Research Article

Stereo Chemical Determination of Stability of Citalopram Enanthiomers: HPLC Chirality Test

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ABSTRACT

Analytical tests for identification, purity and assay of active molecule citalopram – racemic mixture and enantiomer in substance as well as in drug preparations were developed. The methods were optimized on the base of validation procedures in accordance with European Pharmacopoeia, EMA and ICH. Tests are HPLC with using of chiral chromatography column and they are distinguished with excellent reproducibility, accuracy, high sensitivity and stereo selectivity Using developed methods the kinetic profile of chemical stability of citalopram in biological media and ratio of enantiomers were determined depending on kind of solvent mixture, pH and time.

Keywords: Citalopram, Escitalopram, Chirality, Chiral HPLC.

INTRODUCTION

Citalopram (*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran -5-carbonitril) is an antidepressant drug of the selective serotonin reuptake inhibitor (SSRI) class. It has been used to treat major depressions, anxiety and panic disorders and it is prescribed off-label for a number of anxiety conditions.

Citalopram is available in 10 mg, 20 mg, and 40 mg tablets, as well as 10 mg containing oral solutions in more than 30 drug formulations.

Citalopram has one stereo center, to which a 4-fluorophenyl group and an *N,N*-dimethyl-3-aminopropyl group bind. Due to this chirality, the molecule exists in two enantiomeric forms - *S*-(+)-citalopram and *R*-(–)-citalopram. Citalopram is fixed on the pharmaceutical market as a racemic mixture, consisting of 50% (*R*)-(–)-citalopram and 50% (*S*)-(+)-citalopram. But only the (*S*)-(+) enantiomer has the antidepressant effect and high selectivity of serotonin reuptake inhibition.

The (*S*)-(+) enantiomer, which generic name is escitalopram is approved in antidepressant therapy for the treatment of adults with major depressive disorders, generalized anxiety disorders, social anxiety disorders or panic disorder. Whereas citalopram is supplied as a hydrobromide, escitalopram is supplied as an oxalate salt (hydrooxalate)[9]. In both cases, the salt formed of the amine makes these lipophilic compounds water-soluble. Escitlopram can be considered an example of "evergreening"[1] (also called "lifecycle management"[2] pharmaceutical strategy in order to extend the lifetime of a drug, in this case of the citalopram franchise. Escitalopram as enanthiopure compound of the racemic mixture citalopram requires less investment and less time to develop used for the same indications.

Chirality exists everywhere in nature and plays a significant role in a number of aspects of our life. The consideration of the aspect of chirality is very important for the environment, as well as for some industries, more concretely for the pharmaceutical industry. More than half of the medicines used are chiral[7, 8]. On the other hand it's known that for most of them the pharmacological effects are due to one of the enantiomers only[3,4]. The need of enantiomerically pure medicines is of great importance. Inspite of that, only about 25 % of them are introduced as pure enantiomers. There is a difference in the quantity and quality of enantiomers activity. It is possible that the pharmacologically inactive enantiomer shows some unwanted side effects and in some cases even toxic effects can be observed.[5] The problem of the chiral purity of the used medicines attains exceptional importance in the beginning of the thalidoimide tragedy in 1960 which leads to a more strict control and detailed consideration at the approval of newly-developed medicines. The chiral analysis also finds clinical applications, which include monitoring of some metabolic disorders caused by some deseases[6]. Besides, the study of the effects of storage of medicines, especially when one of the enantiomers transforms into a racemic mixture in time, is a necessary condition for their quality.

For identification and quantitation of citalopram there was developed a great variety of analytical methods – spectrophotometric[10, 11], HPLC with UV or diode-array detection[12-15], fluorimetric[16-18], Raman spectrometric, GC/MS[19], CE[20-26] and methods for separation of citalopram, escitalopram and its metabolites based on their chiral properties[27-29]. But in spite of the presence of so many analytical methods in scientific literature it does not exist pharmacopoeia or other validated chirality test for enantiomeric purity of drugs containing citalopram and especially escitalopram.

The aim of this study is the investigation of analytical and chromatographic parameters and conditions to develop HPLC chirality test for simultaneously determination of two enantiomeric forms of citalopram with wide range of application but especially for quality control of drugs and for studying of chemical stability in different media.

MATERIALS AND METHODS

Chromatographic system

The chromatographic procedure was carried out using:

Liquid chromatograph Shimadzu LC – 10, Phenomenex Columns: Chirex 3126 and 250 x 4.60 mm, ODS with particle size 5 µm; Detector SPD 10 AVvp – UV-VIS with fixed analytical wavelengths.

Chromatographic conditions

Isocratic mobile phase, prepared by mixing of filtered and degassed Metanol - Acetonitrile : 55:45 v/v respectively;

- 230 nm analytical wavelengths;

- column temperature 25°C;

- flow rate about 1.5 ml/min.

Reagents

Acetonitril HPLC grade, Methanol HPLC grade, Buffer solution with pH = 2.0, prepared by European Pharmacopoeia 7.0, Reagents; Buffer solution with pH = 7.4, prepared by European Pharmacopoeia 7.0, Reagents; Buffer solution with pH = 9.0, prepared by European Pharmacopoeia 7.0, Reagents, reference substances citalopram (CRS) and escitalopram CRS.

Preparation of reference solutions

Reference solutions were prepared by dissolving and mixing of adequate and equal amounts of CRS substances in mobile phase to obtain solutions with concentration 0.00002 mg/ml.

Test preparation

To a sample containing 0,0010 mg citalopram racemic mixture (RS) was added 50.0 ml buffer solution with pH 2 or 7.4 or 9. The obtained test solution was heated at fixed temperature 37^0 C and continuously stirring. In time aliquot portion of the sample is taken and injected into the chromatograph. The study was prolonged to the obtaining of unchangeable remainder.

RESULTS AND DISCUSSION

The question of chiral purity in the process of generic production of the drug preparation is very debatable because of presence of a patent protection of every single enantiomer. By these reason the development of analytical methods for determination of biological active enantiomers is very important for generic as well as for original producers in order to safe patent rights.

For stereo chemical analysis of citalopram – racemic mixture and enantiomers direct HPLC method with special fixed chiral column was applied. The method is distinguished with wide range based on possibility to achieve the optimal values of analytical parameters, high performance and stereo selectivity.

There was used stationary phase in which to basic packing ODS (octadecylsilan) (S)-valine and (R)-1-(α-naphthyl)ethylamine urea are added. They are playing the role of electron donor substances and they are able to bind at different positions in the packing substance molecule both enantiomers – Fig. 1.

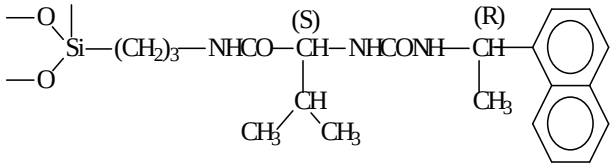

Figure 1: Structure of binding chiral packing.

With these chiral column in solvent mixture methanol – acetonitrile (55 : 45 v/v) was achieved resolution between two enantiomers 0.42 and at obtained limits - 9 µg for limit of detection and 15 µg for limit of quantitation the system suitable test is valid. On fig. 2 is shown chromatogram of (RS) Citalopram in solvent – methanol – acetonitrile (55 : 45 v/v) obtained with column Chirex at 230 nm. In analogue chromatogram (fig. 3) obtained with other column - 4.6 x 150 mm RP-18, ODS, 5 mm particle size the resolution is slight (near 0) and this method can't find an application in identification and purity tests.

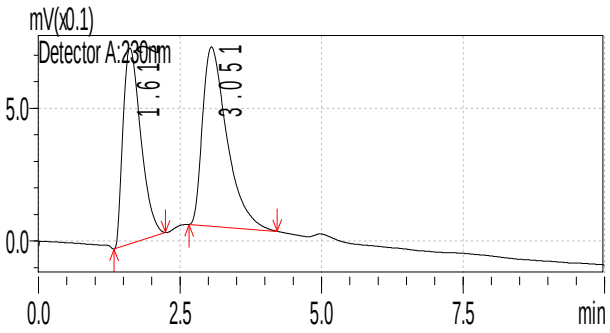

Figure 2: Chromatogram of (RS) Citalopram in solvent – methanol – acetonitril (55 : 45 v/v) obtained with column Chirex at 230 nm.

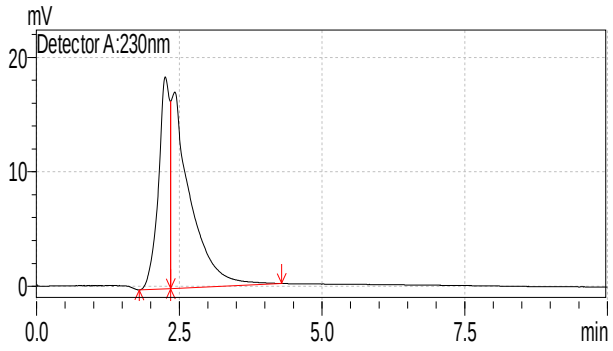

Figure 3: Chromatogram of (RS) Citalopram in solvent – methanol – acetonitrile (55 : 45 v/v) obtained with column 4.6 x 150 mm RP-18, ODS, 5 mm at 230 nm.

Validation of analytical procedure

1. Specificity

Specificity in respect of reagents – "Placebo" solution containing all reagents without active substances was prepared. There are no peaks in the chromatogram obtained from this solution with Rt of R-(–) and S-(+) citalopram.

2. Repeatability

Six (6) equal solutions from homogenous samples containing (RS) citalopram were analyzed by HPLC method. Standard deviation (SD) is about 2040 AU (absorption units) from area and relative SD (RSD) is 5.56 %.

3. Limit of detection (LOD)

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

4. Limit of quantitation (LOQ)

15 µg for citalopram, 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 was found to be about 0.98853 for (RS) citalopram – Fig. 4.

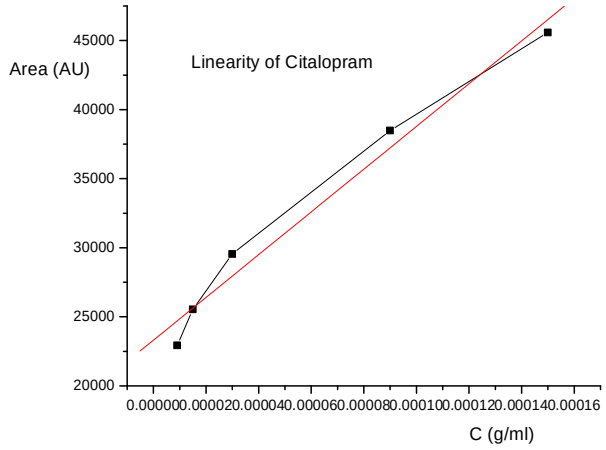

Figure 4: Linearity of citalopram

Y = A + B * X (Citalopram)

Parameter	Value	Error
A	23317.40541	1088.28466
B	1.5468E8	1.3645E7

R	SD	N	P
0.98853	1644.13829	5	0.00147

6. System suitability test

For system suitability test determination some chromatographic parameters such as retention time, resolution, LOD and LOQ were appointed for optimization of conditions at different mobile phases. At mobile phase Methanol/Acetonitrile (55 : 45 v/v) and flow rate about 1ml/min the resolution of enantiomers in (RS) citalopram is better than at the mobile phase Methanol/Acetonitrile (70 : 30 v/v) but the change of flow rate effects higher column efficiency – from 2840 to 6200 theoretical plates which is more suitable in assay and purity tests.

Chemical stability profile

Developed chromatographic procedure includes identification test against reference substances citalopram and escitalopram, purity test and assay. The chemical stability of analytes in different media – solvent mixtures and buffer solutions with appropriate biological pH values - 2, 7.4 and 9 was studied and the ratio of too enantiomers was determinate in dependence on kind of solvents and time. The obtained results as % of *R*-(–) and *S*-(+) citalopram are presented on table 1. The identification is based on comparison of Rt of analyte with those of reference substance. The retention time and the areas of the citalopram peaks corresponded to that observed in the chromatogram of the reference solution.

Table 1: Content in % of *R*-(–) и *S*-(+) citalopram at different solvent media

Solvent mixture	Time (min)	% *R*-(–)-citalopram	% *S*-(+)-citalopram
Mobile phase	0 – 50	52	48
Buffer solution with pH = 2	0 - 120	-	94.11 – 102.27
Buffer solution with pH = 9	0	-	95
	30	50	33
	60	50	27
	90	50	27
	120	44	27
	180	48	22
	210	63	-
	240	52	-
	270	62	-
	300	55	-
	330	65	-
Buffer solution with pH = 7.4	0 - 180	-	94 – 100 %

In acid media and at pH = 7.4 (RS) citalopram has been hydrolyzed for period of 240 min. It spontaneously transforms in *S*-(+)- enantiomer form which is biological active form and the % of this compound to total racemic mixture putted in the buffer solutions is about 100 % (Fig. 5). In organic solvent mixture Methanol/Acetonitrile (55 : 45 v/v) (RS) citalopram is stable in interval of 50 min

and the ratio between both enantiomers is approximately equal and correspond exactly to ratio in the original molecule citalopram – 1: 1. In alkaline media obtained with boric buffer (pH = 9) the both enantiomers appear in the chromatogram but the ratio between than varies in the all observed time interval. At first minute in alkaline media is detected only *S*-(+)- enantiomer (95 %), and after 180 min - only *R*-(-)- enantiomer which has negligible biological action. The total quantity is't responded to putted amount of (RS) citalopram. There were found decomposed related substances – Fig. 6.

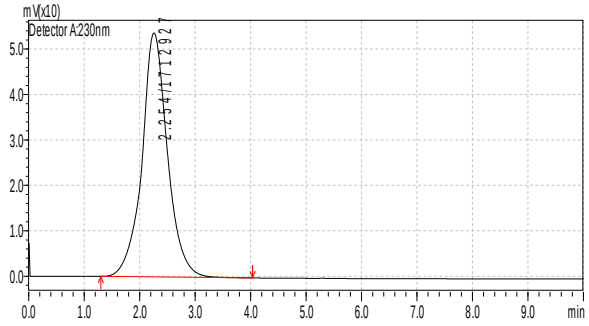

Figure 5: Chromatogram of (RS) citalopram in solvent – buffer solution with pH = 2 and column Chirex.

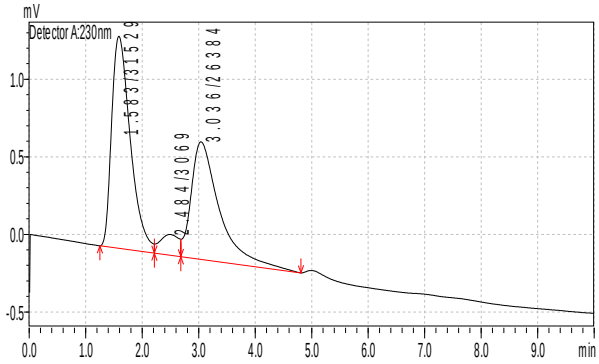

Figure 6: Chromatogram of (RS) citalopram in solvent – buffer solution with pH = 9 and column Chirex.

CONCLUSION

Stereo selective HPLC methods are useful for analytical and toxicological practice and for regulatory institutions for quality control of novel generic drugs. Chiral HPLC method for analysis of racemic mixture citalopram gives also possibilities for answering of very debatable in the area of the patent justice and intellectual property questions about the ingenious of chiral molecule and chances for manifestation of it properties.

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