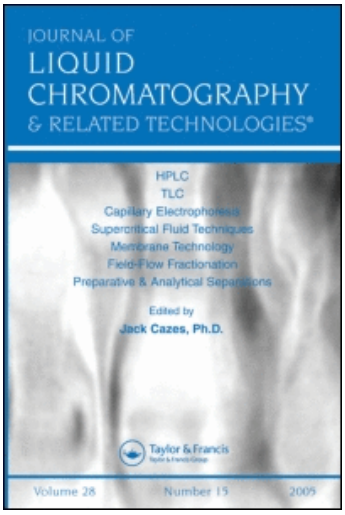

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY AND DRUG DISSOLUTION STUDIES OF FLUOXETINE HYDROCHLORIDE IN CAPSULE FORMULATIONS

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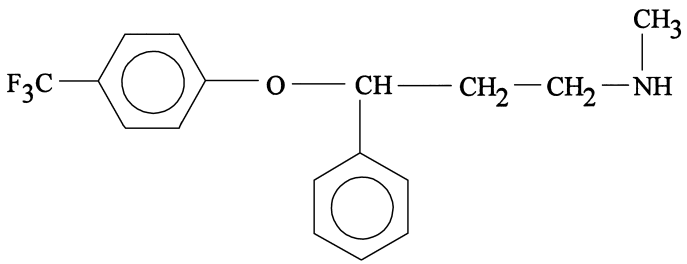
ABSTRACT

A sensitive and simple high performance liquid chromatographic method for the assay of fluoxetine HCl was developed. The procedure is based on the use of the reversed-phase high performance liquid chromatographic method with UV detector. Each analysis required no longer than 6 minutes. The detector response was linear in the range of 0.01-50 μg/mL for fluoxetine HCl. The detection limit was found to be 0.0057 μg/mL. There was no significant difference between interday and intraday stud-

ies for fluoxetine HCl determined for two different concentrations. This method was applied, without any interferences from the excipients, for the determination of the drug in capsules and in drug dissolution studies. This method can be useful in routine quality control analysis of fluoxetine HCl pharmaceutical dosage form.

INTRODUCTION

Fluoxetine[N-Methyl-3-phenyl-3-(α,α,α-trifluoro-p-tolyoxy)-propylamine] is an antidepressant which selectively inhibits the re-uptake of serotonin. It is reported to cause fewer antimuscarinic side-effects than tricyclic antidepressants. Its mode of action in depression is not fully understood. The concomitant administration of a sedative may be required for anxiety and insomnia. Fluoxetine HCl has also been tried in obesity and alcohol abuse.[1-4] Adverse effects reported with fluoxetine HCl include nausea, nervousness, insomnia, and anxiety, headache, tremor, drowsiness, and dry mouth.[5] Fluoxetine HCl is readily absorbed from the gastro-intestinal tract and extensively metabolised in the liver to its primary active metabolite desmethyl fluoxetine HCl and other metabolites.[6]



Structure of Fluoxetine

Fluoxetine has been determined by spectrophotometry,[7] gas chromatography,[8-10] flow injection analysis,[11] capillary electrophoresis,[12] fluorimetry,[13] electrochemistry,[14] and recently by enzymeimmunoassay.[15] Several high pressure liquid chromatographic (HPLC) procedures have been reported for the determination of fluoxetine HCl in biological fluids and in pharmaceutical formulations.[16-23] Recently, Raggi et al.[24] published a report on the analytical methods for the quality control of fluoxetine capsules.

Studies on the bioavailability of drugs from a given dosage form revealed that, in many situations various dosage forms with the same content of the active compound did not give the same therapeutic effect. This is ascribed to differences in physical characteristics of the active compound in formulation

factors or in technological processes used by different manufactures, therefore, resulting in different bioavailability profiles.[25] The dissolution test has been widely used in areas of quality control.

Pharmaceutical or in vitro bioavailability is one of the aspects of drug bioavailability. Among all tests that can be performed on drug solids, dissolution testing is considered to be sensitive, reliable, and rational for predicting in vivo drug bioavailability behaviour.

The main purpose of an oral solid pharmaceutical dosage form is to make available a certain and defined amount of the active substance to the human body, through the gastrointestinal system.[26] The pharmaceutical industry and the regulatory agencies focus on the evaluation of the drug release kinetics from dosage forms.

Up to date, no examination by dissolution rate studies of fluoxetine HCl in capsule dosage forms has appeared in the literature. The objective of the work described in this paper is to develop a simple, sensitive, and direct high performance liquid chromatographic method with UV detector, available for the quantitation of fluoxetine HCl in its pharmaceutical solid dosage forms, for quality control purposes. The developed HPLC method is also compared to the spectrophotometric method as both methods were applied to the in vitro dissolution rate studies of the drug from the capsule dosage forms.

EXPERIMENTAL

Apparatus

The chromatographic apparatus (Waters Assoc., Milford, MA, USA) consisted of model 510 pumps and a model 717 plus autosampler, and a model 481 UV spectrophotometric detector. The chromatograms were analyzed with a chromatographic workstation (Baseline 810). Injection volume of 50 µL was used. Spectrophotometric measurements were carried out using Shimadzu 2100 double beam UV-Vis spectrophotometer.

Dissolution rate and quantitative determination studies were realised at 226.2 nm wavelength. The dissolution rates of fluoxetine from capsules was performed on Caleva 7ST dissolution apparatus (G.B. CALEVA Inc., England).

Chromatographic Conditions

The separation was performed on a reversed-phase Supelcosil LC-18 (250x4.6 mm, 5 µm particle size) column. The mobile phase consisted of a mixture of 0.05 M potassium dihydrogen phosphate : acetonitrile : phosphoric acid (69.5: 30: 0.5) (pH 3.60). The mobile phase was prepared daily, filtered,

sonicated, and degassed before use, and delivered at a flow rate of 1.5 mL/min. The UV detector was set at a wavelength of 226.2 nm (AUFS 0.01).

Chemicals and Reagents

Fluoxetine HCl was kindly supplied by Lilly Drug Inc. (Istanbul, Turkey). The internal standard, verapamil was received from Knoll-Deutsche Drugs Inc. (Istanbul, Turkey). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). All other chemicals (analytical grade) were obtained from Sigma (St. Louis, MO, USA) or Merck. Doubly distilled water was used for preparing solutions.

The potassium hydrogen phosphate buffer solution, prepared with doubly distilled water was filtered through WTP 0.5 μm filters (Whatman, Maidstone, UK). For dissolution studies, working solutions of 0.1 M HCl (pH 1.2), which is adequate to physiological conditions in gastric fluids, were used.

Preparation of the Standard Solutions

Internal standard solution was prepared by dissolving 10 mg verapamil HCl in 10 mL methanol.

Standard Solutions and Calibration Curves

A stock solution of fluoxetine HCl was prepared by dissolving 10 mg fluoxetine HCl in 10 mL methanol. Standard solutions for HPLC were prepared with mobile phase by varying the concentration of fluoxetine in the range of 0.01 μg/mL-50 μg/mL and maintaining the concentration of verapamil (internal standard) at a constant level of 0.25 μg/mL.

For the spectrophotometric method the standard solutions were prepared in 0.1 M HCl (dissolution medium) within the concentration range of the drug 3.5 μg/mL - 34.5 μg/mL. The calibration curve for HPLC analysis was constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration. The results of dissolution rate studies was obtained by plotting the drug concentration against the peak-through amplitude at 226.2 nm in the UV spectrum.

Assay Procedure for Capsules

Not less than ten capsules were accurately weighed, emptied carefully, and the mass of the collected content was determined. The empty shells were

weighed and the net fill weight per capsule was calculated. An amount of powder material equivalent to about 10 mg fluoxetine HCl was accurately weighed, transferred into a 10 mL volumetric flask, diluted with methanol, sonicated for 10 minutes, and then completed to the volume with the same solvent. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate and internal standard, then diluting them with mobile phase in order to obtain a final solution. In this study, five capsule dosage forms which include only fluoxetine HCl and produced by different firms were investigated.

Recovery Experiments

In order to establish the reliability and suitability of the proposed method, recovery experiments were performed. In order to know whether the excipients show any interference with the analysis, known amounts of the pure drug were added to the different pre-analysed formulations of fluoxetine HCl including a constant level of the internal standard, and the mixtures were analysed by the proposed method. After three repeated experiments the recoveries were calculated.

In Vitro Dissolution Studies

Drug release was carried out according to the USP dissolution procedures for the single-entity products with use of a USP paddle-stirrer type of apparatus in 900 mL of 0.1 M HCl (pH 1.2, gastric medium), at a stirring rate of 75 rpm. The temperature of the cell was maintained at 37 ± 0.5°C by use of a thermostatic bath. At each sample interval, an exact volume of a sample was withdrawn from each flask and replaced immediately with an identical volume of fresh medium. A correction factor was included in the calculations to account for the drug lost in the samples. At predetermined time intervals (2,4,6,10,15, 20,30,45,60,75,90 min), the concentrations of fluoxetine HCl in dissolution medium were determined by HPLC using UV detector.

Furthermore, to obtain comparative dissolution rate results, a UV spectrophotometric method at 226.2 nm was also applied. This spectrophotometric method was similar to those described in the USP for the single-entity products. The dissolution test data were obtained from the average of six parallel studies. In dissolution rate study, five capsule dosage forms which were produced by different firms were used.

RESULTS AND DISCUSSION

Method Validation and Development

Figure 1 shows a typical chromatogram obtained from the analysis of a standard fluoxetine HCl solution using the proposed method. Using the

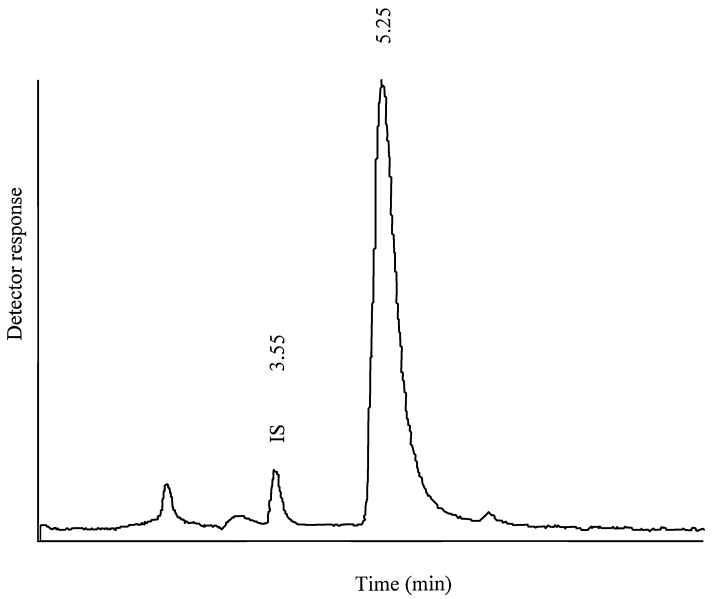


Figure 1. Typical chromatogram of fluoxetine HCl (1 μg/mL) and internal standard verapamil HCl (IS; 0.25 μg/mL) obtained with UV detector.

described chromatographic conditions, fluoxetine HCl and the internal standard verapamil were well separated and their retention times were 5.25 and 3.55 min, respectively. As shown in Figure 1, the substances were eluted, forming well shaped, symmetrical single peaks, well separated from the solvent front.

The relationship between the peak area ratios of fluoxetine HCl to the internal standard and the concentration of drug, was linear over the concentration 0.01 μg/mL -50 μg/mL. The injection volume used was 50 μL. The regression equation was shown in Table 1. The limit of detection of the procedure was shown in Table 1, which was calculated as the blank response plus three times the blank standard deviation divided by the slope of the calibration curve.

Table 2 represents the results obtained for intra- and inter- day variability studies of fluoxetine HCl samples. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference for the assay which was tested within day and between days.

Table 1

Characteristics of Fluoxetine HCl Calibration Plots

Method	Linearity Range (μg/mL)	Equation	r	SE of Slope	SE of Intercept	Detection Limits (μg/mL)
HPLC with UV detector	0.01 - 50	y: 3.47x + 0.076	0.999	0.00043	0.068	0.0057
Spectro-photometric method	3.5 - 34.5	y: 0.036x + 0.011	0.999	0.0008	0.017	0.25

Quantitative Determination

The applicability of the method was tested by analyzing fluoxetine HCl in five different commercial capsules which is produced by different firms. The results of the analysis of fluoxetine capsules (Table 3) indicate that the proposed assay can be used for quantitation and routine quality control analysis of fluoxetine HCl in commercial samples.

Recovery tests confirmed the accuracy of the proposed methods (Table 3). It can be concluded from this table that the proposed method is sufficiently accurate and precise to be applied to pharmaceutical dosage forms within a

Table 2

Intraday and Interday Precision of Fluoxetine HCl Standards

Theoretical Conc. (μg/mL)	Intraday Measured[a] Conc. (μg/mL)		Interday Measured[b] Conc (μg/mL)	
	Mean	RSD%	Mean	RSD%
0.05	0.051	1.29	0.049	1.87
0.5	0.498	0.82	0.499	1.02

[a] Mean values represent five different fluoxetine HCl standards for each concentration. [b] Interday reproducibility was determined from five different runs over a 3 week period.

Table 3

Results of the Determination and the Recovery Analysis of Fluoxetine HCl in Capsule Dosage Forms

Formulation	Labelled Claim (mg)	Mean of Amount Found (mg)[a]	RSD % of Amount Found	Added (mg)	Recovered (mg)[b]	Recovery %	RSD% Recovery
A	20	19.87	1.12	10.0	9.94	99.35	0.23
B	20	19.72	1.09	10.0	9.87	98.63	0.31
C	20	19.99	1.15	10.0	9.91	99.08	0.44
D	20	19.97	0.79	10.0	9.92	99.17	0.50
E	20	19.93	0.91	10.0	9.89	99.88	0.26

[a] Each value is the mean of five experiments. [b] Each value is the mean of three experiments.

short analysis time (< 6 min). This method is very simple and rapid and it does not involve any complicated sample preparation. High percentage recovery data shows that the method is free from the interferences of the excipients used in the formulations. The proposed method can be used for routine analysis of fluoxetine and as an alternative tool for the drug quality control laboratories.

Dissolution Studies for Capsules

The proposed HPLC method was compared with a spectrophotometric method for the quantitation of fluoxetine HCl in dissolution rate samples from the capsules. The characteristics of the calibration plots from standard fluoxetine HCl in 0.1 M HCl (pH 1.2, artificial gastric medium) obtained with the proposed comparative spectrophotometric method is shown in Table 1. Five different capsule formulations which were manufactured by five different pharmaceutical companies were investigated with the paddle dissolution method.

The release profiles of fluoxetine HCl from these capsule dosage forms are illustrated in Figure 2a and 2b, for spectrophotometric and HPLC methods, respectively.

The release data were investigated by using zero order, first order, Hixson-Crowell, and RRSBW.[27,28] All studied kinetics and their related rate constants and determination coefficients and other parameters are summarized in Table 4. According to the investigation of the kinetic assessment of the release data, the most proper release kinetic was found to be RRSBW, because of the highest determination coefficient and lowest sum of weighed squared deviations obtained with this model (Table 4). The release of active material from cap-

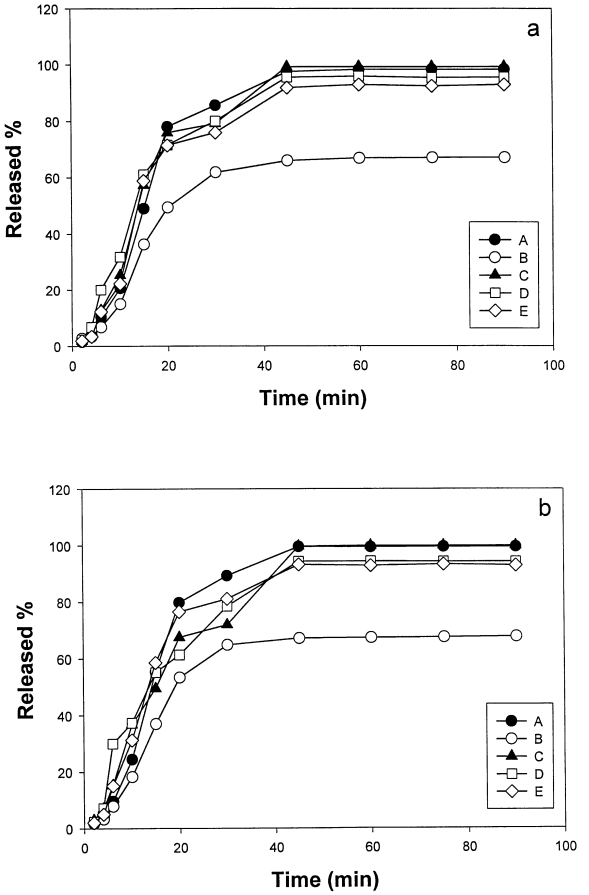


Figure 2. Dissolution profiles obtained from five different capsule formulation with (a) spectrophotometric (b) HPLC method.

sules was attained to 63.2%, at the end of 28 min, except formulation B. The release of fluoxetine HCl from formulation B was attained to 63.2%, at the end of 50 min. This may be due to the use of different excipients in formulation B and/or different manufacturing techniques for this formulation.

According to the RRSBW kinetic, $\beta>1$ is characteristic for a slower initial rate followed by an accelerated approach to the final plateau, i.e. an initial upward curvature and a sigmoid overall appearance.[27] In this study, shape fac-

Table 4

The Kinetic Assessment of Release Data of the Paddle Method

		First Order			Zero Order			Hixson-Crowell			RRSBW			
Method	**Sample**	**kr**	**r^2**	**SWSD**	**kr^0**	**r^2**	**SWSD**	**k**	**r^2**	**SWSD**	**$T_{(min)}$**	**β**	**r^2**	**SWSD**
HPLC	A	4.37	0.881	0.41	13.71	0.692	0.81	1.21	0.845	0.33	21.74	1.64	0.964	0.21
	B	0.82	0.740	0.42	9.18	0.684	0.69	0.48	0.722	0.43	46.97	1.15	0.906	0.66
	C	5.70	0.892	0.18	13.47	0.780	0.58	1.25	0.889	0.23	19.69	1.52	0.980	0.18
	D	2.21	0.872	0.35	11.93	0.741	0.47	0.91	0.848	0.76	24.25	1.23	0.927	0.29
	E	2.04	0.835	0.44	12.15	0.677	0.38	0.88	0.790	0.95	25.81	1.33	0.925	0.13
Spectro-photometry	A	3.30	0.900	0.29	13.77	0.713	0.67	1.08	0.850	0.57	24.31	1.57	0.961	0.10
	B	0.82	0.773	0.35	9.26	0.712	0.60	0.47	0.754	0.36	49.72	1.12	0.914	0.47
	C	4.08	0.878	0.35	13.56	0.726	0.69	1.16	0.862	0.77	22.04	1.55	0.968	0.56
	D	2.43	0.866	0.37	12.37	0.709	0.61	0.95	0.832	0.99	23.75	1.31	0.942	0.29
	E	2.04	0.866	0.34	12.59	0.707	0.30	0.860	0.822	0.72	28.02	1.40	0.930	0.11

kr: Release rate constant of first order kinetic. kr^0: Release rate constant of zero order kinetic. k: Release rate constant of Hixson-Crowell kinetic. r^2: Determination coefficient. SWSD: Sum of weight squared deviations. β: Shape factor. $T_{(min)}$: Value stands for the time for 63.2% release of the drug.

tors (β) obtained from RRSBW was found to be bigger than 1 for all samples and for both analytical methods. The release of fluoxetine HCl from the capsule formulations tested were completed within 40 min in both methods except formulation B.

The results obtained show that, if no strict requirements on dissolution tests of the final product are observed, drugs with a large difference in dissolution characteristics can be found in the market which, accordingly, affect the bioavailability of the active substance. Therefore, it is essential to consider the in vitro dissolution tests as important criteria for the quality of the pharmaceutical dosage formulations if obtained from various sources; and which can judge the suitability of these formulations to deliver the required active substance properly to the patient. Thus, the need of dissolution tests of drug formulations is indispensable to ensure good drug quality control.

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