Arabian Journal of Chemistry (2014) xxx, xxx–xxx

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride

Mahmoud A. Omar [a,*], Osama H. Abdelmageed [a,b], Sayed M. Derayea [a], Tamer Z. Attia [a,c]

[a] *Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt*
[b] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*
[c] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan*

Received 6 January 2013; accepted 16 December 2013

KEYWORDS

Spectrofluorimetric determination;
Antidepressants;
Dansyl chloride;
Dosage forms;
Human plasma

Abstract A reliable, sensitive and selective spectrofluorimetric method has been developed for the determination of certain antidepressant drugs namely sertraline hydrochloride, fluoxetine hydrochloride, paroxetine hydrochloride, amineptine hydrochloride and bupropion hydrochloride in pure forms, pharmaceutical formulation and human plasma. The method is based on the reaction of investigated drugs with 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride) in the presence of 0.5 M sodium carbonate to yield highly fluorescent derivatives, measured at 450 nm (excitation at 347 nm). The different experimental parameters affecting the development and stability of the reaction products were carefully studied and optimized. The calibration plots were constructed over the range of 0.02–0.14 μg mL^{-1}. The proposed method was successfully applied for analysis of cited drugs in dosage forms. The high sensitivity of the proposed method allows the determination of investigated drugs in spiked and real human plasma. Statistical comparisons of the results with the reference methods show an excellent agreement and indicate no significant difference in accuracy and precision.

1. Introduction

Depression is a chronic or recurrent illness that affects both economic and social functions of patients and can eventually lead to suicidal behaviors. Antidepressant medications have been used in the treatment of major depressive disorders (Parfitt, 2002). In the last years, prescriptions of antidepressants have increased dramatically in Egypt. Sertraline, paroxetine, fluoxetine, bupropion and amineptine are commercially

* Corresponding author. Tel./fax: +20 862369075.
E-mail address: momar1971g@yahoo.com (M.A. Omar).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1878-5352 © 2014 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.arabjc.2013.12.015

available antidepressant drugs extensively used in Egypt. The chemical structure of the studied antidepressant drugs in this work is shown in Table 1.

Several methods have been published for the determination of these drugs in bulk or in different pharmaceutical formulations as well as in biological fluids, these methods include volumetric methods (Bueno et al., 2000; Delazzeri, 2005), spectroscopic methods (Basavaiah and Sameer, 2010; Darwish, 2005; Darwish and Refaat, 2006; Mohamed et al., 2007; Onal et al., 2005, 2006; Sameer and Basavaiah, 2011), electrochemical methods (Atta-Politou et al., 2001; Nouws et al., 2006), chromatographic methods (Berzas Nevado et al., 2006; Sbarra et al., 1979, 1981; Tsaconas et al., 1989; Zainaghi et al., 2003; Zhu and Neirinck, 2002) and capillary electrophoretic methods (Labat et al., 2002; Mandrioli et al., 2002).

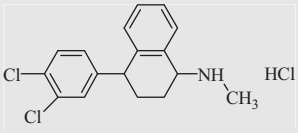
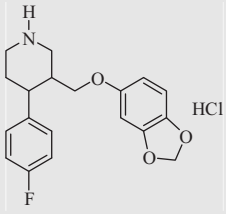
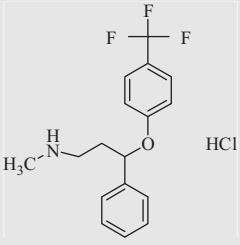
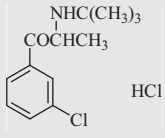
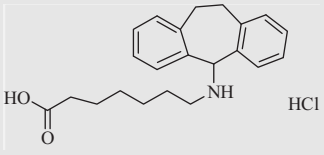
The wide use of these drugs necessitates the development of a rapid, accurate, sensitive, applicable and cheaper method for their determination in pure forms, pharmaceutical formulations, and spiked and real human plasma. So in this study, we describe a novel sensitive spectrofluorimetric method for the determination of these drugs depending on the presence of a secondary amine moiety.

2. Experimental

2.1. Apparatus

- A Perkin Elmer LS 45 Luminescence spectrometer (United Kingdom) connected to an IBM PC computer loaded with the FL WINLAB™ software.
- Spectronic™ Genesys™ 2PC. Ultraviolet/Visible spectrophotometer (Milton Roy Co, USA) with a matched 1 cm quartz cell connected to IBM computer loaded with winspec™ application software.

Table 1 Structural Formula of the studied antidepressant drugs.

Name	Chemical name [1]	Structure
Sertraline hydrochloride	(1S,4S)-4[3,4-dichlorophenyl]-1,2,3,4 tetrahydro-N-methyl-1-naphthylamin	
Paroxetine hydrochloride	(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine hydrochloride	
Fluoxetine hydrochloride	(3*RS*)-N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propane-1-amin hydrochloride	
Bupropion hydrochloride	1-(3-Chlorophenyl)-2-[(1,1-dimethylethyl) amino]-1-propanone hydrochloride	
Amineptine hydrochloride	Dihydro-10,1I-dibenzo[a,&ycloheptenyl-5-amino-7-heptanoic acid	

- Milwakee SM 101 pH meter, Portugal.
- Digital analytical balance (AG 29, Meltter Toledo, Glattbrugg, Switzerland).
- Laboratory centrifuge 4000 c/s (Bremsen ECCO, Germany).

2.2. Materials and reagents

All the chemicals used throughout this work were of analytical reagent grade and their solutions were prepared with double distilled water. Samples of investigated drugs were generously supplied by their respective manufacturers and were used without further purification.

* Sertraline hydrochloride (Pfizer Egypt, S.A.E., Cairo, Egypt), fluoxetine hydrochloride (EIPICO, El Asher Ramadan City, Cairo, Egypt), paroxetine hydrochloride (Pharaonia Pharmaceuticals Pharo Pharma, Alexandria, Egypt), amineptine hydrochloride (Servier Egypt Industries Limited, 6th October City, Giza, Egypt) and bupropion hydrochloride (Adwia Co., El Asher Ramadan City, Cairo, Egypt).
* 5-(Dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride), was purchased from Sigma (St. Louis, USA). A stock solution containing 3.71×10^{-3} M of dansyl chloride was freshly prepared in acetone and was further diluted with the same solvent to obtain 3.71×10^{-5} M solution.
* Sodium carbonate (El Nasr chemical co., Abu Zabbal, Egypt); 0.5 M aqueous solution (pH 10) was prepared by dissolving 13.3 g in 250.0 mL of double distilled water.
* Acetone was purchased from El Nasr chemical co., Abu Zabbal, Egypt.
* Plasma was kindly provided by Minia Hospital for psychiatric medicine and the study was performed and permitted according to institutional guidelines. Plasma samples were kept frozen until assay after gentle thawing.
* Chloroform (Merck, Darmstadt, Germany).

2.3. Pharmaceutical formulations

The following available commercial preparations were analyzed;

Lustral® tablets (Pfizer Egypt, S.A.E., Cairo, Egypt) labeled to contain 50.0 mg sertraline per tablet. Flutin® capsules (EIPICO, El Asher Ramadan City, Cairo, Egypt) labeled to contain 20.0 mg fluoxetine per capsule. Paxetin® tablets (Pharaonia Pharmaceuticals Pharo Pharma, Alexandria, Egypt) labeled to contain 20.0 mg of paroxetine per tablet. Survector® tablets (Servier Egypt Industrial Limited, 6th October City, Giza, Egypt) labeled to contain 100.0 mg of amineptine hydrochloride per tablet. Abstain® tablets (Adwia Co., El Asher Ramadan City, Cairo, Egypt) labeled to contain 150.0 mg of bupropion per tablet.

2.4. Preparation of standard solution

An accurate weight 40.0 mg of hydrochloric salts of each investigated CNS drugs was transferred into a 100-mL separating funnel containing about 20 mL distilled water. The resultant solution was rendered distinctly alkaline by a drop wise addition of 33% w/v aqueous ammonia then the librated free base was extracted with three potions of 20 mL chloroform and the combined chloroformic extracts were filtered through anhydrous sodium sulfate supported on Whitman filter paper into a 100-mL volumetric flak. The filter paper was washed thoroughly with two portions of 5 mL chloroform. The combined extracts and washings were diluted to volume with chloroform to provide a standard solution containing 400.0 μg mL^{-1}. 10.0 mL of chloroform extract was further distilled under vacuum and the oily residue was dissolved in 20.0 mL of mixture of acetone −0.5 M sodium carbonate (3:2) and transferred into a 100 mL volumetric flask then completed to the volume with the same solvent mixture to obtain a standard solution containing 40.0 μg mL^{-1}. This solution was further diluted with the same solvent mixture to prepare working standard solutions containing 0.20–1.40 μg mL^{-1} (200.0–1400.0 ng mL^{-1}). The standard solutions were stable for 7 days when kept in the refrigerator.

2.5. General procedure

Into a series of 10-mL volumetric flasks, 1.0 mL of working standard solution of drugs was transferred over the cited concentration range of 0.20–1.40 μg mL^{-1} and 0.7 mL of 3.71×10^{-5} M of dansyl chloride reagent was added and mixed well. The reaction mixture was left for 25 min, and then completed to the volume with acetone. The fluorescence intensity of the reaction products was measured at 450 nm after excitation at 347 nm. Blank experiment was carried out simultaneously. The relative fluorescence intensity of each sample solution for each investigated drug was accurately measured and plotted against the final concentration of the drug (ng mL^{-1}) to get the calibration graphs.

2.5.1. Determination of the studied drugs in pharmaceutical formulations (tablets and capsules)

A quantity of finely powdered twenty tablets or mixed capsule contents equivalent to 100.0 mg of active component was transferred to a 50-mL volumetric flask, sonicated for about 10 min with about 30 mL double distilled water then the volume was made up with distilled water, mixed well and filtrated. The first portion of the filtrate was discarded; 20 mL of the clear solution was transferred quantitatively to a 100-mL separating funnel. The contents of the funnel were rendered alkaline with a drop wise addition 33% w/v aqueous ammonia solution, and the procedure was completed as described for preparation of the stock standard solutions.

2.5.2. Procedure for spiked human plasma

5.0 mL of drug free human blood sample was taken from healthy volunteers into a heparinized tube, centrifuged at 3000 rpm for 30 min. Then 1.0 mL of the supernatant (plasma) was spiked with 1.0 mL of investigated drugs (2–14 μg mL^{-1}). 2.0 mL of acetonitrile was added as a precipitating agent for protein then centrifuged at 4000 rpm for about 20 min. The supernatant was rendered alkaline by adding 1.0 mL of 33% w/v aqueous ammonia and then extract 3 times with 3×3 mL of chloroform. The combined extracts were evaporated to dryness under vacuum. The residue was dissolved and diluted to volume with a mixture of 6.0 mL of acetone

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

and 4.0 mL of 0.5 M of sodium carbonate solution. Aliquots covering the working concentration range were transferred into 10-mL volumetric flasks. Then the general procedure was followed. A blank value was determined by treating the drug free blood sample in the same manner.

2.5.3. Procedure for real human plasma

For fluoxetine, 20.0 mg was taken orally once daily by three healthy human volunteers for 4 weeks. 5.0 mL of human blood sample was taken by using heperinized tube after an average of 6 h following the last oral administration and then centrifuged at 3000 rpm for 30 min. Then 3.0 mL of plasma obtained was treated with 2.0 mL of acetonitrile as precipitating agent for protein then centrifuged at 4500 rpm for about 20 min. The supernatant was rendered alkaline by adding 1.0 mL of 33% w/v aqueous ammonia and then extract 3 times with 3×3 mL of chloroform. The combined extracts were evaporated to dryness. The residue was dissolved and diluted to volume with a mixture of 6.0 mL of acetone and 4.0 mL of 0.5 M of sodium carbonate solution. Then the general procedure was followed.

For paroxetine, 40.0 mg was taken orally once daily by three healthy human volunteers for 14 days. 10.0 mL of human blood sample was taken by using heperinized tube after an average of 12 h following the last oral administration and then centrifuged at 3000 rpm for 30 min. Then 6.0 mL of plasma obtained was treated with 4.0 mL of acetonitrile as precipitating agent for protein and then centrifuged at 4500 rpm for about 20 min. Then the procedure was followed as described for fluoxetine.

For bupropion, 150.0 mg was taken orally every 12 h by three healthy human volunteers for 14 days. 5.0 mL of human blood sample was taken by using heperinized tube after an average of 6 h following the last oral administration. Then the procedure was followed as described for fluoxetine.

For sertraline, 50.0 mg was taken orally once daily by three healthy human volunteers for 14 days. 5.0 mL of human blood sample was taken by using heperinized tube after an average of 12 h following the last oral administration. Then the procedure was followed as described for fluoxetine.

For Amineptine, 100.0 mg was taken orally twice daily by three healthy human volunteers for 7 days. 5.0 mL of human blood sample was taken by using heperinized tube at 8th day after an average of 1 h following the last oral administration. Then the procedure was followed as described for fluoxetine.

3. Results and discussion

Dansyl chloride, is an important and widely used fluorescence reagent, was first introduced for the determination of some primary and secondary amines, imidazoles, phenol, etc. (Ayad and el-Hay, 1984; Frei-Hausler and Frei, 1973; Pütter, 1979). In the recent reports, dansyl chloride was further used as a fluorogenic reagent for determination of some pharmaceutical compounds (Cruces-Blanco et al., 2000; Houdier et al., 2000; Lucca et al., 2000).

3.1. Fluorescence spectrum

The fluorescence spectra of the sertraline as a representative example for investigated drugs in its reaction with dansyl chloride forming a highly intense yellow fluorescent derivative with emission at 450 nm after excitation at 347 nm are shown in Fig. 1. All other studied drugs exhibited similar spectra at the same excitation and emission wavelengths.

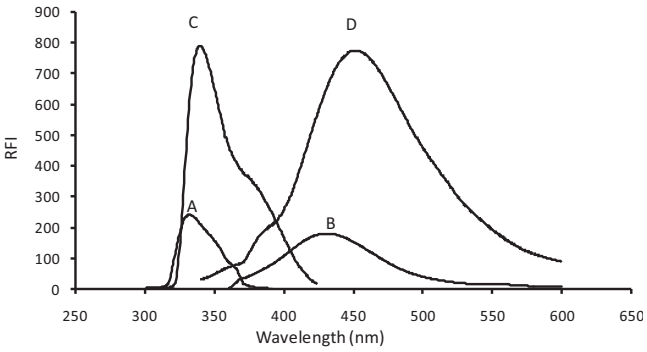


Figure 1 Fluorescence spectra where A and B are excitation and emission spectra of blank, while C and D are excitation and emission spectra of sertraline, as a representative example, (140.0 ng mL^{-1}) with dansyl chloride.

3.2. Optimization of variables

The spectrofluorimetric properties of the fluorescent products as well as the different experimental parameters affecting the development of the reaction product and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include pH, acetone and sodium carbonate ratio, concentration of the reagent, type of buffer, reaction time and dilution solvent

3.2.1. Effect of pH

The influence of pH on the relative fluorescence intensity of the reaction product was studied by using different molar concentrations of sodium carbonate solution (0.10–0.80 M). Maximum fluorescence intensity was obtained upon using 0.5 M sodium carbonate solution. The pH of the reaction mixture was found to be 10. Our experimental finding is in good agreement with previous reports where it was found that the optimum pH for densylation labeling of most amino acids, amines, imidazoles and phenols occurred at pH 9.5–10.5 (Seiler, 1970). The rate of dansylation process was found to increase with increasing the pH value this is due to an increase in the rate of hydrolysis of dansyl chloride into dansyl hydroxide (Seiler, 1970). The latter shows strong fluorescence and hence interferes seriously in the determination. However, under the proposed chosen conditions and wavelengths used, there was no interference arising from any dansyl hydroxide formed, as indicated by the low fluorescence intensity of the reagent. Since HCl is released during the reaction, buffering is always required. It was observed that sodium carbonate solution gave the highest relative fluorescence intensity while borate buffer and Teorell and Stenhagen buffer gave very low fluorescence intensity. So 0.50 M sodium carbonate solution is recommended in our experiment.

3.2.2. Effect of acetone: sodium carbonate ratio

By using different ratios of a mixture of acetone and 0.5 M sodium carbonate solution, it was found that the maximum

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

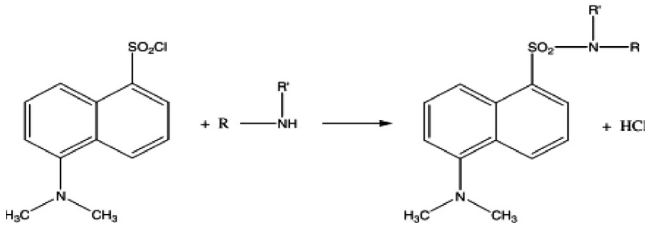


Figure 2 Proposed reaction pathway between dansyl chloride and investigated antidepressant drugs.

relative fluorescence intensity was obtained upon using mixture of acetone and 0.5 M sodium carbonate (3:2) solution.

3.2.3. Effect of concentration of dansyl chloride

The influence of the concentration of dansyl chloride was studied by different volumes (0.1–0.8 mL) of 3.71×10^{-5} M dansyl chloride. It was found that the reaction of dansyl chloride with investigated drugs started upon using 0.1 mL of the reagent in the presence of sodium carbonate (pH 10.0). Increasing the volume of the reagent, produces a proportional increase in the fluorescence intensity of the reaction product up to 0.6 mL and remains constant up to 0.8 mL after that a slight decrease in relative fluorescence intensity (RFI) occurs. Therefore, 0.7 mL of 3.71×10^{-5} M dansyl chloride solution was chosen as the optimal volume of the reagent.

3.2.4. Effect of reaction time

Different time intervals were tested. It was found that after 25 min, the reaction product reaches the highest fluorescence intensity and remains stable at room temperature for about an additional 20 min.

3.2.5. Effect of diluting solvent

Different diluting solvents were tried to dilute the reaction mixture throughout the study. It was found that acetone gave the highest relative fluorescence intensity. Dilution with 0.5 M sodium carbonate solution, water, and acetone–water almost produced very week fluorescence and did not reduce the fluorescence intensity of the blank. While upon using acetone, the fluorescence intensity attained its highest value, this was attributed to the low fluorescence value of the reagent.

3.3. Stoichiometry and mechanism of the reaction

The stoichiometry of the reaction mechanism was studied adopting job's method (Job, 1964) of continuous variation. The molar ratio of dansyl chloride to each of investigated drugs was 1:1. Based on the observed molar reactivity of the reaction, and depending on the presence of secondary amino group and by an analogy to similar reports dealing with the reaction of dansyl chloride with compounds containing secondary amino groups (Pesez and Bartos, 1974), the reaction pathway proposed is presented in Fig. 2.

Table 3 Evaluation of accuracy of the investigated analytical procedure at three concentration levels within the specified range.

Drug	% Recovery [a]		
	20.0 ng mL^{-1}	80.0 ng mL^{-1}	140.0 ng mL^{-1}
Sertraline	100.33 ± 1.06	99.75 ± 0.46	100.41 ± 0.27
Amineptine	99.77 ± 1.11	100.52 ± 0.28	99.99 ± 0.15
Paroxetine	99.64 ± 1.20	100.52 ± 0.46	100.20 ± 0.24
Bupropion	100.06 ± 1.26	101.02 ± 0.60	100.07 ± 0.18
Fluoxetine	100.27 ± 0.94	99.39 ± 0.62	99.45 ± 0.35

[a] Mean of 3 replicates ± SD.

3.4. Validation of the proposed method

3.4.1. Concentration range

Topic Q2A (1994) is established by confirming that the analytical procedure provides a suitable degree of precision, accuracy and linearity when applied to the sample containing amount of analyte within or at the extreme of the specified range of the analytical procedure (The United States Pharmacopoeia XXV and NF XX, 2002; Topic Q2B, 1996). In this work, concentration ranging from 20.0 to 140.0 ng mL^{-1} was studied for the investigated drugs. The whole set of experiments were carried out through this range to ensure the validation of the proposed procedure. Linear calibration graphs were obtained for all the studied drugs by plotting the RFI of the studied drugs versus the drug concentration (ng mL^{-1}) within the specified range. Linearity was indicated by a high correlation coefficient obtained. The correlation coefficients (r) of the formed products were in the range from 0.9990 to 0.9998 indicating good linearity, as shown in Table 2.

3.4.2. Accuracy

The United States Pharmacopoeia XXV and NF XX, 2002 was checked at three concentration levels within the specified range; six replicate measurements were recorded at each concentration level. The results were recorded as percent recovery ± standard deviation, as shown in Table 3. The results obtained show the close agreement between the measured and true values. Meanwhile, a comparison of the obtained results from the analysis of the drug products by the proposed procedure with those obtained from the reported methods

Table 2 Analytical parameters of spectrofluorimetric determination of investigated CNS drugs with dansyl chloride.

Investigated drugs	Linear range (ng/mL)	Intercept (a)	Standard deviation of intercept (Sa)	Slope (b)	Correlation coefficient (r)	LOD (ng/mL)	LOQ (ng/mL)
Sertraline	20.0–140.0	6.14	1.45	4.15	0.9993	1.049	3.497
Amineptine	20.0–140.0	3.43	1.76	3.78	0.9991	1.398	4.659
Paroxetine	20.0–140.0	1.29	1.84	3.49	0.9990	1.582	5.272
Bupropion	20.0–140.0	−1.43	2.41	3.31	0.9995	2.181	7.271
Fluoxetine	20.0–140.0	−13.29	2.38	3.19	0.9998	2.234	7.445

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

Table 4 Statistical analysis of the results obtained using the proposed and reference methods for spectrofluorimetric analysis of authentic samples using dansyl chloride.

Drug	Proposed methods ± SD (n = 5)	Reported method[8–10] ± SD (n = 5)
Sertraline	100.22 ± 0.93 t = 1.62 [a] F = 1.16 [a]	101.21 ± 0.99
Amineptine	100.40 ± 0.68 t = 0.46 F = 3.27	100.69 ± 1.22
Paroxetine	100.01 ± 1.21 t = 0.09 F = 1.06	100.08 ± 1.25
Bupropion	99.88 ± 0.77 t = 0.42 F = 2.65	100.16 ± 1.25
Fluoxetine	100.07 ± 0.78 t = 0.98 F = 2.77	100.74 ± 1.29

[a] Tabulated value at 95% confidence limit; F = 6.338 and t = 2.306.

Table 5 Evaluation of precision using spectrofluorimetric method for determination of investigated drugs with dansyl chloride.

Drug	Amount taken (ng mL^{-1})	% Recovery (±S.D) [a]
Sertraline	20	100.63 ± 0.9587
	80	99.75 ± 0.3863
	140	100.31 ± 0.2887
Amineptine	20	100.04 ± 1.063
	80	100.58 ± 0.4646
	140	99.99 ± 0.1996
Paroxetine	20	99.93 ± 1.147
	80	100.57 ± 0.4452
	140	100.18 ± 0.2462
Bupropion	20	99.81 ± 1.193
	80	100.74 ± 0.6133
	140	100.11 ± 0.3026
Fluoxetine	20	100.16 ± 1.135
	80	99.29 ± 0.7134
	140	99.65 ± 0.5267

[a] Mean of 3 replicates ± SD.

(Basavaiah and Sameer, 2010; Darwish, 2005; Mohamed et al., 2007) revealed that there is no significant difference between them with respect to accuracy as indicated by t- and F-tests, as shown in Table 4.

3.4.3. Precision

The United States Pharmacopoeia XXV and NF XX, 2002 was checked at three concentration levels, eight replicate measurements were recorded at each concentration level; the results are summarized in Table 5. The calculated relative standard deviations were all below 2.2% indicating excellent precision of the proposed method.

3.4.4. Limit of detection

Topic Q2A, 1994; Topic Q2B, 1996) was calculated based on the standard deviation of response and the slope of calibration curve. The limit of detection is expressed as:

$$\mathrm{LOD} = 3\sigma/\mathrm{S}$$

where σ is the standard deviation of intercept. S is the slope of calibration curve.

The results are summarized in Table 2. The calculated detection limits for all the studied drugs were less than 2.234 ng mL^{-1} indicating good sensitivity of the proposed method. According to USP *XXV* validation guidelines (The United States Pharmacopoeia XXV and NF XX, 2002), the calculated LOD values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOD equations were actually detected in these experiments.

3.4.5. Limit of quantitation

Topic Q2A, 1994 was calculated based on standard deviation of intercept and slope of calibration curve. In this method, the limit of quantitation is expressed as:

$$\mathrm{LOQ} = 10\sigma/\mathrm{S}$$

The calculated quantitation limits for all the studied drugs were all less than 7.445 ng mL^{-1}, as shown in Table 2, indicating good sensitivity of the proposed method. According to USP XXV validation guidelines (The United States Pharmacopoeia XXV and NF XX, 2002), the calculated LOQ values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOQ equations were actually quantitated in these experiments.

3.4.6. Specificity and interference

The specificity of the method was investigated by observation of any interference encountered from the common tablet excipients, such as talc, starch, gum acacia, lactose and magnesium stearate. This study indicates that the presence of these excipients did not interfere with the proposed method as proved by the excellent recoveries obtained as shown in Table 6. So the proposed method was found to be selective for the investigated drugs in the presence of these common excipients.

3.5. Application to pharmaceutical dosage forms

The proposed method was applied for the determination of investigated CNS drugs in commercial pharmaceutical dosage forms. The results of proposed method were statistically compared with those of reported methods (Basavaiah and Sameer,

Table 6 Analysis of the investigated drugs (100.0 ng mL^{-1}) in presence of some common excipients using the proposed spectrofluorimetric method with 3.71×10^{-5} M dansyl chloride.

Excipients	Amount Added (mg)	% Recovery[d] ± SD				
		Sertraline	Amineptine	Paroxetine	Bupropion	Fluoxetine
Starch	100	99.33 ± 0.78	99.19 ± 0.72	98.98 ± 0.64	100.80 ± 0.78	99.29 ± 0.89
Lactose	50	99.70 ± 0.51	99.83 ± 0.63	99.25 ± 0.66	100.73 ± 0.71	99.12 ± 0.98
Mg stearate	10	101.25 ± 0.54	99.47 ± 0.71	99.57 ± 0.47	99.86 ± 0.33	100.22 ± 1.04
Gum acacia	10	98.84 ± 0.87	99.42 ± 0.79	99.81 ± 0.85	100.28 ± 0.89	100.98 ± 0.46
Talc	10	100.38 ± 0.59	100.07 ± 0.61	99.11 ± 0.66	100.44 ± 0.91	100.81 ± 1.08

[d] Mean of 3 replicates ± SD.

2010; Darwish, 2005; Mohamed et al., 2007), in respect to accuracy and precision. The obtained mean recovery values of the obtained amount were 99.49–100.67 ± 0.98–1.09%, as shown in Table 7. According to *t*- and *F*-tests, no significant difference was found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates a good level of precision and accuracy.

3.6. Application to spiked human plasma

The high sensitivity attained by the proposed method allowed the determination of the studied drugs in spiked human plasma. The concentrations of investigated CNS drugs were computed from its corresponding regression equations. The obtained mean recovery values of the obtained amount were 97.44–101.61 ± 0.425–1.78%, as shown in Table 8. Thus it can be seen that the proposed method is suitable for the analysis of the investigated CNS in human plasma.

3.7. Analysis of cited drugs in real human plasma

Fluoxetine is metabolized into its active metabolite norfluoxetine (Lemberger et al., 1985). Norfluoxetine concentrations are approximately equal to those of the parent drug during chronic therapy (Brunswick et al., 2002). After a fixed daily dose of fluoxetine (20.0 mg day^{-1}), the concentration of the drug and its active metabolite in the blood continues to grow through the first few weeks of treatment, and their steady concentration in the blood is achieved only after 4 weeks (Pérez et al., 2001). The paroxetine is completely absorbed after oral administration and metabolized in the liver forming three main metabolites: the two isomers (3S,4R)-4-(4-fluorophenyl)-3-[(4-hydroxy-3-methoxyphenoxy)methyl]-piperidine (M1) and (3S,4R)-4-(4-fluorophenyl)-3-[(3-hydroxy-4-methoxyphenoxy) methyl]-piperidine (M2) and (3S,4R)-3-hydroxymethyl-4-(4-fluorophenyl) piperidine (M3) (Hiemke and Hartter, 2000). Steady-state plasma paroxetine concentrations were achieved after approximately 10 days following a 40-mg once daily dose (Mandrioli et al., 2007). The bupropion is mainly metabolized into hydroxybupropion. Steady state plasma level was achieved within 5 days while its metabolites within 8 days in healthy volunteers following a 150-mg dose of the extended-release tablet every 12 h (Briggs et al., 1993). The sertraline is mainly metabolized into N-desmethylsertraline. Steady state plasma concentration level for sertraline and its metabolite was achieved after approximately one week of a 50-mg once-daily dosing (Mandrioli et al., 2006; Package Insert, 1992). Amineptine is mainly metabolized by beta-oxidation of the side chain, its principle metabolites have the same structure as the parent compound except that its side chain is reduced to five carbon atom (Lachatre et al., 1989). Steady state plasma level was achieved at 8th day following two 100.0 mg doses per day (Rop et al., 1990).

% recovery of investigated CNS drugs and their metabolites in plasma was calculated by using the following equation

$$\%\,\text{Recovery}_{\text{invivo}} = (\text{concentration}_{\text{found}}/\text{concentration}_{\text{taken}}) \times 100$$

where% $\text{recovery}_{\text{in vivo}}$ is % recovery for drug in real human sample.$\text{Concentration}_{\text{found}}$ is concentration of the drug found in the real human sample.$\text{Concentration}_{\text{taken}}$ is concentration of the drug reported in the real human sample.

Table 7 Statistical analysis of the results obtained using the proposed spectrofluorimetric and reported methods for analysis of the investigated drugs in pharmaceutical dosage forms.

Drug	Pharmaceutical dosage forms	Proposed methods ± SD (n = 5)	Reported method[8–10] ± SD (n = 5)
Sertraline	Lustral® tablets	100.67 ± 1.03 t = 0.92 [a] F = 1.81 [a]	101.20 ± 0.77
Amineptine	Ramixol® tablets	100.04 ± 1.09 t = 0.20* F = 2.13*	101.18 ± 1.58
Paroxetine	Paxetin® tablets	99.49 ± 1.05 t = 0.45 F = 1.99	99.86 ± 1.49
Bupropion	Abstain® tablets	100.03 ± 0.99 t = 0.96 F = 1.35	100.68 ± 1.16
Fluoxetine	Flutin® capsules	100.01 ± 0.98 t = 1.02 F = 1.40	100.59 ± 0.82

[a] Tabulated value at 95% confidence limit; F = 6.338 and t = 2.306.

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

Table 8 Application of the proposed method for determination of studied CNS drugs in spiked human plasma.

Drug	20 ng mL^{-1}	% Recovery [a] 80 ng mL^{-1}	140 ng mL^{-1}
Sertraline	97.44 ± 1.784	98.96 ± 1.573	99.13 ± 1.13
Amineptine	101.54 ± 1.32	101.61 ± 0.946	98.84 ± 0.691
Paroxetine	101.23 ± 1.39	99.85 ± 1.04	98.53 ± 0.653
Bupropion	99.72 ± 1.06	101.49 ± 0.837	99.90 ± 0.592
Fluoxetine	98.86 ± 1.15	99.08 ± 0.773	98.21 ± 0.452

[a] Mean of 3 replicates ± SD.

Table 9 % recoveries after application of the proposed method for determination of investigated CNS drugs and their metabolites in real human plasma sample.

Drug	Intraday assay		Interday assay	
	Concentration $_{Found}$ [a]	% Recovery$_{invivo}$	Concentration $_{Found}$ [a]	% Recovery$_{invivo}$
Sertraline	26.20	81.24	25.57	79.30
	29.93	92.81	26.83	83.19
	27.35	84.81	28.91	89.64
Mean ± SD		86.29 ± 5.92		84.04 ± 5.22
Fluoxetine	23.98	80.75	26.24	88.35
	25.06	84.37	24.24	81.62
	25.51	85.90	22.68	76.37
Mean ± SD		83.67 ± 2.65		82.11 ± 6.01
Paroxetine	18.55	83.13	18.12	81.15
	20.58	92.23	20.36	91.24
	21.61	96.82	19.55	87.62
Mean ± SD		90.73 ± 6.97		86.67 ± 5.11
Bupropion	79.26	88.31	74.69	83.21
	81.34	90.62	80.01	89.14
	84.89	94.58	86.01	95.82
Mean ± SD		91.17 ± 3.17		89.39 ± 6.31
Amineptine	71.32	90.95	70.62	90.05
	75.63	96.44	74.58	95.10
	73.85	94.17	68.17	86.95
Mean ± SD		93.85 ± 2.76		90.69 ± 4.12

[a] mean of three determinations (investigated drugs with its metabolites in plasma samples) ng mL^{-1}.

% Recoveries after application of the proposed method for the determination of the investigated CNS drugs in the real human plasma sample by intra and inter day assay are shown in Table 9.

3.8. Determination of stability constant

The stability constant of the formed product was calculated using the following equation (Sawyer et al., 1984):

$$K_s = (A/A_{ex}C_x)/[(C_m - A/A_{ex}C_x)(C_L - nA/A_{ex}C_x)^n$$

where K_s is the stability constant of the formed product; $N = X/(1-X)$ where X is the mole fraction of the dansyl chloride at the maximum of the continuous variation curve; A/A_{ex} is the ratio of the observed relative fluorescence intensity to that indicated by the tangent for the same wavelength; C_m is the molar concentration of the dansyl chloride and C_L is the molar concentration of the investigated drugs.

$$C_x = C_L/n$$

The calculated stability constants for the formed fluorescent product of the investigated drugs ranged from 33.19×10^7 to 53.54×10^7 as shown in Table 10 indicating good stability of the formed product. The high stability constants of the formed products may account for their high relative fluorescence intensity.

Table 10 The calculated stability constant of the reaction of the investigated drugs with dansyl chloride.

Drug	$K_s \times 10^7$	Log K_s
Sertraline	53.54	8.73
Amineptine	39.01	8.59
Paroxetine	33.19	8.52
Bupropion	49.94	8.69
Fluoxetine	37.81	8.58

4. Conclusion

The proposed spectrofluorimetric method has the advantage of being a novel, fast, highly sensitive and low cost method for

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

determination of the investigated antidepressant drugs in pure forms, pharmaceutical formulations, spiked and real human plasma without any interference from common excipients present or other components that may be likely in denaturized plasma, and with minimum detection limits. Therefore, the developed method is suitable for a routine analysis of the investigated antidepressant drugs in quality control and clinical laboratories.

Acknowledgements

The authors express their gratitude to Dr. Monsef Mafouz a consultant psychiatrist and manager of Minia hospital for psychiatric medicine (Minia, Egypt) for providing the plasma samples.

References

Atta-Politou, J., Skopelitis, I., Apatsidis, I., Koupparis, M., 2001. In vitro study on *fluoxetine* adsorption onto charcoal using potentiometry. Eur. J. Pharm. Sci. 12 (3), 311–319.

Ayad, M.M., El-Hay, M.H., 1984. Spectrofluorimetric micro-determination of imidazoline derivatives using 1-dimethylaminonaphthalene-5-sulphonyl chloride. The Analyst 109 (11), 1431–1434.

Basavaiah, K., Sameer, A.M.A., 2010. Use of charge transfer complexation reaction for the spectrophotometric determination of bupropion in pharmaceuticals and spiked human urine. Thai J. Pharm. Sci. 34 (4), 134–145.

Berzas Nevado, J.J., Villaseñor Llerena, M.J., Guiberteau Cabanillas, C., Rodríguez Robledo, V., Buitrago, S., 2006. Sensitive capillary GC–MS-SIM determination of selective serotonin reuptake inhibitors: reliability evaluation by validation and robustness study. J. Sep. Sci. 29 (1), 103–113.

Briggs, G.G., Samson, J.H., Ambrose, P.J., Schroeder, D.H., 1993. Excretion of bupropion in breast milk. Ann. Pharmacother. 27 (4), 431–433.

Brunswick, D.J., Amsterdam, J.D., Fawcett, J., Quitkin, F.M., Reimherr, J.F., Beasley, C.M., 2002. Fluoxetine and norfluoxetine plasma concentrations during relapse-prevention treatment. J. Affect. Disord. 68 (2–3), 243–249.

Bueno, F., Bergold, A.M., Froehlich, P.E., 2000. Assay of fluoxetine hydrochloride by titrimetric and HPLC methods. Boll. Chim. Farm. 139 (6), 256–259.

Cruces-Blanco, C., Carretero, A.S., Peinado, S.F., Gutierrez, A.F., 2000. Spectrofluorimetric determination of methyl paraben in pharmaceutical preparations by means of its chloride derivative. Microchim. Acta 134 (1–2), 107–111.

Darwish, I.A., 2005. Development and validation of spectrophotometric methods for determination of fluoxetine, sertraline, and paroxetine in pharmaceutical dosage forms. J. AOAC Int. 88 (1), 38–45.

Darwish, I.A., Refaat, I.H., 2006. Spectrophotometric analysis of selective serotonin reuptake inhibitors based on formation of charge-transfer complexes with tetracyanoquinodimethane and chloranilic acid. J. AOAC Int. 89 (2), 326–333.

Delazzeri, L., 2005. Development of methods for the quality control of bupropion hydrochloride and paroxetine hydrochloride in compounding pharmacies. Caderno de Farmácia 21, 37–38.

Frei-Hausler, M., Frei, R.W., 1973. An investigation of fluorigenic labelling of chlorophenols with dansyl chloride. J. Chromatogr. 84 (1), 214–217.

Hiemke, C., Hartter, S., 2000. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol. Ther. 85 (1), 11–28.

Houdier, S., Perrier, S., Defrancq, E., Legrand, M., 2000. A new fluorescent probe for sensitive detection of carbonyl compounds: sensitivity improvement and application to environmental water samples. Anal. Chim. Acta 412, 221–233.

Job, P., 1964. Advanced Physicochemical Experiments, second ed. Oliner and Boyd, Edinburgh. Ann. Chem. 1936 (16), 97, p. 54.

Labat, L., Deveaux, M., Dallet, P., Dubost, J.P., 2002. Separation of new antidepressants and their metabolites by micellar electrokinetic capillary chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 773 (1), 17–23.

Lachatre, G., Piva, C., Riche, C., Dumont, D., Defrance, R., Mocaer, E., Nicot, V., 1989. Single-dose pharmacokinetics of amineptine and of its main metabolite in healthy young adults. Fundam. Clin. Pharmacol. 3 (1), 19–26.

Lemberger, L., Bergstrom, R.F., Wolen, R.L., Farid, N.A., Enas, G.G., Aronoff, G.R., 1985. Fluoxetine: clinical pharmacology and physiologic disposition. J. Clin. Psychiatry 46 (3), 14–19.

Lucca, A., Gentilini, G., Lopez-Silva, S., Soldarini, A., 2000. Simultaneous determination of human plasma levels of four selective serotonin reuptake inhibitors by high-performance liquid chromatography. Ther. Drug Monit. 22 (3), 271–276.

Mandrioli, R., Pucci, V., Visini, D., Varani, G., Raggi, M.A., 2002. Rapid methods for determination of fluoxetine in pharmaceutical formulations. J. Pharm. Biomed. Anal. 29 (6), 1127–1134.

Mandrioli, R., Saracino, M.A., Ferrari, S., Berardi, D., Kenndler, E., Raggi, M.A., 2006. HPLC analysis of the second-generation antidepressant sertraline and its main metabolite N-desmethylsertraline in human plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 836 (1–2), 116–119.

Mandrioli, R., Mercolini, L., Ferranti, A., Furlanetto, S., Boncompagni, G., Roggi, M.A., 2007. Determination of the antidepressant paroxetine and its three main metabolites in human plasma by liquid chromatography with fluorescence detection. Anal. Chim. Acta 591 (2), 141–147.

Mohamed, G.G., El-Dien, F.A., Mohamed, N.A., 2007. Utility of 7,7,8,8-tetracyanoquinodimethane charge transfer reagent for the spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides. Spectrochim. Acta A Mol Biomol. Spectrosc. 68 (5), 1244–1249.

Nouws, H.P., Delerue-Matos, C., Barros, A.A., Rodrigues, J.A., 2006. Electroanalytical determination of paroxetine in pharmaceuticals. J. Pharm. Biomed. Anal. 42 (2), 341–346.

Onal, A., Kepekçi, S.E., Oztunç, A., 2005. Spectrophotometric methods for the determination of the antidepressant drug paroxetine hydrochloride in tablets. J. AOAC Int. 88 (2), 490–495.

Onal, A., Kepekçi, S.E., Cetin, S.M., Ertürk, S., 2006. Spectrophotometric determination of certain antidepressants in pharmaceutical preparations. J. AOAC Int. 89 (4), 966–971.

Package Insert, Zolofi@, Pfizer Inc., 1992. Through Analytical Profile of Drug Substances, vol. 25, p. 443.

Parfitt, K., 2002. Martindale: The Complete Drug Reference, 33rd ed. Pharmaceutical Press, London, UK.

Pérez, V., Puiigdemont, D., Gilaberte, I., Alvarez, E., Artigas, F., 2001. Augmentation of fluoxetine's antidepressant action by pindolol: analysis of clinical, pharmacokinetic, and methodologic factors. J. Clin. Psychopharmacol. 21 (1), 36–45.

Pesez, M., Bartos, J., 1974. Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs. Marcel Dekker Inc., New York.

Pütter, J., 1979. A fluorometric method for the determination of praziquantel in blood-plasma and urine. Eur. J. Drug Metab. Pharmacokinet. 4 (3), 143–148.

Rop, P.P., Spinazzola, J., Bresson, M., Conquy, T., Viala, A., 1990. Determination of amineptine and its main metabolite in plasma by high-performance liquid chromatography after solid-phase extraction. J. Chromatogr. 532 (2), 351–361.

Sameer, A.A.M., Basavaiah, K., 2011. Application of ion association titration for the assay of bupropion hydrochlorides in pharmaceuticals. Chem. Ind. Chem. Eng. Q 17 (3), 299–306.

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015

Sawyer, D.T., Heinman, W.R., Beebe, J.M., 1984. Chemistry Experiments for Instrumental Methods. J. Wiley & Sons Inc., New York.

Sbarra, C., Negnm, P., Fanelh, R., 1979. Quantitative analysis of amineptine (S-1694) in biological samples by gas chromatography-mass fragmentography. J. Chromatogr. 162 (1), 31–38.

Sbarra, C., Castelh, M.G., Noseda, A., Fanelh, R., 1981. Pharmacokinetics of amineptine in man. Eur. J. Drug Metab. Pharmarmacokinet. 6 (2), 123–126.

Seiler, N., 1970. Use of the dansyl reaction in biochemical analysis. Methods Biochem. Anal. 18, 259–337.

The United States Pharmacopoeia XXV and NF XX, 2002. American Pharmaceutical Association, Washington, DC.

Topic Q2A, 1994. Text on validation of analytical procedure. In: International Conference on Harmonization (ICH).

Topic Q2B, 1996. Validation of analytical procedure. In: Methodology, International Conference on Harmonization (ICH).

Tsaconas, C., Padteu, P., D'Athts, P., Mocaer, E., Bromet, N., 1989. Gas chromatographic-mass spectrometric assessment of the pharmacokinetics of amineptine and its main metabolite in volunteers with liver impairment. J. Chromatogr. 487 (2), 313–329.

Zainaghi, I.A., Lanchote, V.L., Queiroz, R.H., 2003. Determination of paroxetine in geriatric depression by high-performance liquid chromatograph. Pharmacol. Res. 48 (2), 217–221.

Zhu, Z., Neirinck, L., 2002. High-performance liquid chromatography-mass spectrometry method for the determination of paroxetine in human plasma. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 780 (2), 295–300.

Please cite this article in press as: Omar, M.A. et al., Spectrofluorimetric protocol for antidepressant drugs in dosage forms and human plasma through derivatization with dansyl chloride. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2013.12.015