Egyptian Journal of Forensic Sciences (2016) xxx, xxx–xxx

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Egyptian Journal of Forensic Sciences

journal homepage: http://www.journals.elsevier.com/egyptian-journal-of-forensic-sciences

Simple spectrophotometric methods for determination of fluoxetine and clomipramine hydrochlorides in dosage forms and in some post-mortem biological fluids samples

Emam A. Ali [a,*], Ahmed M. Adawy [a], Mohamed F. El-Shahat [b], Alaa S. Amin [c]

[a] Forensic Chem Lab, Forensic Medicine Authority, Ministry of Justice, Cairo, Egypt
[b] Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt
[c] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

Received 14 November 2015; revised 22 April 2016; accepted 10 May 2016

KEYWORDS

Fluoxetine hydrochloride;
Clomipramine hydrochloride;
Phenol red;
Bromocresol green;
Metanil yellow;
Post-mortem urine and blood

Abstract Sensitive, simple and rapid spectrophotometric methods for micro determination of fluoxetine hydrochloride (FLU) and clomipramine hydrochloride (CLO). The methods are based on the reaction between the examined drugs and acid dyes, namely; bromocresol green (BCG), phenol red (PhR) and metanil yellow (MY) producing yellow ion-pair complexes followed by their extraction with methylene chloride and measured at 412, 407 and 409 nm for FLU with BCG, PhR and MY, respectively; whereas for CLO at 409, 406 and 407 nm, respectively. All variables that affect the performance of the proposed methods were carefully studied and optimized. Beer's law was obeyed in the concentration ranges 0.86–24.32 μg/mL, 8.64–41.30 μg/mL, 0.86–34.76 μg/mL for FLU and 1.75–24.55 μg/mL, 7.0–50 μg/mL, 1.65–34.78 μg/mL for CLO using BCG, PhR and MY respectively. The methods were validated in terms of accuracy and precision. The proposed methods were successfully applied to the determination of fluoxetine hydrochloride and clomipramine hydrochloride in pure samples, pharmaceutical formulations, spiked post-mortem urine and blood samples.

1. Introduction

Fluoxetine hydrochloride (Fig. 1a) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is chemically designated as N-Methyl-γ-[4-(trifluoromethyl)phenoxy] benzenepropanamine hydrochloride.[1] Fluoxetine is readily absorbed after oral administration and is extensively metabolized in the liver by demethylation. The primary active metabolite is norfluoxetine, which is excreted via the kidneys. Further metabolism can occur by O-dealkylation producing p-trifluoromethyl phenol and hippuric acid. 80% of a drug

* Corresponding author.
Peer review under responsibility of The International Association of Law and Forensic Sciences (IALFS).

http://dx.doi.org/10.1016/j.ejfs.2016.05.007

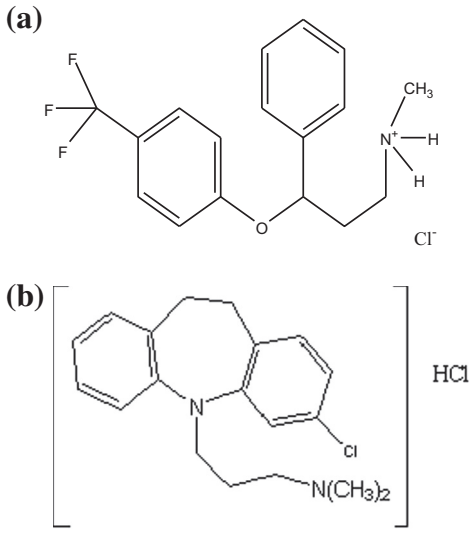

Figure 1 (a) Fluoxetine HCl $C_{17}H_{18}$ F_3NO = 309.3. (b) Clomipramine HCl $C_{19}H_{23}Cl$ N_2·HCl = 351.

dose is excreted in urine with less than 10% as the unchanged parent drug and 15% excreted in feces.[1–3]

Several techniques have been reported for determination of fluoxetine hydrochloride like spectrophotometry,[4,5] liquid chromatography (HPLC),[6–10] liquid chromatography/tandem mass spectrometry (LC–MS)[11] gas–mass chromatography, GC–MS,[12–14] potentiometric,[15] capillary electrophoresis,[16] and fluorescence.[17]

Clomipramine hydrochloride (Fig. 1b), chloro-10, 11-dihydro-N,N-dimethyl-5H-dibenz[b,f]azepine-5-propanamine hydrochloride.[1]

It is a cyclic antidepressant (CA) which is used to treat several psychiatric disorders, generalized anxiety disorder, depression, panic disorder, obsessive–compulsive disorder, eating disorders and attention deficit hyperactivity disorder.[2] Clomipramine hydrochloride is rapidly and completely absorbed after oral administration, but undergoes extensive first-pass N-demethylation to the major active metabolite, monodesmethyl clomipramine. Clomipramine and monodesmethyl clomipramine are further metabolized by 8-hydroxylation, N-oxidation, and conjugation. About 10–15% of a dose is excreted in the urine in 24 h, of which less than 0.2% is unchanged clomipramine or monodesmethyl clomipramine.[1–3]

Several methods described for the determination of clomipramine hydrochloride (CLO) including spectrophotometric[18,19] HPLC,[20–23] GCMass,[24–26] LC–MS,[27,28] chemiluminometric [29] and electrochemical.[30,31]

For screening of antidepressants in forensic blood samples, the strategies ought to be applicable for simultaneous determination of as many antidepressants, as possible in whole blood which is the most common sample matrix in forensic analysis.[32]

Although these sophisticated techniques are available for the determination of these drugs, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The aim of the present study is to develop a simple, accurate, economical, sensitive and less-time consuming spectrophotometric method for the determination of the fluoxetine hydrochloride (FLU) and clomipramine hydrochloride (CLO) in dosage forms and spiked post-mortem urine and blood samples. The methods are based on the reaction between these drugs and acid dyes, bromocresol green (BCG), phenol red (PhR) and metanil yellow (MY) producing a yellow ion-pair complexes followed by their extraction with methylene chloride.

2. Material and methods

2.1. Instrument

A SHIMADZU 1601 spectrophotometer with 1.0 cm quartz cell was used for recording all spectrophotometric measurements. Consort model P400 was used for adjustment of the pH. Temperature adjustment during experiments was carried out with controlled temperature water bath (MLW) Model, W11-TGL, GBR. Automatic pipettes were used to measure the very small volumes whereas glass micropipettes and burets were used to measure the large volumes.

2.2. Chemicals and reagents

Fluoxetine hydrochloride (FLU) and fluoxetine capsules (20 mg/cap) were provided from the Misr Pharmaceutical Company (Egypt). Clomipramine hydrochloride (CLO) and Anafranil tablets (25 mg/tab) were provided by the Novartis Pharma Company (Egypt). Chemicals used are (suppliers) as follows:

Phenol red, bromocresol green, metanil yellow, phosphoric acid, boric acid, sodium hydroxide, and acetic acid are products of Merck chemical company while sodium sulfate anhydrous is a product of the BDH chemical company and used without further purification. The most common solvents are chloroform (Lab-Scan product), methylene chloride, carbon tetrachloride, benzene (Sigma Aldrish products), petroleum ether, toluene, n-hexane and cyclohexane (Merck products). Doubly distilled water was used throughout for preparations of all aqueous solutions.

2.3. Preparation of buffer solution

Britton–Robinson (B.R.) buffer solution was prepared by mixing equal volumes of 0.4 M of three acids [phosphoric, acetic and boric acids]. A series of buffer solutions of pH 2.0–12 were prepared by adding an appropriate volume of 1.0 M sodium hydroxide as recommended by Britton.[33]

2.4. General procedure

In 100 ml separating funnel, 4.0 mL (1.0×10^{-3} M) of reagents (BCG, MY) and 5.0 mL (5.0×10^{-3} M) of PhR were added to different volumes of solution containing (1.0×10^{-3} M) of (FLU and CLO) and 3.0 mL of the optimum buffer solution and the volume was made up to 10 mL with bidistilled water and mixed well. The yellow formed ion-pairs were extracted by a separating funnel with 10 mL methylene chloride; the contents were shaken well for two minutes. Filter the methylene chloride layer over anhydrous sodium sulfate, then complete to 10 mL with the same solvent.[34,35] The absorbance of

Please cite this article in press as: Ali EA et al. Simple spectrophotometric methods for determination of fluoxetine and clomipramine hydrochlorides in dosage forms and in some post-mortem biological fluids samples, *Egypt J Forensic Sci* (2016), http://dx.doi.org/10.1016/j.ejfs.2016.05.007