



SEPARATION AND IDENTIFICATION OF ANTIDEPRESSANT DRUGS IN HUMAN PLASMA BY SPE-TLC METHOD

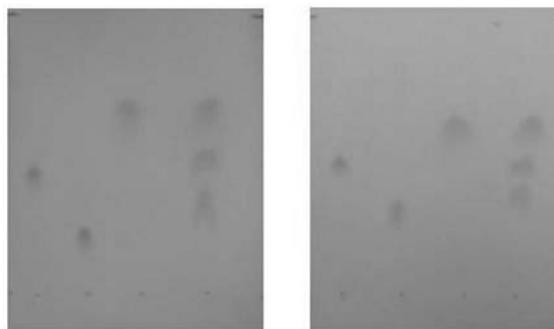
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Development and validation of thin layer chromatography (TLC) and solid phase extraction (SPE) for the separation and identification of simple mixtures of antidepressant drugs (fluvoxamine maleate, paroxetine and sertraline) was achieved by using 2-propanol-dichloromethane (DCM) (70:30, v/v) at 30±1 °C. R_f values were 0.44, 0.22 and 0.68 for fluvoxamine maleate, paroxetine and sertraline, respectively on normal silica plates. The metal ions Fe(II), Ni(II), Cu(II) and Zn(II) of 0.1% concentration, were used to study their effect on the separation of the analytes under study. The R_f values of these drugs on impregnated plates were in the range of 0.18 to 0.64. The separation of these drugs on plain and impregnated plates was compared and it was found that the best separation was on Ni(II) impregnated plate with compact spots. The limits of detection of these drugs were also calculated and found to be 0.1-0.2 ngmL⁻¹ for both plain and impregnated plates. The values of the resolution data (R) for fluvoxamine maleate, paroxetine and sertraline were greater than 1.0 and, hence, complete separation. The solid phase extraction conditions of these drugs were phosphate buffer (50 mM, pH 9.0) with 0.10 mL min⁻¹ as flow rate. The best eluting solvent was methanol containing 0.1% acetic acid for all anti-depressant; with 0.1 mLmin⁻¹ flow rate. The percentage recoveries of fluvoxamine maleate, paroxetine and sertraline drugs were 41%, 35% and 33%, respectively. The values of standard deviation, correlation coefficient and confidence level for the TLC (R_f), SPE (percentage recovery) and UV-Visible spectrometric methods for fluvoxamine maleate, paroxetine and sertraline were in a range of ±0.14-0.33, 0.9992-0.9998 and 95.00-98.75% respectively.



INTRODUCTION

Nowadays, depression is becoming a common problem worldwide, which is a recurrent mood disorder; that affect the normal life of human beings. According to World Health Organization, approximately 121 million people are facing this

problem. It is estimated that this disease will be the second leading one for both sexes by the end of 2020. For the treatment of depression various types of antidepressants are used.¹⁻³ Among the most commonly used anti-depressant drugs are selective serotonin reuptake inhibitors (SSRI) viz fluvoxamine maleate, paroxetine and sertraline

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(Fig. 1). Fluvoxamine maleate, paroxetine and sertraline are a class of potent and selective inhibitors of serotonin reuptake, in synapse and presynapse with little effect on other monoamine reuptake mechanisms or other neurotransmitter receptors. Fluvoxamine (5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone-*o*-(2-aminoethyl)oxime) effects α_1 receptors,⁴ with neuromodulatory role in the brain, (relieving anxiety, stress, depression, learning and cognitive processes, neuroprotection and antipsychotic activity).⁵ Paroxetine ((3*S*-*trans*)-3-[(1,3-benzodioxol-5-yl)oxy)methyl]-4-(4-fluorophenyl)-

piperidine) enhances serotonergic neurotransmission by prolonging serotonin activity at its postsynaptic receptors.⁶⁻⁹ Sertraline [((1*S*,4*S*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine)] effects the monoamine reuptake mechanisms or other neurotransmitter receptors, with the exception of the dopamine transporter, which is not considered to be of therapeutic consequence.¹⁰ In spite of their essential role for controlling depression, these medications result into some side and toxic effects. Therefore, monitoring of these drugs in plasma is an essential need.

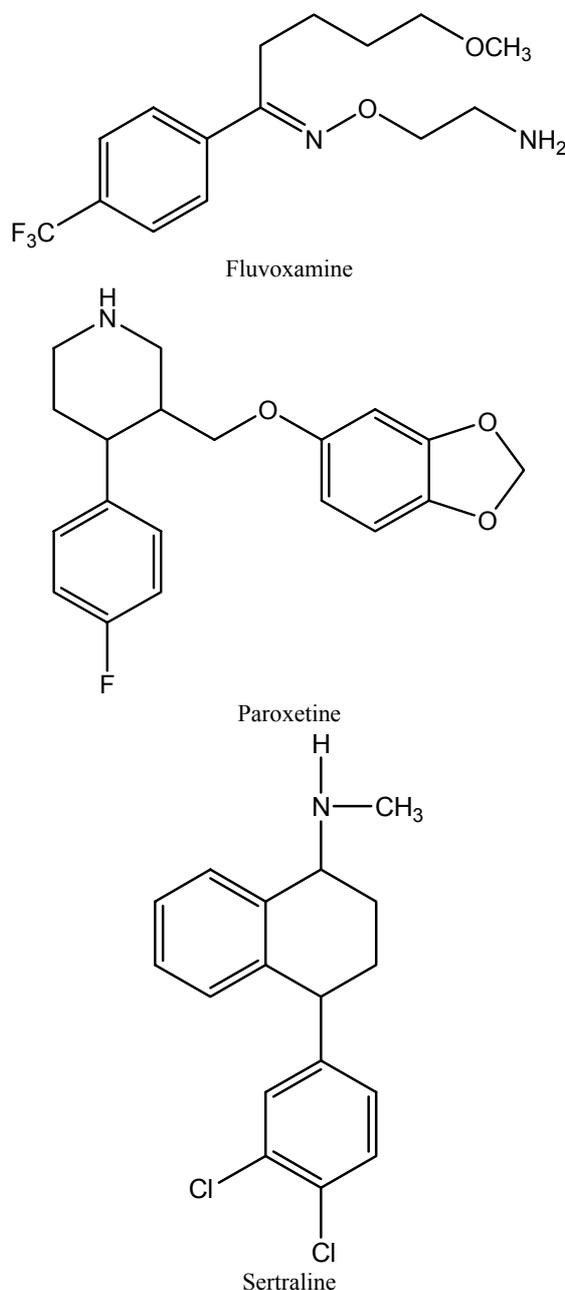


Fig. 1 – Chemical structure of anti-depressant drugs.

A thorough search of literature indicates only few analytical methods for monitoring of these drugs in biological fluids using high performance liquid chromatography (HPLC) technique.¹¹⁻¹³ However, thin layer chromatography (TLC) is a classical modality of chromatography but still has its importance due to its unique features such as being selective, rapid and relatively inexpensive in nature with ease of operation compared to HPLC. Furthermore, TLC method development is the real demand for these drugs in developing countries.¹⁴⁻¹⁶ It has been observed that about 80 percent chromatographers are using solid phase extraction (SPE) as the versatile method of sample preparation.¹⁷ Literature survey also indicates that no method is available for monitoring of these drugs in human plasma by using SPE-TLC combination, which is the need of developing and under developing countries. In view of these facts, attempts have been made to develop a relatively inexpensive, selective and reproducible SPE-TLC methods for analyses of the reported antidepressant in human plasma.

EXPERIMENTAL

1. Chemicals, reagents and instruments

Fluvoxine (50 mg) tablets (Batch No. AD71489); containing fluvoxamine maleate; were purchased from Sun Pharmaceutical Industries Bari Brahma Jammu (J & K). Pari CR tablets; (Batch No. AXG7003R) containing 2.5mg/tablet paroxetine were obtained from Ipca Laboratories Ltd., Mumbai. Daxid (100 mg) tablets (Batch No. 520-72105E) with sertraline as active ingredient were supplied by Pfizer Limited Navi Mumbai. Methanol, *n*-Hexane, 1-butanol, dichloromethane and 2-propanol of HPLC grade were supplied by Merck, India. Ninhydrin GR (2, 2-dihydroxyindan-1,3-dione), acetone, iodine and silica gel G, disodium hydrogen phosphate and *o*-phosphoric acids were purchased from Merck, India. Sulphuric acid and glacial acetic of AR grade acids were obtained from Qualigens, India. Standard solutions (0.10 mg mL⁻¹) of these drugs were prepared in methanol. These drugs were detected on TLC by using a reagent; developed by dissolving 500 mg ninhydrin GR (2, 2-dihydroxyindan-1,3-dione) in 20 mL acetone. Purified water was prepared using a Millipore Milli-Q (Bedford, MA, USA) water purification system, C₁₈ cartridges were purchased from Waters USA. Solid phase extraction unit of Varian USA, pH meter of Control Dynamics (model APX 175 E/C), Spectrophotometer of Perkin Elmer (model EZ201) and centrifuge (model C854/49/06) of Remi were used. Fresh frozen Human plasma (mfg. License No. 504) was purchased from Rotary Blood bank, New Delhi India.

2. Extraction of drugs from commercial tablets

Fluvoxamine maleate, paroxetine and sertraline were extracted from commercially available tablets. Five tablets of

each drug were weighed and crushed to powder, separately and respectively. The powdered drugs were extracted with methanol (100 mL) with sonication and at 50°C. Methanol was separated by centrifugation at 10000 rpm (11180 g). The residue was extracted two more times with same amount of methanol separately. All the methanol fractions were combined together to get 300 mL. This methanol was evaporated under vacuum on water bath to 15 mL, which was allowed to crystallize in freeze at 10°C. The mother liquor was decanted and the crystals were washed with a little amount of *n*-hexane. The purities of the extracted drugs were ascertained by recording their melting points and UV spectra.

3. Solid phase extraction

The experiments were carried out by mixing 1 mL of each drug (1 mgmL⁻¹) into 5.0 mL human plasma individually and separately. The spiked samples were vortexed for 2 minutes, kept for 30 minutes, mixed with 15 mL acetone and centrifuged at 10000 rpm (11180 g) for 5 minutes to separate the supernatant. The supernatant was evaporated to dryness and the residue was re-dissolved in 10 mL phosphate buffer (50 mM, pH 9.0). A Sep-Pak C₁₈ cartridge (1 mL Waters, USA) was pre-conditioned with 2 mL methanol followed by 5 mL distilled water. Spiked sample were passed through cartridge with 0.1 mLmin⁻¹ flow rate followed by cartridge washing with 2 mL triple distilled water at same flow rate. The cartridge was dried by passing hot air and the drugs were eluted by 10 mL methanol containing 0.1% acetic acid at 0.1 mLmin⁻¹ flow rate. The eluted methanol was concentrated under vacuum to 0.5 mL. This solution was used for TLC studies. Solid phase extraction method was been developed by various factors such as flow rate of buffer, eluting solvent, pH of eluting solvent and buffers have been varied to achieve the maximum extraction of these drugs.

4. Preparation of TLC plates

TLC plates (10 cm x 15 cm x 0.5 mm) were prepared in the laboratory by spreading slurry of silica gel G (50 g) in 100 mL triple distilled water. These plates were dried overnight in an oven at 80°C. For impregnated TLC plates, silica gel slurry was prepared with triple distilled water containing 0.10 g of FeSO₄.7H₂O, CoSO₄.7H₂O, NiCl₃.6H₂O, Cu(CH₃COO)₂.H₂O and ZnSO₄.7H₂O in 100 mL distilled water individually and respectively. The plates were activated by heating in an oven at 100°C.

5. Thin layer chromatography

TLC plates were developed in a paper lined rectangular glass chamber (25 x 25 x 12 cm.) containing mobile phase and pre-equilibrated for 30 minutes. Various mobile phases combination such as 1-butanol-dichloromethane and 2-propanol-dichloromethane of different ratio have been developed and used to achieve the separations of the above cited drugs. Different impregnated TLC plates of FeSO₄.7H₂O, CoSO₄.7H₂O, NiCl₃.6H₂O, Cu(CH₃COO)₂.H₂O and ZnSO₄.7H₂O have been used for separation of these drugs. The optimization of chromatographic separations of these drugs has been attained by using different combinations of solvents in mobile phases and concentrations of impregnating reagents in TLC. Besides, the mobile phases and the amount loaded have also been optimized to achieve the maximum separation. The developed simple methods have been used to analyze the contents of selective serotonin reuptake inhibitors (SSRIs) in human plasma.

6. Qualitative analysis of antidepressants

TLC plates were spotted of these drugs at 10.0 ng level with the help of graduated capillaries (10.0 μL) and the chromatograms were developed in TLC chamber pre-equilibrated with solvents for 30 minutes. The chromatograms were developed up to 10.0 cm for 20 minutes by using 2-propanol-DCM (70:30, v/v) at 30 ± 1 °C. After development the plates were dried at 80°C and the spots were detected by the developing reagent on heating plates at 80°C for 15 minutes. Solvent system was optimized by varying the concentrations of 2-propanol and DCM. The analyses of fluvoxamine maleate, paroxetine and sertraline; after extraction by SPE; were also carried out by TLC using the same developed method. The R_f values were calculated. The qualitative determination of these drugs in plasma was ascertained by comparing the R_f values of these drugs with those of standards.

7. Quantitative analysis of antidepressants

For quantitative analyses the spots (10 μL) from five TLC plates were scratched and dissolved in methanol (5.0 mL), which was concentrated to 1.0 mL for determining their concentrations by UV-Vis Spectrophotometer at 282 nm for fluvoxamine maleate and paroxetine and 290 nm for sertraline. The calibration curves were linear for all drugs from 0.1 to 0.01 mg mL^{-1} concentrations. For validation, all the experiments were carried out for five times under the identical experimental conditions and the validation data were calculated by using Microsoft excel software.

RESULTS AND DISCUSSION

1. Extraction of drugs from commercial tablets

Fluvoxamine maleate, paroxetine and sertraline were extracted from commercially available tablets. Five tablets of each drug were weighed, crushed to powder and extracted with methanol. The percentage recoveries of these drugs were in the range up to 98%.

2. Solid phase extraction (SPE)

Fluvoxamine maleate, paroxetine and sertraline were mixed with human plasma to carry out their biological interactions with human plasma proteins. A fraction of fluvoxamine maleate, paroxetine and sertraline were bound with proteins while the remaining free unbound fractions were extracted from plasma by SPE. The optimization of SPE was achieved by different concentrations and pHs of phosphate buffer, flow rate of plasma sample and eluting solvents. The optimum experimental conditions for phosphate buffer were 50 mM, pH 9.0, and 0.1 mL min^{-1} as flow rate. The elution of fluvoxamine maleate, paroxetine and sertraline

from C_{18} was achieved using various solvents such as methanol, ethanol, ethyl acetate, dichloromethane. These solvents were used as pure solvents or containing different amounts of acetic acid or trifluoroacetic acid. As a result of an extensive experimentation the best eluting solvent was methanol containing 0.1% acetic acid for fluvoxamine maleate, paroxetine and sertraline; with 0.1 mL min^{-1} flow rate. The percentage recoveries of fluvoxamine maleate, paroxetine and sertraline in human plasma were 41%, 35% and 33% and chromatogram were shown in (Fig. 2).

3. Thin layer chromatography

R_f values of fluvoxamine maleate, paroxetine and sertraline were obtained by dividing the distance travelled by these drugs by the solvent front (10.0 cm). The results are given in Table 1, which indicates that the R_f value of fluvoxamine maleate, paroxetine and sertraline were 0.44, 0.22 and 0.68. The spots of paroxetine and sertraline showed a slight tailing (Fig. 2). Therefore, attempts have been made to separate these drugs on metal ion impregnated silica gel plates. The metal ions used were Fe(II), Ni(II), Cu(II) and Zn(II) of 0.1% concentration, separately. The mobile phase used was 2-propanol-DCM (70:30, v/v). The results of these findings are given in Table 1. The R_f values of the above cited drugs were in the order of sertraline > fluvoxamine maleate > paroxetine. The R_f value of sertraline was found to be highest and it may be due to the absence of any oxygen atom, which could have interacted with silanol groups through hydrogen bonds. R_f value of fluvoxamine was higher than paroxetine because the presence of two and three oxygen atoms in the molecules; having strong hydrogen bonding with silanol group in paroxetine than fluvoxamine. R_f values of these drugs on impregnated plates were in the range of 0.18 to 0.64. A comparison of R_f values indicated that the best separation was on Ni(II) impregnated plates. Furthermore, a comparison of separation on Ni(II) impregnated plate was carried out with plain plate. It has been observed that the best separation was on Ni(II) impregnated plate as spots were quite compact. The compact spot on Ni(II) impregnated plate may be due to the complex formation of these drugs with metal ion. Of course, these drugs also form complexes with other metal ions but the behavior of Ni(II) ion complexes resulted in to compact spots. The limits of detection of these drugs were also calculated and found to be 0.1-0.2 ng mL^{-1} on both plain and Ni(II)

impregnated plates. TLC chromatograms of these three drugs on plain and nickel impregnated plates are shown in (Fig. 2) indicating better separation of these drugs on nickel metal ion impregnated plates.

The resolution data (resolution factor) for the reported drugs were calculated by the following equation:

$$R(\text{Resolution Factor}) = \frac{d}{r_1 + r_2}$$

where,

r_1 = radius of spot 1

r_2 = radius of spot 2

d = distance between two spots

The values of the resolution data for like fluvoxamine maleate, paroxetine and sertraline are given in Table 2. The separation of the drug is considered complete if the resolution value is 1.0 or more. This table indicates that for all the three analytes the value of R is greater than 1.0 and, hence, the complete separation.

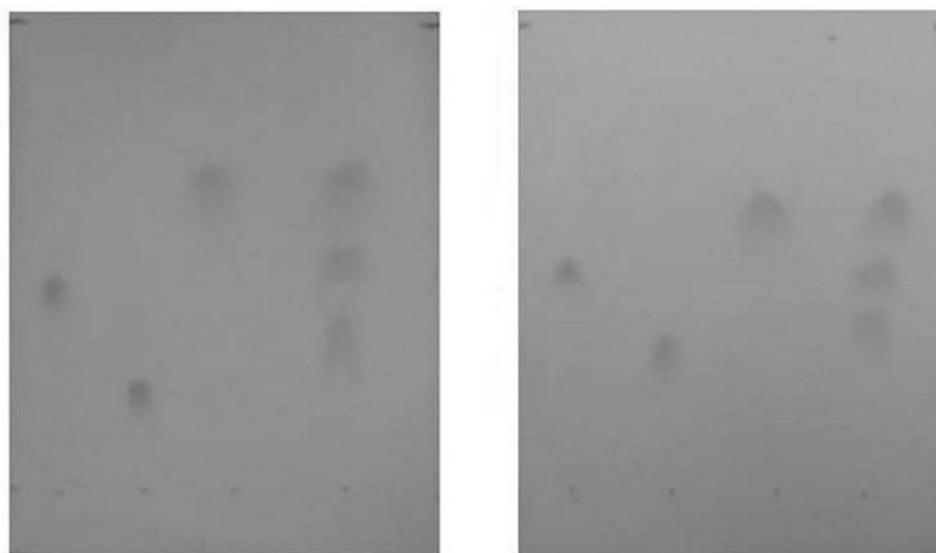


Fig. 2 – TLC Chromatograms, (a): normal and (b): Ni (II) impregnated silica gels.

Table 1

R_f values and detection limits of antidepressant drugs in standard solutions and plasma samples

Antidepressant Drugs	R_f Values on Plain Silica Gel	R_f Values on Plain Impregnated Silica Gel				LOD (ng)
		Fe(II)	Ni(II)	Cu(II)	Zn(II)	
Fluvoxamine	0.44	0.39	0.50	0.24	0.18	0.2
Paroxetine	0.22	0.24	0.32	0.23	0.18	0.2
Sertraline	0.68	0.51	0.64	0.50	0.44	0.1

Table 2

Resolution data of antidepressant drugs

	Fluvoxamine	Paroxetine
Sertraline	R	R
Paroxetine	R	

Table 3

Validation data for antidepressant drugs under study

Compounds	Standard Deviation	Correlation Coefficient	Confidence Level (%)
Fluvoxamine			
R_f	±0.15-0.26	0.9996	98.00
% Recovery	±0.25-0.31	0.9995	98.50

Table 3 (continued)

Linearity test	±0.18-0.29	0.9997	98.70
Limit of Detection	±0.11-0.14	0.9998	98.75
Limit of Quantification	±0.12-0.16	0.9998	98.75
Paroxetine			
R _f	±0.16-0.27	0.9995	97.00
% Recovery	±0.27-0.33	0.9994	98.00
Linearity test	±0.19-0.30	0.9996	98.10
Limit of Detection	±0.14-0.17	0.9997	98.15
Limit of Quantification	±0.13-0.17	0.9997	98.25
Sertraline			
R _f	±0.14-0.24	0.9994	95.00
% Recovery	±0.24-0.30	0.9992	96.00
Linearity test	±0.17-0.28	0.9994	98.00
Limit of Detection	±0.13-0.16	0.9995	98.00
Limit of Quantification	±0.11-0.15	0.9994	98.10

n=5

Validation of the methods

The validation of SPE, TLC and UV-Visible spectrometric methods data was carried out by usual methods through Microsoft Excel Program. The results of the validation parameters are given in Table 3. Validation data of fluvoxamine maleate, paroxetine and sertraline for TLC (R_f), SPE (percentage recovery) and UV-Visible spectrometric methods show that the values of standard deviation for these methods were ±0.15 to 0.31, ±0.16 to 0.33 and ±0.14 to 0.30, respectively. The standard deviation of limits of detection (LOD) and limit of quantification (LOQ) on TLC for fluvoxamine maleate, paroxetine and sertraline were ±0.11 to 0.16, ±0.13 to ±0.17 and 0.11 to 0.16, respectively. The values of correlation coefficients (R) for TLC, SPE and UV-Visible spectrometric methods were 0.9995 to 0.9998, 0.9994 to 0.9997 and 0.9992 to 0.9995, respectively for different parameters such as R_f, % recovery, linearity test, limit of detection and limit of quantification. The confidence levels were 98.00 to 98.75%, 97.00 to 98.25% and 95.00 to 98.10 %, for TLC, SPE and UV-Visible spectrometric methods respectively. These validation parameter values show good reproducibility of the reported methods.

CONCLUSIONS

The results in this paper indicated that the developed SPE-TLC method is relatively inexpensive, reproducible, selective and efficient, which can be used to analyse these antidepressants in human plasma samples. These techniques also provide appreciable percentage recoveries of fluvoxamine maleate, paroxetine and sertraline from the human plasma samples.

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