ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 477–481

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Investigation and analytical application of the reactions of eriochrome cyanine R with fluvoxamine and fluoxetine

B. Starczewska *, H. Puzanowska-Tarasiewicz, K. Baranowska

Institute of Chemistry, University of Bialystok, Al. Pilsudskiego 11/4, 15-443 Białystok, Poland

Received 27 September 1999; received in revised form 4 February 2000; accepted 19 February 2000

Abstract

Eriochrome cyanine R (ECR) reacts with fluvoxamine (FXM) and fluoxetine (FXT) forming coloured ion-association compounds. The composition of the compounds, studied by spectrophometric methods showed that the molar ratio ECR:FXM = 1:2 and ECR:FXT = 1:2. The formation and extraction conditions of the compounds were established. The compounds were characterised by UV, VIS, and IR spectrometry. It was found that the compounds are insoluble in water but quantitatively extracted into buthanol. Under the optimal experimental conditions fluvoxamine and fluoxetine were determined in the range 2–40 μg/ml and 2–20 μg/ml, respectively. The relative standard deviation is about ±2%.

Keywords: Fluvoxamine; Fluoxetine; Eriochrome cyanine R; Spectrophotometry

1. Introducton

Fluvoxamine and fluoxetine are antidepressants belonging to the class of selective serotonin reuptake inhibitors. The chemical structure and metabolism of fluvoxamine and fluoxetine differs from that of classical tricyclic antidepressants.

Reported method for the determination of fluvoxamine and fluoxetine are based mainly on high performance liquid chromatography [1–6] and gas chromatography [7–9]. Most procedures are based on prepurification of fluvoxamine and fluoxetine by liquid–liquid extraction before they are submitted to separation by chromatographic procedures and detection by various detectors (UV, fluorescence, electrochemical detector, nitrogen–phosphorus detector, mass spectrometry). This literature review shows that most of methods allow quantitative determination of fluvoxamine and fluoxetine in plasma, in lower ng/ml range, and that they are suitable for therapeutic drug monitoring purposes of this class of compounds.

Alhaider et al. [10] and Atmaca [11] proposed chloranil and 2, 4, 6-trinitrobenzene sulphonic

* Corresponding author. Tel.: +48-85-7457580; fax: 48-85-7457581.

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00323-X

acid for the spectrophotometric determination of fluvoxamine. Komorowski proposed [12] the NMR technique for quantification of fluoxetine.

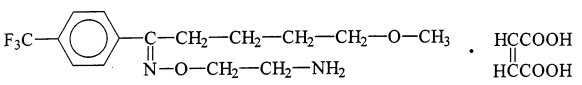
Review of the methods for the determination of fluvoxamine and fluoxetine shows that the number of extractive-spectrophotometric determination of these compounds are very limited although their usefulness for the determination of FXM and FXT in pharmaceuticals and body fluids.

In this work simple extractive-spectrophotometric procedures based on the reaction of eriochrome cyanine R with fluvoxamine and fluoxetine were described. The proposed methods are based on more useful reagents and simpler procedures than others spectrophotometric methods [10,11].

2. Experimental

2.1. Reagents

Fluvoxamine maleate (Eli Lilly, USA)



Fluoxetine hydrochloride (Duphar Scient. Off, Riyadh)

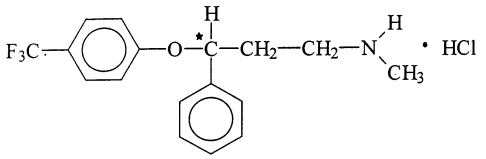


Table 1
Results of the extractive-spectrophotometric determination of fluvoxamine maleate using eriochrome cyanine R, $\lambda = 518$ nm[a]

Amount of FXM [μg/ml]	Mean absorbance ($n = 4$)	Interval	SD	RSD%	$\mu = x \pm t_{0.95}S$
2	0.108	0.01	0.002	1.85	0.11 ± 0.02
5	0.145	0.01	0.002	1.38	0.15 ± 0.01
8	0.200	0.01	0.004	2.00	0.20 ± 0.02
10	0.226	0.01	0.005	2.21	0.22 ± 0.02
12	0.247	0.01	0.004	1.62	0.24 ± 0.01
15	0.299	0.01	0.003	1.00	0.30 ± 0.01
18	0.335	0.02	0.006	1.79	0.34 ± 0.01
20	0.379	0.02	0.007	1.85	0.38 ± 0.02
25	0.437	0.02	0.008	1.83	0.44 ± 0.02
30	0.533	0.01	0.003	0.56	0.53 ± 0.005
35	0.599	0.03	0.013	2.17	0.60 ± 0.02
40	0.667	0.02	0.007	1.05	0.67 ± 0.02

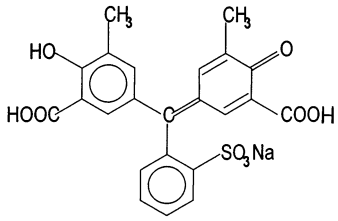
[a] SD, standard deviation; RSD, relative standard deviation.

Table 2
Results of the extractive-spectrophotometric determination of fluoxetine hydrochloride using eriochrome cyanine R, $\lambda = 520$ nm

Amount of FXT [μg/ml]	Mean absorbance($n = 4$)	Interval	SD	RSD%	$\mu = x \pm t_{0,95}S$
2	0.140	0.01	0.003	2.14	0.14 ± 0.01
5	0.299	0.01	0.003	1.00	0.30 ± 0.01
8	0.470	0.02	0.006	1.28	0.47 ± 0.01
10	0.533	0.01	0.004	0.75	0.53 ± 0.01
12	0.656	0.01	0.005	0.76	0.66 ± 0.01
15	0.783	0.01	0.005	0.64	0.78 ± 0.01
18	0.936	0.02	0.009	0.96	0.94 ± 0.02
20	1.007	0.03	0.013	1.30	1.00 ± 0.03

A standard stock solution of FXM or FXT was prepared by dissolving 10 mg (base) in 5 ml of ethanol and diluting with water up to 10 ml. Working solutions of FXM or FXT (4.6×10^{-4} and 5.78×10^{-4} M, respectively), were made every day from standard solutions. These solutions were stored at room temperature.

Eriochrome cyanine R (Lachemia, Chemapol Praha, Czechoslovakia) an aqueous 1×10^{-2} M solution (it was stored under refrigeration).



All chemicals and solvents were of analytical grade.

2.2. Apparatus

A Hewlett-Packard Model 8452 diode-array spectrophotometer;
a Spekol-11 spectrophotometer (Carl Zeiss, Jena, Germany);
a Nicolet spectrophotometer ST–IR, Magna 550-series II; and
a Bruker apparat AC 200 F NMR spectrometer

3. Results and discussion

Eriochrome cyanine R belongs to triphenylmethane dyes which are useful in analytical practice as sensitive spectrophotometric reagents for the determination of a large number of metal ions [13]. These compounds react also with metals ions in the presence of surfactants [14–18] and with some drugs (e.g. chloropromazine, imipramine) forming binary or ternary complexes [19–22].

We found that eriochrome cyanine R reacts in acidic media with fluvoxamine and fluoxetine forming a pink ion-association compounds insoluble in water but fairly soluble in organic solvents (e.g. methanol, ethanol, acetone). They are quantitatively extracted into buthanol. Extracts are coloured and very stable. These properties may be applied for the extractive-spectrophotometric determination of fluvoxamine and fluoxetine.

3.1. General procedure and optimalization of variables for extractive-spectrophotometric determination of fluvoxamine and fluoxetine

In a 25-ml separatory funnels add suitable amounts of fluvoxamine or fluoxetine and 4–8-excess of eriochrome cyanine R ($C = 4.6 \times 10^{-4}$ M). Then add 1.5 ml of 1×10^{-3} M acetic acid (for FXM) or 0.5 ml of 5×10^{-4} M sulphuric acid (for FXT) and dilute to 10 ml with water. Shake the mixture well and extract with two 5-ml portions of buthanol. Transfer the extracts to 10-ml standard flasks and dilute to the mark with organic solvent. Measure the absorbance at 518 and 520 nm for FXM and FXT respectively against the reagents blank.

According the described procedures, the influence of various experimental parameters on the absorbance of the extracts of compounds of ECR with FXM and ECR with FXT was studied.

It was found that the absorbance of the extracts is maximal:

1. for 1.5×10^{-4} M solution of CH_3COOH and 2–5-excess of eriochrome cyanine R in the ECR–FXM system; and
2. for 2.5×10^{-5} M solution of H_2SO_4 and 5–12-excess of eriochrome cyanine R in the ECR–FXT system.

The coloured extracts were stable for about 2 h.

Under the described optimal conditions (procedure), the linear calibration relationship between absorbance and concentration of drugs were tested. The results obtained and the statistical evaluation of precision are given in Table 1 and Table 2.

These results show that Beer's law was obeyed over the concentration range of 2–40 μg/ml for fluvoxamine maleate and 2–30 μg/ml for fluvoxetine hydrochloride. The extractive-spectrophotometric methods were characterised by the molar

absorptivities 6.5×10^3 and 1.7×10^4 dm^3 mol^{-1} cm^{-1}, respectively. The calibration curves were described by equations:

$y = 0.0149x + 0.07$ for FXM

($RSD_a = 1.15\%$, $RSD_b = 4.96\%$)

and $y = 0.0483x + 0.06$ for FXT

($RSD_a = 2.05\%$, $RSD_b = 2.1\%$).

The proposed methods can be applied for the determination of fluvoxamine and fluoxetine in some commercial tablets.

In order to evaluate the selectivity of the developed method for the analysis of pharmaceutical preparations, the effect of the presence of several species which can occur in real samples with FXM or FXT was investigated. A level of interferent was considered to be acceptable if the error was not larger than 5%.

Many associated substances such as glucose, lactose, NaCl, formaldehyde, *p*-hydroxybenzoic acid and did not interfere. The most significant interference was from ascorbic acid and Na_2SO_3 at concentrations of 10 μg. However, they do not occur at such high concentrations in commercial preparations.

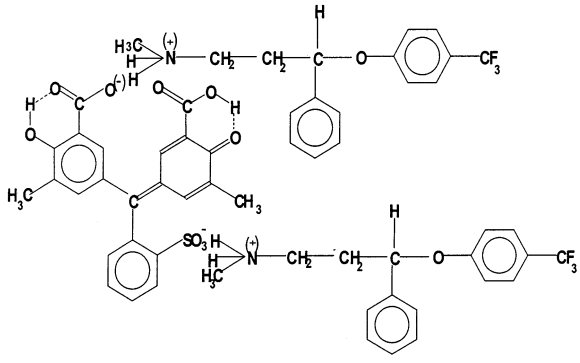
3.2. The composition and structure of the compounds

The composition of the compounds was studied by Job's method of continuous variation and by spectrophotometric titration. The obtained results showed that the molar ratio ECR:FXM = 1:2 and ECR:FXT = 1:2.

The absorption spectra in UV–VIS region of the compounds formed in the ECR–FXM and ECR–FXT systems were examined. It was found that the characteristic for eriochrome cyanine R bands of absorption at 220, 306, and 519 nm for the fluvoxamine maleate (at 208 and 246 nm) and for the fluvoxamine (at 220, 226 and 264 nm) were preserved in the compounds spectra. These observations seem to suggest that the investigated compounds can be classified as ion-association complexes.

The ion-association character of the formed compounds was confirmed by IR spectroscopy. IR spectra of the compounds occurred in the range 400–1700 cm^{-1} are the sum of the reagents (ECR, FXM or FXT) spectra. Significant changes in the IR spectra of the compounds are observed in the region 2300–3700 cm^{-1}. The absorption bands characteristic for vibrations of the N–H group in fluvoxamine and fluoxetine at 2400–2700 cm^{-1} are shifted to longer wavelengths in the spectra of compounds with decrease of their intensity. This suggests that compounds are formed with participation of the nitrogen atom from the tertiary amine group in the aliphatic chains of the FXM maleate and FXT hydrochloride.

From the analysis of the spectra obtained in the UV–VIS, IR regions it can be supposed that structure, e.g. for the compound ECR with FXT is the following:



This formula conforms the spectrophotometric results (Job's and spectrophotometric titration methods) and the molar ratio ECR:FXT = 1:2.

It is known from literature [13] that eriochrome cyanine R forms ECR^{2-} anion at pH 3.5–6.0. We suggest that between FXT or FXM cations and ECR anion occurs the electrostatic interaction with formation of ion-association compounds.

References

[1] J. Orsulak, T. Kennley, J. Debus, G. Crowley, R. Wittman, Clin. Chem. 34 (1988) 1875.
[2] P. Thomare, J. Chromatogr. B. 583 (1992) 217.
[3] B. Parodi, Pharmazie 48 (1993) 678.
[4] G. Misztal, B. Paw, Acta Polon. Pharm. 53 (1996) 177.
[5] C.E. Eap, P. Baumann, J. Chromatogr. B. 686 (1996) 51.

[6] S. Atmaca, Pharmazie 50 (1995) 300.
[7] J. Nash, J. Bopp, Clin. Chem. 28 (1982) 2100.
[8] V. Dixtit, H. Nguyen, J. Chromatogr. 563 (1991) 379.
[9] R. Lautz, Z. Farid, J. Koons, B. Tenbarge, R. Bopp, J. Chromatogr. 614 (1993) 175.
[10] A. Alhaider, Anal. Lett. 26 (1993) 887.
[11] S. Atmaca, Pharmazie 49 (1994) 458.
[12] R. Komorowski, Anal. Chem. 66 (1994) 1024A.
[13] N. Pollakova-Moukova, D. Gotzmannova, V. Kuban, L. Sommer, Coll. Czech. Chem. Commun. 46 (1981) 354.
[14] Xn. Yanju, Chen Xingguo, Hu Zhide, Anal. Lett., 20 (1987) 1001.
[15] Z. Marczenko, M. Jarosz, Analyst 109 (1984) 35.
[16] M. Jarosz, Chem. Anal. 31 (1986) 553.
[17] M. Jarosz, Analyst 111 (1986) 681.
[18] K. Kania, F. Buhl, Chem. Anal. 35 (1990) 775.
[19] J. Valero, Ann. Quim. Ser. B 81 (1985) 372.
[20] J. Karpinska, B. Starczewska, Pharmazie 54 (1999) 41.
[21] B. Starczewska, J. Trace Microprob. Tech. 16 (2) (1998) 151.
[22] B. Starczewska, J. Karpinska, Anal. Lett. 29 (15) (1996) 2475.