

Validated TLC Densitometric Method for the Quantification of Paroxetine Hydrochloride in Solid Dosage Form

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A simple, specific, accurate and precise high performance thin layer chromatography method has been developed for the estimation of paroxetine hydrochloride in tablet dosage forms. The quantification was carried out at 296 nm. Developed method was validated in terms of linearity, accuracy, precision, repeatability and specificity. Limit of detection and limit of quantification of paroxetine hydrochloride were found to be 60 ng/spot and 160 ng/spot, respectively. The linearity range for paroxetine hydrochloride was found to be 160-960 ng/spot with correlation coefficient of 0.995. Content of paroxetine hydrochloride in two tablet dosage forms was found to be 100.10 and 100.20%, respectively.

Paroxetine hydrochloride [(-)-*trans*-5-(4-*p*-flourophenyle-3-piperidylmethoxy)-1,3-benzodioxole hydrochloride], is a selective serotonin reuptake inhibitor (SSRI). It is used in the treatment of depression, anxiety disorder (obsessive-compulsive disorder), premenstrual syndrome and sexual dysfunction¹⁻⁵. Various analytical methods have been reported for the estimation of paroxetine hydrochloride *viz.* HPLC⁶⁻¹⁴, GC¹⁵, HPLC-GC¹⁶, GC-MS¹⁷, LC-MS¹⁸⁻²⁰, capillary electrophoresis²¹, capillary liquid chromatography²² and voltammetric²³ in biological fluid and pharmaceutical dosage forms. Chiral HPLC method was reported for chiral purity determination of paroxetine²⁴. In the present study a sensitive TLC densitometric method using HPTLC for the quantification of paroxetine hydrochloride from tablet dosage forms have been developed.

All the chemicals used in the experiment were of analytical grade. Paroxetine hydrochloride was procured as gift sample from Intas Pharmaceuticals Ltd., Ahmedabad. Paroxetine hydrochloride tablet dosage form was purchased from a local pharmacy.

The chromatography estimation was performed using the following conditions: stationary phase was precoated silica gel 60 F₂₅₄ aluminum sheets (10×10

cm, E. Merck, Cat No. 1.05554.0007) and the mobile phase used was ethyl acetate: acetic acid: water in the ratio of 7.5:1.5:1 v/v. The chamber saturation time employed was 15 min and the developing distance used was 7 cm. Scanning wavelength for paroxetine hydrochloride was 296 nm with slit dimensions of 5.0 x 0.45 mm and scanning speed of 10 mm/s were employed. Spotting parameters used were, 5 mm bandwidth, 10 mm space between two bands and spraying rate 10 s/μl.

Paroxetine hydrochloride (20 mg) was accurately weighed and transferred to 25 ml volumetric flask and volume was made up to the mark with methanol to give a standard stock solution of 0.8 mg/ml. The aliquots (0.2 to 1.2 ml) of stock solution were transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol to obtain working standard solution containing 16, 32, 48, 64, 80 and 96 μg/ml of paroxetine hydrochloride, respectively.

Standard solutions of paroxetine hydrochloride (10 μl) were applied in triplicate on TLC plate. The plate was developed in a solvent system of ethyl acetate: acetic acid: water in the ratio of 7.5:1.5:1 v/v up to distance of 7 cm. After development, the plates were dried in hot air and scanned at 296 nm. The peak areas were recorded. Calibration curve of paroxetine

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TABLE 1: ANALYSIS OF PAROXETINE HYDROCHLORIDE BY PROPOSED METHOD

Sample	Label claim (mg/tablet)	Amount found (mg/tablet) ^a	% Assay ^a	Amount added (mg) ^b	Recovery (%)	Average recovery
01	20.0	20.02 ± 0.84	100.1 ± 0.34	10.0	100.82	100.8%
				15.0	102.07	
				20.0	99.80	
02	20.0	20.04 ± 0.21	100.2 ± 0.09	-	-	-

^aMean±standard deviation of five determinations. ^bRecovery study was performed on one formulation only.

hydrochloride was obtained by plotting peak area vs concentration of paroxetine hydrochloride applied.

Twenty tablets of paroxetine hydrochloride were crushed and ground to fine powder. A powder equivalent to 20 mg of drug was transferred to a conical flask and extracted with methanol (4×25 ml) by sonication. The extracts were filtered through Whatman No. 1 filter paper and the residue was washed with sufficient amount of methanol. The extract and its washings were pooled, transferred to a 100 ml volumetric flask and the final volume was made up to 100 ml with methanol to give a sample solution of 200 µg/ml. A fixed volume of 5 µl of working standard solutions (80 µg/ml) and 5 µl of sample solutions were spotted as sharp bands on the TLC plate and the plate was developed as mentioned above. The band of the drug was scanned at 296 nm.

The method was validated for precision (repeatability and reproducibility), accuracy, and linearity (sensitivity)²⁵. Limit of detection and limit of quantification were recorded. Instrumental precision was checked by repeated scanning of same spot of paroxetine hydrochloride (400 ng) seven times and was expressed as coefficient of variance (% CV). The repeatability of the method was affirmed by analyzing 400 ng/spot of standard solution of paroxetine hydrochloride individually (n=7) and was expressed as coefficient of variance (% CV). Reproducibility of the method was studied by analyzing aliquots of standard solutions of paroxetine hydrochloride (400 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % CV. Accuracy of method was tested by performing recovery studies at three different levels for paroxetine hydrochloride viz. after addition of 50, 75 and 100 % drug in the sample and estimated as described above. The percent recovery as well as average percent recovery for paroxetine hydrochloride was calculated.

Of the various mobile phases tried, the one containing

ethyl acetate:acetic acid: water in the ratio of 7.5:1.5:1 v/v was found to be suitable for paroxetine hydrochloride ($R_f = 0.49$). Limit of detection and limit of quantification of paroxetine hydrochloride was found to be 60 ng/spot and 160 ng/spot, respectively. Estimation was possible by using the same concentration of sample solution (400 ng/spot). The identity of the paroxetine hydrochloride in the sample extracts was confirmed by overlying the UV absorption spectra with that of the reference standard using Camag TLC Scanner III. The purity of the band in the sample extract was confirmed by comparing the absorption spectra recorded at start, middle and end position of the band. The linearity range for paroxetine hydrochloride was found to be 160-960 ng/spot with a correlation coefficient of 0.995. The inter-day and intra-day precision expressed as % CV indicates that the proposed method is quite precise and reproducible. The average of percent recoveries at three different levels was found to be 100.8% (Table 1). The content of paroxetine estimated in the sample extracts of dosage forms, by the proposed method was found to be in 100.10% and 100.20%, respectively (Table 1). The validation parameters of this method have been shown in Table 2.

Paroxetine hydrochloride is freely soluble in methanol and gave very good resolution in the proposed mobile phase hence this property of compound was utilized to develop the TLC densitometric method for the simple and rapid quantification of paroxetine hydrochloride. Proposed method for the quantification of paroxetine

TABLE 2: METHOD VALIDATION PARAMETERS

Parameters	Results
Linearity range	160-960 ng/spot
Correlation coefficient	0.995
Limit of detection (LOD)	60 ng/spot
Limit of quantification (LOQ)	160 ng/spot
Accuracy	100.8%
Precision (%CV)	
Repeatability of application (n=7)	0.72
Repeatability of measurement (n=7)	0.43
Inter day (n=3)	0.89-2.77
Intra day (n=3)	0.64-2.38
Specificity	Specific

hydrochloride from solid dosage forms was found to be simple, specific, sensitive, accurate and precise and may be used for routine quality control.

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