the mixture of the above substances [2, 3, 9, 11, 17, 19, 20, 25, 27, 29, 31]. The favorites are HPLC methods with different detectors because of higher performance and sensitivity than other methods used in pharmaceutical practice. On the other hand, the combination with other drugs, nutrition and supplements may cause changing of action because of production of associated complexes and salts. It is important to follow the stability and influence of same solutions and substances over its contamination in model mixtures and drug preparations as well as their derivatives and related substances [7, 32, 33].

The purpose of this study is the investigation of analytical parameters at different chromatographic conditions and development of a procedure containing HPLC methods for quality control of citalopram, venlafaxine and sibutramine – pure substances, in drug preparations and in model binary and triple mixtures.

Experimental

Chromatographic system:

The chromatographic procedure was carried out using:

Liquid chromatograph Shimadzu LC – 10 Advp equipped with 4.6 x 150 mm column RP-18, ODS with particle size 5 μm; Detector SPD 10 AVvp – UV-VIS with fixed analytical wave lengths.

Chromatographic conditions:

Isocratic mobile phases, prepared by mixing of filtered and degassed acetonitrile:water 55:45 and 70:30 v/v respectively;

- 230 nm analytical wavelengths;
- column temperature 25°C;
- flow rates about 1.0 and 2.0 ml/min.

Reagents

Acetonitril HPLC grade, distilled water R (Reagents (R), European Pharmacopoeia 7.0), Methanol HPLC grade, reference substances venlafaxine HCl, citalopram and sibutramine (CRS).

Test preparation

– Test solutions were prepared by dissolving and mixing adequate and equal amounts of substances in mobile phase to obtain solutions with concentration in ratio 9 μg – 2 mg/ml.

– Reference solutions were prepared by the same manner from CRS.

Results and discussion

HPLC methods for binary mixtures containing venlafaxine HCl and citalopram and triple mixtures containing venlafaxine HCl, citalopram and sibutramine were developed and validated. Analytical parameters specificity, repeatability, limit of detection, limit of quantitation and linearity were studied and determined in accordance with ICH criteria for validated procedures.

Validation of HPLC method for quantitative simultaneously determination of venlafaxine HCl and citalopram

1. Specificity

Specificity in respect of reagents – "Placebo" solution containing all reagents without active substances was prepared. There were no peaks in the chromatogram obtained from this solution with Rt of venlafaxine HCl and Rt of citalopram.

2. Repeatability

Six (6) equal solutions from homogenous samples containing venlafaxine HCl and citalopram were analysed by HPLC method. Standard deviation (SD) and relative SD (RSD) were found. The results are presented on Table 1.

3. Limit of detection:

9 μg for citalopram and 10 μg for venlafaxine HCl, established on the base of ratio noise–signal – 1:3.

4. Limit of quantitation:

15 μg for citalopram and 40 μg for venlafaxine HCl , established on the base of ratio noise–signal – 1:10.

5. Linearity:

The analytical parameter linearity was studied in concentration ratio 9 μg – 2 mg. The accordance between the Area of peaks, measured in absorption units (AU) and concentrations in g/ml is proportional in the intervals. The correlation coefficients were found to be about 0.99148 for venlafaxine HCl and 0.98853 for citalopram.

6. System suitability test

For system suitability test, determination of some chromatographic parameters such as retention time, resolution and column efficiency as number of theoretical plates were appointed for optimization of conditions. The results are shown on table 2. At the first mobile phase (methanol/acetonitrile (55:45 v/v) and flow rate about 1 ml/min, the resolution of binary mixture between venlafaxine HCl and citalopram is better than at the second (methanol/acetonitrile (70:30 v/v) (fig. 4) but the change of flow rate effects a higher column efficiency which is more suitable in assay and purity tests. For identification and assay tests of triple mixture more acceptable is flow rate about 2 ml/min at the same mobile phase because the resolution and column efficiency are definitely better (fig. 5).

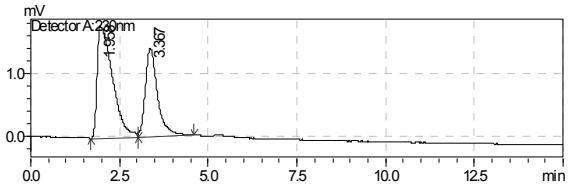

Fig. 4. *Chromatogram of binary mixture containing venlafaxine HCl and citalopram at mobile phase methanol/acetonitrile (55:45 v/v) and flow rate 1 ml/min*

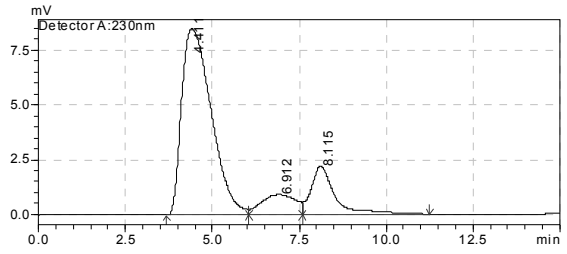

Fig. 5. *Chromatogram of triple mixture containing venlafaxine HCl, citalopram and sibutramine at mobile phase methanol/acetonitrile (70:30 v/v) and flow rate 2 ml/min*

Table 1. *Repeatability of samples containing venlafaxine HCl and citalopram*

№	Area of Venlafaxine HCl (AU) (0.004 g/ml)	Area of Citalopram (AU) (0.001 g/ml)	$\bar{X}$	SD (AU)	RSD (%)
1	37934	35179	For venlafaxine HCl: 39109.8(3) For citalopram: 36625.5	or venlafaxine HCl: 1398.2022 For citalopram: 2039.3716	For venlafaxine HCl: 3.57 For citalopram: 5.56
2	37646	39539			
3	41577	35179			
4	39754	39480			
5	39766	32184			
6	37982	35192			

Table 2. *Retention time, flow rates, resolution of citalopram toward other substances and column efficiency at different mobile phases for binary mixture containing venlafaxine HCl and citalopram and triple mixture containing venlafaxine HCl, citalopram and sibutramine*

Mobile phase	Flow rate	Retention time (min)	Resolution	Number of theoretical plates
Methanol/acetonitrile (55:45v/v)	1 ml/min	1.95 for venlafaxine HCl 3.36 for citalopram	1.66	750 for venlafaxine HCl 2840 for citalopram
Methanol/acetonitrile (70:30 v/v)	1 ml/min	1.12 for venlafaxine HCl 1.67 for citalopram	0.98	410 for venlafaxine HCl 2970 for citalopram
Methanol/acetonitrile (70:30 v/v)	2 ml/min	4.71 for venlafaxine HCl 8.09 for citalopram	1.38	270 for venlafaxine HCl 6200 for citalopram
Methanol/acetonitrile (70:30 v/v)	1 ml/min	2.01 for venlafaxine HCl 2.97 for citalopram 3.48 for sibutramine	0.79 0.91	380 for venlafaxine HCl 2210 for citalopram 330 for sibutramine
Methanol/acetonitrile (70:30v/v)	2 ml/min	4.41 for venlafaxine HCl 6.91 for citalopram 8.11 for sibutramine	1.34 1.31	580 for venlafaxine HCl 3390 for citalopram 1020 for sibutramine

Conclusion

HPLC procedure for quality control of antidepressants venlafaxine HCl, citalopram and sibutramine was performed at different chromatographic conditions. The methods are validated in respect of purposes of pharmaceutical and toxicological practices for binary mixtures containing venlafaxine HCl and citalopram and triple mixtures containing venlafaxine HCl, citalopram and sibutramine.

Acknowledgement. This work was supported by Grant №13 from 2011 year from the Council of Medicinal Science at Medical University – Sofia

References

1. Asafu-Adjaye, E. B. et al. Validation and application of a stability-indicating HPLC method for the in vitro determination of gastric and intestinal stability of venlafaxine. – J. Pharm. Biomed. Anal., **43**, 2007, № 5, 1854-1859.
2. Bartolinčić, A. et al. Development of practical and accurate HPLC methods for enantioselective analysis of fluoxetine and citalopram. – Chem. Anal. (Warsaw), **51**, 2006, № 4, 509.
3. Bymaster, F. P. et al. Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. – Neuropsychopharmacology, **25**, 2001, № 6, 871-880.
4. CBC News, March 26, 2010 (http:/www.cbc.ca) health story 6 herbal-dietnatural htm.
5. Cephalon gets six-month Provigil patent extension. Philadelphia Business Journal. 2006-03-28. Retrieved 2007-07-21. – www.bizjournals.com/philadelphia/
6. Currie, J. The Marketization of Depression: The Prescribing of SSRI Antidepressants to Women. – http://www.whp-apsf.ca/pdf/SSRIs.pdf
7. Da Fonseca, P. et P.S. Bonato. Chiral HPLC analysis of venlafaxine metabolites in rat liver microsomal preparations after LPME extraction and application to an in vitro biotransformation study. – Anal. Bional. Chem., **369**, 2010, № 2, 817-824.
8. De Silva R. et R. Hanwella. Pharmaceutical patents and the quality of mental healthcare in low and middle-income countries. – Psychiatrist, **31**, 2007, 121-123.
9. Faridbod, F. et al. Citalopram analysis in formulation and urine by a novel citalopram potentiometric membrane sensor. – J. Food Drug Anal., **17**, 2009, № 4, 264-273.
10. Flegal, M., D. Carroll, J. Kuczmarski et L. Johnsom. Overweight and obesity in the United States: prevalence and trends, 1960-1994. – Int. J. Obes. Relat. Metab. Disord, **22**, 1998, № 3, 39-46.
11. Greiner, C. et al. Determination of citalopram and escitalopram together with their active main metabolites desmethyl(es-)citalopram in human serum by column-switching high performance liquid chromatography (HPLC) and spectrophotometric detection. – Journal of Chromatography B, **848**, 2007, № 2, 391-394.
12. Huskamp, H. A. et al. Generic Entry, Reformulations, and Promotion of SSRIs. – Pharmacoeconomics, **26**, 2008, № 7, 603-616.
13. Heal, D. J., A. Prow et J. Martin. Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from d-amphetamine and d-fenfluramine. – Int. J. Obes. Relat. Metab. Disord., **22**, 1998, № 1, S18-22.
14. Torp-Pedersen, C. et al. Cardiovascular responses to weight management and sibutramine in high-risk subjects: an analysis from the SCOUT trial. – Eur. Heart J., **28**, 2007, № 9, 2915-2919.
15. Lichtenberg, F. R. et T. J. Philipson. The Dual Effects of Intellectual Property Regulations: Within- and Between- Patent Competition in the US Pharmaceuticals Industry. – National Bureau of Economic Research. NBER

WORKING PAPER SERIES October 2002 – http://www.nber.org/papers/w9303
16. Liu, W., H. Cai et H. Li. High performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) method for simultaneous determination of venlafaxine and its three metabolites in human plasma. – J. Chromatogr. B. Analy.t Technol. Biomed. Life. Sci., **850**, 2007, № 1-2, 405-411.
17. Manova, M., A. Stoimenova, A. Savova, P. Peikov et G Petrova. Biotechnol. Utilization and price trends in some reimbursed cardiovascular medicines. – Biotechnol. & Biotechnol. Eq. **25**, 2011, № 2, 2424-2431.
18. Millán, S. et al. Stereoselective determination of demethyl- and didemethyl-citalopram in rat plasma and brain tissue by liquid chromatography with fluorescence detection using precolumn derivatization. – Biomed. Chromatogr., **22**, 2008, № 3, 265-271.
19. Mitkov, J., I. Pencheva et Al. Zlatkov, Synthesis and chromatografic ivestigations of some ester derivatives of caffeine-8-thioglycolic acid. – 13th pamhellenic pharmaceutical congress, Athens, Greece, full text CD. – Eur. J. Drug Metabolism and Pharmacokinetics, **32**, 2007, special issue.
20. Müller. D., W. Weinmann et M. Hermanns-Clausen. Chinese slimming capsules containing sibutramine sold over the Internet: a case series. – Dtsch. Ärztebl. Int., **106**, 2009, № 6, 218-223.
21. Mullins, C. D. et al. Comparison of first refill rates among users of sertraline, paroxetine, and citalopram. – Clin Ther., 2006, № 2, 297-305; discussion 296.
22. Muth, E. A. et al. Antidepressant biochemical profile of the novel bicyclic compound Wy-45 ,030, an ethyl cyclohexanol derivative. – Biochem. Pharmacol., **35**, 1986, № 24, 4493-4497.
23. Pakes, A. et M. Simpson. Patent renewal data. – In: Brookings Papers on Economic Activity. Microeconomics. Clifford Winston and Martin Neil Baily, eds., The Brookings Institution, 1989, 331-410.
24. Rao, R. N., A. N. Raju et R. Narsimha. Isolation and characterization of degradation products of citalopram and process-related impurities using RP-HPLC. – J. Sep. Sci., **31**, 2008, № 10, 1729-38.
25. Roox, K., J. Pike, A. Brown et S. Becker. Patent-related barriers to market entry for generic medicines in the European Union: a review of weaknesses in the current European Patent System and their impact on the market access of generic medicines. Brussels: European Generic Medicines Association. 2008.
26. Baldania, S. et al. RP-HPLC estimation of venlafaxine hydrochloride in tablet dosage forms. – Indian J. Pharmaceutical Sci., **70**, 2008, № 1, 124-128.
27. Simoens, S. et S. De Coster. Potential savings from increased substitution of generic for originator medicines in Europe. – Journal of Generic Medicines, **4**, 2006, 43-45
28. Somasekhar, V., D. Gowrisankar et H. N. Shivakumar. Development and Validation of a Rapid RP-HPLC Method for the Determination of Venlafaxine Hydrochloride in Pharmaceutical Dosage forms using Experimental Design. – E-Journal of Chemistry, **6**, 2009, № 4, 1091-1102, http://www.e-journals.net
29. Vidyavathi, M. et al. Rapid HPLC determination of venlafaxine in microbial biotransformation studies. – Current Trends in Biotechnology and Pharmacy, **3**, 2009, № 1, 64-70
30. Yardley, J. P. et al. 2-Phenyl-2-(1-hydroxycycloalkyl)ethylamine derivatives: synthesis and antidepressant activity. – J. Med. Chemistry, **33**, 1990, № 10, 2899-905.
31. Zlatkov, Al., P. Peikov, G. Momekov, I. Pencheva et B. Tsvetkova. Synthesis, Stability and Computational Study of Ester Derivatives of Theophylline-7-acetic Acid with Antiproliferative Activity. – Der Pharma Chemica, **2**, 2010, № 6, 197-210.
32. Zlatkov, Al. et P. Peikov. Synthesis and chemometric study of some benzhydrylpiperazine derivatives. – 14th Panhellenic Pharmaceutical Congress, Athens, Greece, May, 12-14,2009, full text CD. – Eur. J. Drug Metabolism and Pharmacokinetics, **34**, 2009.

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