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Study and analytical application of ion-pair formation in the system fluoxetine-pyrocatechol violet and fluvoxamine-pyrocatechol violet

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Received October 8, 2002, accepted November 11, 2002

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Pharmazie 58: 245–248 (2003)

Pyrocatechol violet (PCV) reacts in aqueous media with fluoxetine (FLX) and fluvoxamine (FLV) forming coloured ion-association complexes, which are insoluble in water but quantitatively extracted into chloroform-n-butanol mixture. The composition of the compounds, studied by spectrophotometric methods showed that the molar ratio PCV : FLX and PCV : FLV is 1 : 1. The compounds were characterized by UV-VIS, IR and NMR spectrometry. Under optimal experimental conditions fluoxetine and fluvoxamine were determined in the range 1.3–18.0 μg/ml and 2.6–39.1 μg/ml, respectively. The proposed methods have been succesfully applied to the determination of these drugs in pharmaceuticals and natural samples.

1. Introduction

Fluoxetine (FLX) and fluvoxamine (FLV) belong to the class of the new antidepressive drugs of the second-generation, which potently inhibit neuronal reuptake of serotonin (SSRI). Both are used for the treatment of major depression, obsessive-compulsive disorder, bordeline personality, anorexia, bulimia, autism, alcoholism [1, 2].

The most widely used analytical methods of determination of FLX and FLV in biological samples are HPLC [3–12] and GC [13–15]. Most procedures are based on prepurification of FLX and FLV by liquid-liquid and solid-phase extraction before the compounds are submitted to separation and detection by various detectors (UV, fluorescence, electrochemical detector, nitrogen-phosphorous detector, mass spectrometry). Fluoxetine together with fluvoxamine have been determined by some electroanalytical methods [16–20]. Alhaider et al. [21] and Atmaca [22] proposed chloranil and 2,4,6-trinitrobenzene sulphonic acid for the spectrophotometric determination of FLX and FLV. Khan [23] proposed potassium iodide and benzoyl peroxide to spectrophotometric assay of fluoxetine. A review shows that the number of extractive-spectrophotometric determinations of these compounds is very limited [24].

In this paper, we present an easy, simple and sensitive extractive-spectrophotometric procedure based on the reaction of pyrocatechol violet with FLX and FLV. This work is a continuation of our previous studies on using triphenylmethane dyes for the determination of antidepressant drugs [25–28]. The proposed methods can be recommended for routine analysis of these components in pharmaceutical preparations and biological samples.

2. Investigations, results and discussion

Reaction and extraction of the coloured compounds FLX and FLV with PCV depend on the acidity of the medium, PCV concentrations and the nature of the organic solvents. Of the various acids tested, sulphuric acid and acetate acid were found to be most suitable in the case of FLX and FLV, respectively. In acidic medium the dye pyrocatechol violet appears in anion form H_3R^- [29]. In medium of those acids the absorbance of extracts of studied compounds is maximum and stable.

The effect of the pyrocatechol violet concentration was also studied. The maximum and stable absorbance for both systems was obtained for a 6–12 and 8–12-fold excess of PCV with respect to FLX and FLV, respectively. The compounds can be quantitatively extracted by the mixture of chloroform-n-butanol. The extracts are stable for about 6 h. The logarithm of the constans of extraction ($\log K_{ex}$) for FLX-PCV and FLV-PCV systems was calculated [30] and found to be 4.59, 4.96, respectively. The studied complexes were quantitatively investigated from aqueous to organic phase. Their properties have been

Table 1: Analytical data

Parameters	Values	
	FLX-PCV system	FLV-PCV system
Analytical wavelength λ_{max} (nm)	445	445
Beer's law range (ppm)	1.3–18.0	2.6–39.1
Molar absorptivity ($l \cdot mol^{-1} \cdot cm^{-1}$)	$1.99 \cdot 10^4$	$2.14 \cdot 10^4$
Sandell's sensitivity ($\mu g \cdot cm^2$/0,001 A)	0.015	0.021
Correlation coefficient (R)	0.9996	0.9998
Relative standard deviation, RSD (%)	1.53	0.37
Limit of quantitation LQ (μg/ml)	0.88	2.04
Limit of detection LD (μg/ml)	0.033	0.61
Regresion equation (Y*)		
Slope (b)	0.0597	0.0337
Intercept (a)	0.0101	0.0494

$Y^* = a + bc$, where c is the concentration of analyte in ppm

exploited for the extractive-spectrophotometric determination of FLX and FLV. Beer's law was obeyed in the range 1.3–18.0 μg/ml for FLX and 2.6–39.1 μg/ml for FLV. In Table 1 analytical data of elaborated methods are shown. It can be stated, that the proposed methods are accurate, precise and sensitive.

The proposed methods were applied to the determination of FLX and FLV in pharmaceuticals and biological samples. The results are shown in Table 2.

In order to evaluate the selectivity of the developed methods for the analysis of pharmaceutical preparations, the effect of the presence of several species which can occur in real samples with FLX or FLV was investigated. A level of interference was considered to be acceptable if the error was not larger than ±5% in the absorbance of FLX-PCV and FLV-PCV systems. Associated substances such as NaCl (1000-fold), glucose, sucrose (200-fold), starch and acetyl salicylic acid (50-fold), citric acid and ascorbic acid (20-fold) did not interfere.

The solid phase extraction (SPE) procedures allow the selective isolation of FLX and FLV from human serum and albumin. The SPE reported here are simple and rapid to carry out and do not require an evaporation step. In every instance, the selected elution solvents provided the cleanest samples which could be directly analysed by the proposed extractive-spectrophotometric methods. The recovery values for FLX and FLV were calculated by comparison of the actual content of the drugs in the extracts with those theoretically expected. In every instance, the recoveries of drugs from C_{18} bonded silica columns were surprisingly high, uniform and stable in the range 91–101%. The results are shown in Table 3.

Table 2: Results of the determination of FLX in *Prozac*® and FLV in *Fevarin*® tablets

Sample	Found (mg)	Relative error (%)	S	$\pm t_{0,95} \cdot S_{\bar{x}}$	RSD %
1 Tablet containing 20 mg of FLX	19.96 19.80 19.64 19.70	−0.2 +1.0 −1.8 −1.5	0.1664	0.231	0.84
1 Tablet containing 50 mg of FLV	50.32 49.86 50.23 49.55 49.27	+0.64 −0.28 +0.46 −0.90 −1.46	0.4445	0.5527	0.89

Table 3: Results of the extractive-spectrophotometric determination of FLX in serum and FLV in albumin after SPE isolation

Taken (μg)	Found by described method (μg)	Absolute recovery (%)	S	$\pm t_{0,95} \cdot S_{\bar{x}}$	RSD (%)
FLX 3.09	3.13 3.02 2.93 2.90 2.97	101.29 97.82 94.84 93.85 96.1	0.0903	0.1123	3.02
FLV 11.0	11.05 11.11 11.93 10.05 10.07	100.45 101.0 99.36 91.36 91.55	0.0551	0.0624	0.49

Table 4: Values of λ_{max} of the compounds forming in FLX-PCV and FLV-PCV systems

Compd.	Region	
	$UV_{\lambda_{max}}$	$VIS_{\lambda_{max}}$
FLX	210, 226, 264	–
FLV	206, 246	–
PCV	202, 278	445
FLX-PCV	238, 278	445
FLV-PCV	212, 252	445

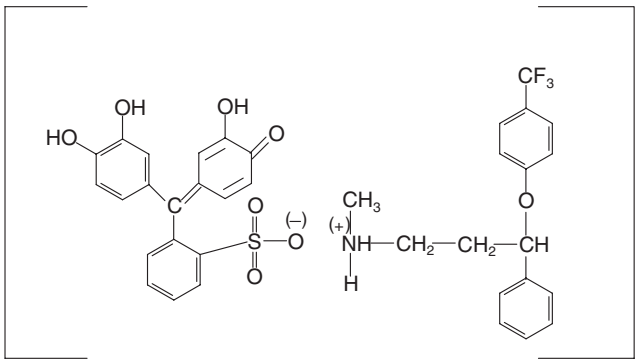
The stoichiometric ratios of drugs to PCV in each of the studied complexes were determined using the Job's method of continous variation and by spectrophotometric titration. It was established that molar ratio of dye-pyrocatechol violet is 1 : 1 for both compounds. It was expected that H_3R^- form of pyrocatechol violet is taking part in the reaction with a monopositive cation of FLX and FLV.

The absorption spectra of the compounds in the UV-VIS region were examined. In Table 4 the results of these experiments are summarized. It was found that the characteristic bands of absorption for the reagent were preserved in the compound spectra. These observation suggests that the investigated compounds can be classified as ion-association complexes.

The ion-association character of the formed complexes was confirmed by IR spectra (KBr discs) in the region 400–4000 cm^{-1}. The spectra of the compounds in the region 600–1800 cm^{-1} are the sum of the spectra of reagents. The absorption bands of the N–H bond at 2400–2900 cm^{-1} of FLX and FLV, characteristic for the first-and second-amines [31], in the spectra of the corresponding complexes either disappeared or were reduced to a small hump. This suggests that compounds are formed with participation of the nitrogen atom from the amine group of the side aliphatic chain of FLX and FLV with H_3R^- form of pyrocatechol violet.

For the purpose of confirmation of structure of studied complexes, the ^{13}C NMR spectrum was made. $DMSOd_6$ was used as a solvent. The results for the system FLX-PCV are given in Table 5.

It can be stated that in the spectrum of compounds the aromatic systems of reagents (studied drugs and PCV) are preserved. It is possible to conclude that the conjunction between FLX and FLV and pyrocatechol violet has an ion-association character. From the analysis of spectra it can be supposed that structure, e.g. for the compound PCV with FLX is the following:



FLX-PCV

Table 5: Values of the chemical shift (^{13}C NMR) for the e.g. FLX-PCV compound

Investigated compound	Signal				
FLX	30.01	71.80–74.60	129.92	136.92	
PCV			116.01–135.47	140.59–145-73	151.47–172-26
FLX-PCV	30.24	70.40	116.42–137.55	138.75–158.57	167.08

3. Experimental

3.1. Reagents

A standard stock solution of fluoxetine (FLX) hydrochloride was made of a commercial product (Eli Lilly) by dissolving 100 mg in 25 ml of methanol and diluting with water up to 100 ml. A standard stock solution of fluvoxamine (FLV) maleate was made of a commercial product (Duphar Scient. Off in Riyadh and Solvay Pharmaceutical B.V., Holland) by dissolving 50 mg in 30 ml of ethanol and diluting with water up to 100 ml. Pyrocatechol violet solution 1.0×10^{-2} M was prepared using a commercial product (P.O. Ch-Gliwice) which was cleaned by the precipitation from 2.0 M HCl medium. The solutions were kept in an amber coloured bottle at room temperature. All chemicals and solvents were of analytical grade and the solutions were prepared with doubled distilled water.

3.2. Apparatus

Hewlett Packard model 8452 diode-array spectrophotometer. Specol-11 spectrophotometer (Carl Zeiss, Jena, Germany). FTIR- spectrometer Magna 550, II serie, Nicolet. VEB Analytic Dresden PHMK 77/1046. SPE – System 12G, J. T. Baker.

3.3. General procedure

It was found that PCV reacts with FLX and FLV forming a yellow ion-association compound insoluble in water but fairly soluble in organic solvents (e.g. methanol, acetone, chloroform, n-butanol). The formation and extraction of the coloured compounds of FLX and FLV with PCV depends on the acidity, the nature of organic solvents used and excess of dye respect to studied drugs. These factors were investigeted as follows.
Suitable amounts of FLX or FLV, 1.0 ml of 1.0×10^{-2} M H_2SO_4 for FLX and 2.0 ml of 1.0×10^{-1} M CH_3COOH for FLV and 8–12-fold excess of pyrocatechol violet with respect to FLX and FLV were mixed in 25-ml separatory funnels and diluted to 10 ml with distilled water. The mixture was shaken and extracted into chloroform-n-butanol mixture. The extracts were combined in 10-ml volumetric flasks and diluted to the mark with the mixture of above organic solvents. The absorbance was measured at 445 nm against the reagents blank. The optimal conditions for the formations and extraction of the compounds studied are summarized in Table 6.

3.4. Extractive-spectrophotometric determination of fluoxetine and fluvoxamine

Into a 25-ml separatory funnel containing 0.2 to 2.7 ml of 2.0×10^{-4} M of FLX hydrochloride were placed 1.0 ml of 1.0×10^{-2} M H_2SO_4, 7–8-fold excess of PCV with respect to FLX and diluted to 10 ml with distilled water. The mixture was shaken and extracted with two portions of extractions solvents consisted of chloroform-n-butanol (2 : 1, v/v) succesive 5 ml. The extracts were combined in a 10-ml volumetric flasks and diluted to the mark with a mixture of chloroform-n-butanol (2 : 1, v/v). The absorbance was measured at 445 nm against the reagents blank. The same procedure was elaborated for the determination of FLV. Into a separatory funnel placed up 0.2 to 3.0 ml of 3.0×10^{-4} M of fluvoxamine maleate, 2.0 ml 1.0×10^{-1} M CH_3COOH and 9-fold excess of pyrocatechol violet with respect to FLV and diluted to the 10 ml with distilled water. The solution was extracted once for 1 min with 10 ml of chloroform-n-butanol mixture (1 : 1, v/v). The extract were transferred to 10-ml volumetric flasks and diluted to the mark with the same mixture of organic solvents (1 : 1, v/v). The absorbance was measured at 445 nm against the reagents blank. Under the described experimental conditions (procedure), the standard calibration graphs for FLX and FLV were constructed.

Table 6: Optimal conditions for the formation and extraction of compounds of fluoxetine and fluvoxamine with pyrocatechol violet

Parameters	FLX-PCV system	FLV-PCV system
Medium	$[H_2SO_4]$ $1.0 \cdot 10^{-3}$ mol/l	$[CH_3COOH]$ $1.0 \cdot 10^{-2}$ mol/l
Organic solvent	$CHCl_3 + C_4H_9OH$ (2 + 1)	$CHCl_3 + C_4H_9OH$ (1 + 1)
Excess of dye PCV	6–12	8–12
Analytical wavelength λ_{max} (nm)	445	445
The time of extraction (min)	1	1
Multiplicity of extraction	2-fold (two times with 5.0 ml)	1-fold (once with 10 ml)

3.4.1. Tablets

5 tablets of Prozac® each containing 20 mg of FLX hydrochloride were powdered. An accurately weighed portion, equivalent to about 20 mg of FLX, was transfered into 100-ml volumetric flasks, dissolved in 20 ml of methanol and diluted to the mark with distilled water, shaked well and filtered. To analysis 0.5 ml of fluoxetine aqueous solution was taken.
Two tablets of Fevarin® each containing 50 mg of FLV maleate were powdered. An accurately weighed portion, equivalent to about 50 mg of FLV, was transferred into 100-ml volumetric flasks dissolved in 35 ml of ethanol and diluted to the mark with water. After shaking and filtering, 1.0 ml of this solution was taken to the determination.
Determination of the contents of FLX and FLV in tablets was carried out the same way.

3.4.2. Serum

The isolation of fluoxetine hydrochloride from serum was carried out by a solid-phase extraction (SPE) procedure. For this purpose the 1 ml reversed-phase cartridges C_{18} (Bakerbond spe) were used. 0.5 ml of blank serum samples were mixed with 1.0 ml 2.0×10^{-4} M of FLX, 0.5 ml of methanol and buffered with 0.5 ml of 0.2 M borate buffer at pH 9.0. The extraction columns were activated subsequently once with 2 ml of methanol, 1 ml of water and 3 ml of 0.2 M borate buffer pH 9.0. The column must not become dry before sample application. Afterwards the buffered serum sample was slowly applied onto the conditioned column. The sample was passed

Table 7: SPE procedure for the isolation of FLX from human serum and FLV from 5% albumin using C_{18} cartridges

The followed steps of SPE process	FLX-PCV system	FLV-PCV system
	Reagent	
Condition of sorbent	2.0 ml methanol 1.0 ml water 3.0 ml 0.2 M borate buffer pH 9.0	2.0 ml acetonitrile 2.0 ml water 1.0 ml 0.1 M acetate buffer pH 3.18
Load	2.5 ml diluted serum	2.0 ml diluted albumin
Rinse	1.0 ml acetonitrile-water mixture (1 : 1, v/v)	0.4 ml chloroform-n-butanol mixture (1 : 1, v/v)
Elute	5×1.0 ml chloroform-n-butanol mixture (2 : 1, v/v)	5×1.0 ml chloroform-n-butanol mixture (1 : 1, v/v)

slowly through the column under a mild vacuum (−200 mm Hg). The column was then washed with 1 ml of acetonitrile-water mixture (1 : 1, v/v) to elute interferences from the cartridge and dried completely after the washing. The elution of FLX was achieved by rinse with five one ml portions of chloroform-n-butanol mixture (2 : 1, v/v). The elutes were collected into a glass tube and transfered to a 25-ml separatory funnel and next proceeded according to the procedure described in calibration graph section.

3.4.3. Albumin

To the isolation of FLV maleate from a 5% solution of albumin (Biomed, Warsaw) the SPE procedure was used. The SPE method was performed on 1 ml reversed-phase cartridges (Bakerbond spe).
0.5 ml of albumin sample was spiked with 1.0 ml 2.53×10^{-4} M of FLV and buffered with 0.5 ml of 0.1 M acetate buffer pH 3.18. The octadecyl extraction column was conditioned subsequently by 2.0 ml of acetonitrile, 2.0 ml of water, 1.0 ml of acetate buffer pH 3.18 and twice with 1.0 ml of acetonitrile-water mixture (1 : 1, v/v). After loading the whole spiked albumin sample onto the activated column, the cartridge was washed with 0.4 ml of chloroform-acetone mixture (2 : 1, v/v). Afterwards, the column was dried completely before the eluting step. Elution was done with five one ml portions of chloroform-n-butanol mixture (2 : 1, v/v) to a glass tube and then transfer to a 25-ml separatory funnel. Finally, the elute was subjected to an extractive-spectrophotometric determination according to the procedure described in calibration graph section.
The SPE procedures for isolation of both studied drugs from serum and albumin, respectively are described in Table 7.

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