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Spectrofluorimetric determination of certain antidepressant drugs in human plasma

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Abstract

Background: Certain antidepressant drugs namely Sertraline hydrochloride, Fluoxetine hydrochloride, Paroxetine hydrochloride, Thioridazine hydrochloride and Amineptine hydrochloride were studied throughout this work using spectrofluorimetric method.

Methods: The spectrofluorimetric method is based on the charge-transfer reaction of these drugs as n-electron donors with 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π-electron acceptor. The drug-TCNQ complexes showed excitation maxima ranged from 290-301 nm and emission maxima ranged from 443-460 nm.

Results and discussion: The different experimental parameters affecting the formation and stability of the complexes were carefully studied and optimized. The calibration plots were constructed over the range of 50-450 ng mL-1 for Fluoxetine and Sertraline, 50-550 ng mL-1 for Paroxetine, 50-650 ng mL-1 for Thioridazine and 50-750 ng mL-1 for Amineptine. The proposed method was validated according to ICH and USP guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Conclusion: A simple, reliable, sensitive and selective spectrofluorimetric method has been developed for determination of certain antidepressant. The proposed method was successfully applied to the analysis of the cited drugs in dosage forms. The high sensitivity of the proposed method allows determination of investigated drugs in spiked and real human plasma.

Keywords: Antidepressant drugs,7,7,8,8-tetracyanoquinodimethane (TCNQ), Dosage forms, Human plasma, Spectrofluorimetric determination

Background

Depression: a common mental disorder 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 to treat all forms of major depressive disorders (Parfitt & Martindale 2002). In the last years prescription of antidepressants has increased dramatically in Egypt. Sertraline, paroxetine, fluoxetine and amineptine are extensively used as antidepressant drugs in Egypt while thioridazine is a potent antipsychotic agent which is used in treatment of depression accompanied with anxiety. The chemical structures of the studied drugs in this work are shown in Table 1. Several methods have been published for 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; Basavaiah et al. 1999), Spectroscopic methods (Onal et al. 2005; Darwish & Refaat 2006; Patel et al. 2009; Darwish 2005; Mohamed et al. 2005; Mohamed et al. 2007), Electrochemical methods (Nouws et al. 2006; Atta-Politou et al. 2001), Chromatographic methods (Zainaghi et al. 2003; Nevado et al. 2006; Meiling et al. 2002; Sbarra et al. 1979, 1981; Tsaconas et al. 1989) and Capillary electrophoretic methods (Labat et al. 2002; Mandrioli et al. 2002).

The wide use of these drugs necessitates the development of simple, accurate, sensitive, applicable and cheaper method for their determination in pure forms, pharmaceutical formulations, spiked and real

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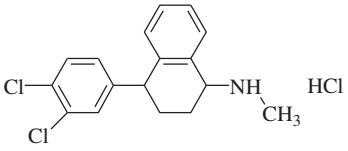
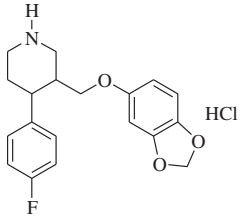
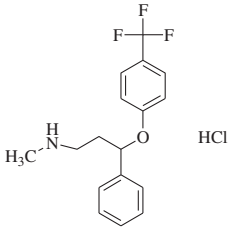
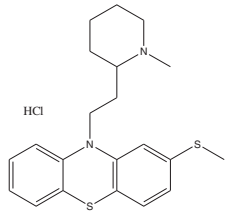
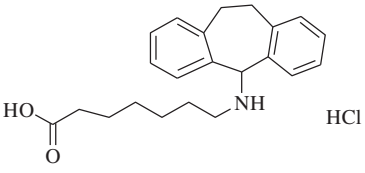
Table 1 Structural formula of the studied drugs

Name	Chemical name	Structure
Sertraline Hydrochloride	(1S,4S)-4 [3,4-dichlorophenyl]- 1,2,3,4 tetrahydro-N-methyl-1-naphthylamine	
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-amine hydrochloride	
Thioridazine Hydrochloride	10-[2-(1-methylpiperidin-2-yl) ethyl] -2-methylsulfanyl-phenothiazine hydrochloride.	
Amineptine Hydrochloride	dihydro-IO,1 I-dibenzo[a,&ycloheptenyl-5-amino-7-heptanoic acid	

human plasma. So this study describes a simple and very sensitive spectrofluorimetric method for determination of these drugs depending on the formation of charge-transfer complexes.

Experimental and Methods

Apparatus

- A perkin Elmer LS 45 Luminescence spectrometer (United Kingdom) connected to an IBM PC computer loaded with FL WINLABTM software.
- SpectronicTM GenesysTM 2PC. Ultraviolet/Visible spectrophotometer (Milton Roy Co, USA) with matched 1 cm quartz cell connected to IBM computer loaded with winspecTM application software.
- Milwakee SM 101 pH meter (Portugal).
- Digital analytical balance (AG 29, Meltter Toledo, Glattbrugg, Switzerland).
- Laboratory centrifuge 4000 rpm (Bremsen ECCO, Germany).

Materials and reagents

All materials were of analytical reagent grades and the 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 was kindly provided by Pfizer Egypt, S.A.E., Cairo, Egypt. Fluoxetine hydrochloride was kindly provided by EIPICO, El Asher Ramadan City, Cairo, Egypt. Paroxetine hydrochloride was kindly provided by Pharaonia Pharmaceuticals Pharo Pharma, Alexandria, Egypt. Amineptine hydrochloride was kindly provided by Servier Egypt Industries Limited, 6th October City, Giza, Egypt and thioridazine hydrochloride was supplied by Delta Pharm, S.A.E, El Asher Ramadan City, Cairo, Egypt.

7,7,8,8-tetracyanoquinodimethane (TCNQ) (Sigma Chemical Co., USA) was prepared as 1×10^{-3} M in acetonitrile. Solution was found to be stable for at least one week at 4°C. Acetonitrile, diethyl ether and methanol (Riendel-De-Haen AG, Germany). Chloroform, 1, 2 Dichloromethane, Ethanol and 33% W/V ammonia solution (El Nasr chemical Co., Abu Zabbal, Egypt).

Plasma was kindly provided by EL-Minia Hospital of Psychiatric medicine and kept frozen until assay.

Pharmaceutical formulations

The following available commercial preparations were analyzed; Lustral® tablets (Pfizer Egypt, S.A.E., Cairo, Egypt) labeled to contain 50 mg sertraline per tablet. Flutin® capsules (EIPICO, El Asher Ramadan City, Cairo, Egypt) labeled to contain 20 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. Thiozine® tablets (Delta Pharm, S.A.E, El Asher Ramadan City, Cairo, Egypt) labeled to contain 100 mg of Thioridazine hydrochloride per tablet.

Preparation of standard solutions

An accurately weighed 20.0 mg salt of each investigated drugs, was transferred into 125-mL separating funnel containing about 20 mL of distilled water. The resultant solution was rendered distinctly alkaline with dropwise addition of 33% w/v aqueous ammonia solution. The librated free base was extracted with three potions of 5 mL chloroform. The combined chloroformic extracts were filtered through anhydrous sodium sulfate supported on Whitman filter paper. 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 stock standard solution containing 200.0 μg mL^{-1}. This solution was further diluted with the same solvent to prepare working standard solutions containing 0.50 - 4.50 μg mL^{-1} of fluoxetine and sertraline, 0.50- 5.5 μg mL^{-1} of paroxetine, 1.0-6.5 μg mL^{-1} of thioridazine and 1.0-7.5 μg mL^{-1} of amineptine. The standard solutions were stable for seven days when kept in the refrigerator.

General analytical procedure

Into a series of 10 mL volumetric flasks, 1.0 mL of working standard solution of each drug was transferred over the cited concentration. One mL of 1×10^{-3} M TCNQ solution was added and mixed well. The reaction mixture was allowed to stand at room temperature (25.0 ± 5°C) for 40 min for fluoxetine, sertraline and paroxetine; 35 min for thioridazine and 45 min for amineptine then completed to the mark with chloroform. The fluorescence intensity of the complexes was measured at 443, 447, 447, 450 and 458 nm after excitation at 290, 291, 291, 295 and 301 nm for fluoxetine, sertraline, paroxetine, amineptine and thioridazine respectively. Blank experiment was carried out simultaneously. The relative fluorescence intensity of each sample solution for each investigated drugs was accurately measured and plotted against the final drug concentration (ng mL^{-1}) to get the calibration graphs.

Procedure for pharmaceutical formulations (tablets and capsules)

A quantity of finely powdered twenty tablets or mixed capsules contents equivalent to 100.0 mg of active component was transferred to 50-mL volumetric flask, sonicated for about 10 minute with about 30 mL double distilled water. The volume was made up with distilled water, mixed well and filtered. The first portion of the filtrate was discarded; twenty mL of the clear solution was transferred quantitatively to a 125 mL separating funnel. The contents of the funnel were rendered alkaline with dropwise addition 33% w/v aqueous ammonia solution, and the procedure was completed as described under preparation of the standard solutions.

Procedure for spiked human plasma

5.0 mL of drug free human blood sample was taken from three healthy volunteers into a heparinized tubes, centrifuged at 3000 rpm for 30 minutes then 1.0 mL of the drug free plasma (supernatant) was spiked with 1.0 mL of investigated drugs containing 5.0-45.0 μg mL^{-1} of fluoxetine and sertraline, 5.0- 55.0 μg mL^{-1} of paroxeine, 10.0- 65.0 μg mL^{-1} of thioridazine and 10.0-75.0 μg mL^{-1} of amineptine. 2.0 mL of acetonitrile was added as 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 the librated free base three times with 3×3 mL of chloroform. The combined chloroformic extracts were filtered through anhydrous sodium sulfate supported on Whitman filter paper. The filter paper was washed thoroughly with two portions of

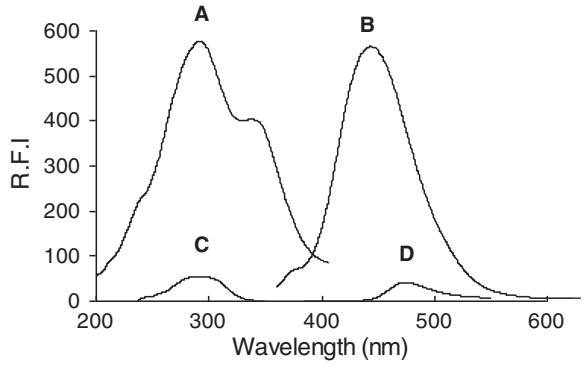


Figure 1 Fluorescence spectra of the produced CT-complex of fluoxetine (450 ngml^{-1}) with 1 × 10^{-3} M TCNQ where; A) Excitation spectrum B) Emission spectrum, C) Excitation spectrum of blank and D) Emission spectrum of blank.

5 mL chloroform. The combined extracts and washings were diluted to volume with chloroform. Aliquotes covering the working concentration range was 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.

Procedure for real human plasma

For fluoxetine, 20.0 mg was taken orally once daily by three healthy human volanteers for 4 weeks. 5.0 mL of human blood sample was taken by using heperanized tube after an average of 6 hrs following the last oral administration and centrifuged at 3000 rpm for 30 minute. 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 minute. The supernatant was rendered alkaline by adding 1.0 mL of 33% w/v aqueous ammonia followed by extraction with 3 × 3 mL of chloroform. The combined extracts were diluted to volume with chloroform; then the general procedure was followed.

For paroxetine, 40.0 mg was taken orally once daily by three healthy human volanteers for 14 days. 10.0 mL of human blood sample was taken by using heperanized tube after an average of 12 hrs following the last oral administration and centrifuged at 3000 rpm for 30 minute. 6.0 mL of plasma obtained was treated with 4.0 mL of acetonitrile as precipitating agent for protein then centrifuged at 4500 rpm for about 20 minute; then the procedure was followed as described for fluoxetine starting from "The supernatant was rendered alkaline by. . .".

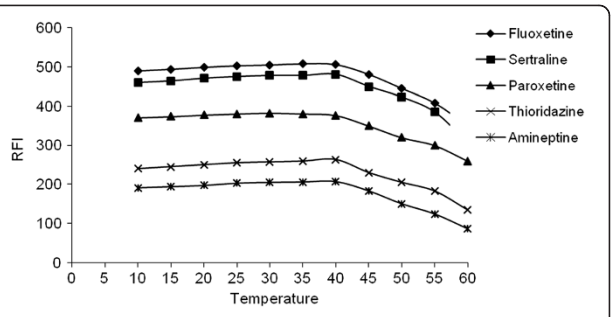


Figure 3 Effect of temperature on the RFI of the reaction product using TCNQ and 350 ng mL-1 of the studied drugs.

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

For thioridazine, 100.0 mg was taken orally four times daily by three healthy human volunteers for 7 days. 5.0 mL of human blood sample was taken by using heperanized tube at 8 th day, 3 hrs after the last morning oral administration; then the procedure was followed as described for fluoxetine.

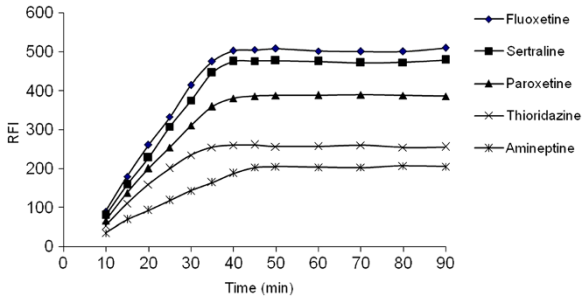


Figure 2 Effect of reaction time on the RFI of the reaction product using TCNQ and 350 ng mL-1 of the studied drugs.

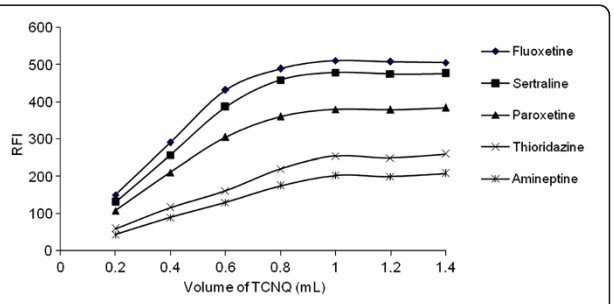


Figure 4 Effect of 1 × 10-3 M TCNQ volume on its reaction with 350 ng mL-1 of the studied drugs.

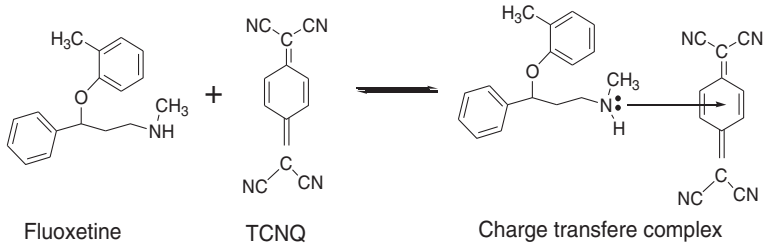


Figure 5 The suggested reaction pathway between fluoxetine as representative example of the studied drugs with TCNQ.

Results and discussion

The aim of this work is to establish a simple, sensitive, reliable, selective and cheap spectrofluorimetric method for the analysis of investigated drugs in pure forms, pharmaceutical formulations, spiked and real human plasma. The developed method was based on reaction of investigated drugs with 7,7,8,8-tetracyanoquinodimethane (TCNQ) to form highly fluorescent product, measured fluorometrically.

Fluorescence spectrum

Solutions of the studied drugs have very weak native fluorescence intensity, however in presence of TCNQ, the fluorescence intensity increases substantially. The formed CT complexes between the investigated drugs and TCNQ were probably through the lone pair of electron donated by the N atom in investigated drugs (n-donor) to TCNQ (π-acceptor). The fluorescence intensity of the reaction product was measured at 443, 447, 447, 450 and 458 nm after excitation at 290, 291, 291, 295 and 301 nm for fluoxetine, sertraline, paroxetine, amineptine and thioridazine respectively. Figure 1 shows the fluorescence spectra of the reaction product of fluoxetine as a representative example of investigated drugs TCNQ.

Optimization of variables

The spectrofluorimetric properties of the fluorescent product as well as the different experimental parameters affecting the development and stability of the CT-complex were carefully studied and optimized. Each factor was changed individually while the others were kept constant. The studied factors include diluting solvent, reaction time, temperature and concentration of the reagent.

In order to select the suitable solvent for CT-complex formation, the reaction of TCNQ with studied drugs was carried out in different solvents. The studied solvents are chloroform, acetonitrile, ethanol, methanol and 1,2-dichloroethane. It was found that chloroform was considered to be the best solvent for the fluorescence development proved by the highest RFI observed relative to other solvents.

The fluorescence intensity of the formed CT-complex was monitored at different time intervals. It was found that complete fluorescence developments were attained after 40 minutes for fluoxetine, sertraline and paroxetine; after 35 min for thioridazine and after 45 min for amineptine (Figure 2). The fluorescence intensity remained stable for at least 2 hours.

The effect of temperature on the formed charge transfer complexes was studied in the range of 10 - 60°C. All the formed complexes were stable up to 40°C. At temperature higher than 40°C, the RFI decreases probably due to dissociation of the complex. Thus, the determinations of studied drugs were carried out at 25 ± 5°C (Figure 3).

Different volumes of 1×10^{-3} M of TCNQ reagent were used ranging from 0.2 to 1.4 mL. It was observed that the relative fluorescence intensity (RFI) increases by increasing volume of TCNQ and reaches its maximum values at 1 mL of 1×10^{-3} M of TCNQ after which no further increase in RFI was observed. So 1 mL of 1×10^{-3} M of TCNQ was chosen as an optimum concentration for further investigation (Figure 4).

Table 2 Analytical parameters of spectrofluorimetric determination of investigated drugs with TCNQ

Investigated drugs	Linear range ng mL^{-1}	Intercept (a)	Standard deviation of intercept (Sa)	Slope (b)	Correlation coefficient (r)	LOD ng mL^{-1}	LOQ ng mL^{-1}
Fluoxetine	50-450	−0.20	2.58	1.45	0.9995	5.35	17.85
Sertraline	50-450	−2.18	2.82	1.36	0.9998	6.23	20.77
Paroxetine	50-550	0.63	2.45	1.10	0.9997	6.68	22.26
Thioridazine	100-650	−1.47	2.68	0.71	0.9992	11.37	37.90
Amineptine	100-750	−0.43	2.37	0.57	0.9990	12.48	41.61

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

Drug	50.0 ng mL^{-1}	Recovery %[a] 250.0 ng mL^{-1}	450.0 ng mL^{-1}
Fluoxetine	100.94 ± 1.29	99.80 ± 0.51	100.41 ± 0.28
Sertraline	99.84 ± 1.69	98.91 ± 0.55	100.14 ± 0.30
	50.0 ng mL^{-1}	**Recovery % [a] 250.0 ngmL^{-1}**	**550.0 ngmL^{-1}**
Paroxetine	100.92 ± 1.28	100.09 ± 0.67	100.06 ± 0.31
	100.0 ngmL^{-1}	**Recovery % [a] 350.0 ngmL^{-1}**	**650.0 ngmL^{-1}**
Thioridazine	100.44 ± 1.57	101.31 ± 0.95	100.48 ± 0.41
	100.0 ngmL^{-1}	**Recovery % [a] 450.0 ngmL^{-1}**	**750.0 ngmL^{-1}**
Amineptine	100.14 ± 1.87	98.81 ± 0.72	100.47 ± 0.44

[a] Mean of Six replicate measurements.

Stoichiometry and Mechanism of the reaction

The stoichiometric of the reaction mechanism was studied adopting the job's method (Job 1964) of continuous variation. The molar ratio of TCNQ to each of investigated drugs was 1:1. The reaction pathway proposed in Figure 5 is presented.

Validation of the proposed method

Concentration range (Topic Q2A 1994) was established by confirming that the analytical procedure provided 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 (Topic Q2B 1996; The United States Pharmacopoeia XXV and NF XX 2002). In this work, concentration ranging from 50 to 450 ng mL^{-1} (for fluoxetine and sertraline), 50 to 550 ng mL^{-1} (for paroxetine), 100 to 650 ng mL^{-1} (for thioridazine) and 100 to 750 ng mL^{-1} (for amineptine) were studied. The whole set of experiments were carried out within 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 high correlation coefficient obtained. The correlation coefficients (r) of the formed products were in the range 0.9990 to 0.9998 indicating good linearity, as shown in Table 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 expressed as percent recovery ± standard deviation (Table 3). The obtained results show the close agreement between the measured and true values. Meanwhile, comparison of the obtained results from the analysis of the drug products by the proposed procedure with those obtained from the reported methods (Darwish 2005; Mohamed et al. 2005, 2007) revealed that their is no significant difference between them with respect to accuracy as indicated by *t*- and F- tests (Table 4).

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 below 2.2% indicating excellent precision of the proposed procedure at both levels of repeatability and intermediate precision.

Limit of detection (Topic Q2A 1994) was calculated based on standard deviation of response and the slope of calibration curve (Topic Q2B 1996). The limit

Table 4 Statistical analysis of the results obtained using the proposed procedures and reference method for spectrofluorimetric analysis of authentic samples using TCNQ

Drug	% Recovery ± SD		t-value [b]	F-value [b]
	Proposed methods	Reported method [#]		
Fluoxetine	100.34 ± 0.89	99.95 ± 1.08	0.62	1.44
Sertraline	99.61 ± 1.57	99.67 ± 1.61	0.06	1.05
Paroxetine	99.29 ± 1.02	99.38 ± 1.20	0.13	1.40
Thiridazine	100.32 ± 1.24	100.39 ± 1.30	0.09	1.09
Amineptine	100.37 ± 1.04	100.57 ± 1.69	0.23	2.63

[b]Tabulated value at 95% confidence limit; F = 6.338 and *t* = 2.306.
[#]References (Darwish 2005; Mohamed et al. 2005, 2007).

Table 5 Evaluation of precision of the proposed spectrofluorimetric method for the determination of the investigated drugs

Drug	Conc. μg/ml	Mean[c]	S.D	RSD
Fluoxetine	50	100.84	1.33	1.32
	250	98.81	0.62	0.63
	450	100.47	0.41	0.41
Sertraline	50	99.77	1.50	1.50
	250	98.87	0.50	0.51
	450	100.17	0.28	0.27
Paroxetine	50	100.71	1.32	1.31
	250	100.14	0.69	0.69
	550	100.08	0.28	0.28
Thioridazine	100	100.86	1.58	1.57
	350	101.31	0.76	0.75
	650	100.46	0.37	0.37
Amineptine	100	100.10	1.84	1.84
	450	98.77	0.65	0.66
	750	100.53	0.39	0.39

[c]mean is average of eight determinations.

of detection was expressed as (The United States Pharmacopoeia XXV and NF XX 2002):

$$\mathrm{LOD} = 3\sigma/S \quad (1)$$

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 12.48 ng mL^{-1} indicating good sensitivity of the proposed method.

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 (The United States Pharmacopoeia XXV and NF XX 2002):

$$\mathrm{LOQ} = 10\sigma/S \quad (2)$$

The calculated quantitation limits for all the studied drugs were all less than 41.61 $ngmL^{-1}$, as shown in Table 2, indicating good sensitivity of the proposed method. So it can be applied for analysis of drug in biological fluids.

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.

Application to pharmaceutical dosage forms

The proposed method was applied for determination of investigated drugs in commercial pharmaceutical dosage forms. The results were statistically compared with those of reported methods (Darwish 2005; Mohamed et al. 2005, 2007), in respect to accuracy and precision. The obtained mean recovery values were 100.62-101.01 ± 0.66-1.39%, as shown in Table 7. According to t- and F- tests, no significant difference was found between the proposed and reported methods at 95% confidence level. This indicates good level of precision and accuracy.

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 their corresponding regression equations. The obtained mean recovery values of the obtained amount were 99.76-100.39 ± 1.33 - 1.99% (Table 8).

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. 2002a). After a fixed daily dose of fluoxetine (20.0 mg day^{-1}), the

Table 6 Analysis of the investigated drugs (100.0 ng mL^{-1}) in presence of some common excipients using the proposed spectrofluorimetric method

Excipients	Amount Added μgmL^{-1}	% Recovery [d] ± SD				
		Fluoxetine	Sertraline	Paroxetine	Thioridazine	Amineptine
Starch	50	100.07 ± 0.61	99.19 ± 0.72	99.85 ± 0.87	100.80 ± 0.78	101.29 ± 0.79
Lactose	50	98.21 ± 0.91	99.83 ± 0.63	98.78 ± 0.61	100.73 ± 0.71	98.12 ± 0.45
Mg stearate	50	101.25 ± 0.54	98.24 ± 1.25	101.58 ± 1.61	99.86 ± 0.33	101.62 ± 1.07
Gum acacia	50	99.25 ± 0.59	99.76 ±1.01	98.82 ± 0.86	100.28 ± 0.89	101.98 ± 0.49
Talc	50	100.38 ± 1.58	100.18 ±0.25	98.21 ± 0.67	99.70 ± 0.51	99.81 ± 0.34

[d]Average of three determinations.

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 method ± SD (n = 5)	Reported methods [8-10] ± SD (n = 5)
Fluoxetine	Neurazine® tablets	100.94 ± 1.390	100.53 ± 1.46
		t = 0.45 [e] F = 1.09 [e]	
Sertraline	Thiozine® tablets	100.18 ± 1.15	100.13 ± 1.68
		t = 0.06 F = 2.13	
Paroxetine	Stellasil® tablets	100.01 ± 1.196	99.98 ± 0.95
		t = 0.04 F = 1.59	
Thioridazine	Tryptizol® tablets	100.62 ± 0.663	100.22 ± 1.07
		t = 0.71 F = 2.59	
Amineptine	Survector® capsules	100.14 ± 1.26	99.88 ± 1.59
		t = 0.29 F = 1.59	

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

concentration of the drug and its active metabolite in the blood continued to grow through the first few weeks of treatment, and their steady concentration in the blood is achieved only after four weeks (Pérez et al. 2001; Brunswick et al. 2002b). 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), (3S,4R)-4-(4-fluorophenyl)-3-[(3-hydroxy-4-methoxyphenoxy)methyl]-piperidine(M2) and (3S,4R)-3-hydroxymethyl- 4-(4-fluorophenyl) piperidine (M3) (Hiemke & Hartter 2000). Steady-state plasma paroxetine concentrations were achieved after approximately 10 days following 40-mg once daily dose (Mandrioli et al. 2007). Thioridazine is mainly metabolized into mesoridazine and sulphoridazine. Steady-state plasma thioridazine concentrations were achieved after approximately 7 days following four 100-mg doses per day (Vanderheeren & Muusze 1977). Sertraline is mainly metabolized into N-desmethylsertraline. Steady state plasma concentration level for sertraline and its metabolite were achieved after approximately one week of a 50-mg once-daily dosing (Package Insert, Zolofi@, Pfizer Inc 1992; Mandrioli et al. 2006). Amineptine is mainly metabolized by beta-oxidation of the side chain, its principle metabolites has 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 is achieved at 8 th day following two 100.0 mg doses per day (Rop Pok et al. 1990).

According to the reported metabolic pathway of all the cited drugs; the proposed method can be used specifically for determination of fluoxetine and sertraline only in presence of their metabolites in plasma because the metabolic products are considered as compounds containing primary

Table 9 % Recoveries after application of the proposed method for determination of investigated CNS drugs in real human plasma sample

Drug	Intraday assay	Interday assay
	% $Recovery_{invivo}$	% $Recovery_{invivo}$
Fluoxetine		
Mean ± SD	91.53 ± 5.11	86.28 ± 5.27
Sertraline		
Mean ± SD	88.83 ± 5.38	85.50 ± 5.947
Paroxetine		
Mean ± SD	76.76 ± 4.82	75.28 ± 7.94
Thioridazine		
Mean ± SD	91.53 ± 5.11	92.26 ± 2.73
Amineptine		
Mean ± SD	86.29 ± 6.44	87.41 ± 3.87

Table 8 Application of the proposed method to the determination of studied drugs in spiked human plasma

Concentration ($ngmL^{-1}$)	% Recovery [f]				
	Fluoxetine	Sertraline	Paroxetine	Thioridazine	Amineptine
50	97.79	100.12	102.40	99.01	100.84
100	98.62	97.68	99.20	99.36	101.64
150	103.03	103.22	97.54	100.05	102.39
250	99.11	98.56	99.46	97.21	98.84
350	98.42	100.76	98.22	101.64	99.31
450	101.10	99.85	101.55	99.40	99.56
550	-	-	99.72	99.25	101.61
650	-	-	-	102.19	98.76
750	-	-	-	-	100.61
Mean ± SD	99.68 ± 1.99	100.03 ± 1.92	99.73 ± 1.73	99.76 ± 1.565	100.39 ± 1.33

[f]Mean of three replicate measurements.

amino group (as norfluoxetine and norsertraline); which should not interfere upon application of the suggested procedure, while for thioridazine, paroxetine and amineptine, their metabolites can interfere with the determination of the parent drugs because they contain the same function group (secondary or tertiary amine moiety) as well.

So % recovery of fluoxetine and sertraline in plasma were calculated by using the following equation

$$\%\text{Recovery}_{\text{in vivo}} = (\text{concentration}_{\text{found}}/\text{concentration}_{\text{taken}}) \times 100 \quad (3)$$

Where,

% Recovery $_{\text{in vivo}}$ is % recovery for drug in real human sample.
Concentration $_{\text{found}}$ is concentration of the drug founded in real human sample.
Concentration $_{\text{taken}}$ is concentration of the drug reported in real human sample.
While % recovery of thioridazine, paroxetine and amineptine and their metabolites in plasma were calculated by using the same equation

Where,

% Recovery $_{\text{in vivo}}$ is % recovery for drug and their metabolites in real human sample.
Concentration $_{\text{found}}$ is concentration of the drug and their metabolites founded in real human sample.
Concentration $_{\text{taken}}$ is reported concentration of the drug and their metabolites in real human sample.
% Recoveries after application of the proposed method for determination of investigated CNS drugs in real human plasma sample by intra and inter day assay are shown in Table 9.

Conclusion

The proposed spectrofluorimetric method has the advantage of being simple, highly sensitive and low cost method for determination of the investigated antidepressant drugs in pure forms, pharmaceutical formulations, without any interference from common excipients present and with minimum detection limits. Furthermore the proposed method was successfully applied for analysis of the cited drugs in spiked and real human plasma. Therefore, the developed method can be considered as suitable for routine analysis of investigated antidepressant drugs in quality control and clinical laboratories. Also it is suitable for selective determination of fluoxetine and sertraline only without their metabolites in human plasma.

Competing interests

All authors declare that there is no competing of interest.

Authors' contributions

Dr Mahmoud M. Omar and Dr. Sayed M. Derayea proposed the idea and design the experimental section. Dr Tamer Z. Attia, Dr. Sayed M. Derayea and Dr Mahmoud M. Omar carried out the experimental parts and participated in sequence alignment and drafted the manuscript. All authors participated in preparation of the discussion and result section. Dr Osama H. Abdelmageed and Dr. Tadayuki Uno revised the final manuscript. Finally all authors read and approved the final manuscript.

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