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Development and Validation of Densitometry Estimation of Trifluoperazine Hydrochloride in Dosage Form

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Abstract: A simple, sensitive and validated high performance thin layer chromatographic method has been developed for the estimation of Trifluoperazine Hydrochloride in pure drug and its formulation. Aluminium plates precoated with Silica gel G 60 F_{254} was used as stationary phase and acetonitrile: ether: 10% ammonia solution (8:1:3 drops v/v) was used as mobile phase. The experimental parameters like band size of the spot application, chamber saturation time, solvent front migration, slit width etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with Rf value 0.42±0.07. Quantification was carried out by the use of Densitometric absorbance mode at 345 nm. The proposed HPTLC method was quantitatively evaluated in terms of precision, repeatability, accuracy and calibration correlation proving its utility in routine analysis of its dosage form.

Key words: HPTLC · Trifluoperazine Hydrochloride

INTRODUCTION

Trifluoperazine Hydrochloride, 10-[3-(4-methylpiperazin-1-yl) propyl]-2 tirfluoro methyl phenothiazine dihydrochloride used as antipsychotic. Trifluoperazine is a typical anti-psychotic drug of the phenothiazine group. It has a central anti-adrenergic, anti-dopaminergic and minimal anti-cholinergic activity. It mainly acts on dopamine receptor. Mode of action includes inhibiting dopamine D2 receptors in the brain possessing á-adrenergic blocking, antiemetic and some anticholinergic activity [1]. Literature review shows that there are developed methods for including UV, Fluorometric, RP-HPLC, HPTLC, GC-MS [2-9] methods. Author of the article and his research team has development different pharmaceutical dosage form [10-17]. The present study described the development and validation of a simple, specific, accurate and precise HPTLC method for the determination of Trifluoperazine Hydrochloride in pharmaceutical dosage form.

MATERIALS AND METHODS

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals Corporation ltd. Mumbai, India. Deionized and ultrapure water used in all experiments was obtained from Milli – Q system (Millipore).Silica gel 60F_{254} TLC plates (20×10 cm and 10×10 cm, layer thickness 0.2mm, Merck, Germany) were used as stationary phase. Tablets were purchased from local market. Toluene, Glacial acetic acid, Ethyl acetate were used of AR Grade were used for preparing the mobile phase. A Camag HPTLC system containing Camag Linomet IV semiautomatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner – 3 with WINCAT software version. Solutions of the test substance were applied to silica gel 60 F_{254} TLC plates. The plate was placed in a chromatographic tank previously saturated for 10 min with developing mobile phase; acetonitrile: ether: 10% ammonia solution (8:1:3 drops v/v). The plate was developed by normal vertical developing tank at ambient temperature for a distance of 110 mm. the spots were detected under a UV lamp and scanned densitometrically at 345 nm.

Preparation of Standard Stock Solution: A stock solution of Trifluoperazine Hydrochloride (1mg/ml) was prepared by dissolving the authentic sample in methanol. This solution was used to construct a calibration plot by applying 0.5,1, 1.5,2, 2.5 and 3 µl (equivalent to 50-300 ng

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per band) to a TLC plate. The data of peak area versus drug concentration was treated by linear least square regression to obtain the calibration graphs. The plate was developed in twin trough glass chamber under operating conditions described above. After development the plate was air dried and evaluated densitometrically at wavelength of 345 nm. Calibration curve was constructed by plotting peak areas versus concentration and the regression equation was calculated.

Prewashing of Plates: HPTLC was performed on 10×10cm precoated silica gel 60F_{254} precoated plates from E.Merck. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried and activated for 40 minutes at 130 degree centigrade with the plates being placed between two sheets of glass to prevent deformation of the aluminium during heating.

Sample Application: The standard and formulation samples of Trifluoperazine Hydrochloride were spotted on precoated TLC plates in the form of narrow bands of lengths 6mm, with 10mm from the bottom and left margin and with 9mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 200 to 600 ng spot^{-1}.

Method Validation: The developed methods was validated in terms of linearity and range, limit of detection, limit of quantification, recovery study and inter day study, intra day study and study by different analysts. The linear regression data in HPTLC showed a good linear relationship over a concentration range 20-300 ng/spot for Trifluoperazine Hydrochloride. The correlation coefficients obtained were 0.9994 for Trifluoperazine Hydrochloride. The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (200, 400 and 600 ng spot^{-1} Trifluoperazine Hydrochloride) of the drug in six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days. The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses three times on the same day and on three different days for three different concentrations of Trifluoperazine Hydrochloride (200, 400 and 600 ng spot^{-1}). The results are reported in terms of relative standard deviation (RSD).Accuracy of the method was determined by standard addition method in which the known amount of standard Trifluoperazine Hydrochloride solutions were added to pre analyzed tablet solution. These amounts corresponded to 50, 100 and 150% of the amounts claimed on the label. In order to determine LOD and LOQ, concentrations in the lower part of the linear range of the calibration curve were used. Stock solution of Trifluoperazine Hydrochloride (10 μl) was prepared and different volume of stock solution in the range 200 to 600 ng spot^{-1} were spotted in triplicate. The amount Trifluoperazine Hydrochloride by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.) / b and quantification limit was calculated by (10×A.S.D.) / b, where "b" corresponds to the slope obtained in the linearity study of method. Ruggedness of the proposed method was estimated by changing different conditions like mobile phase composition (± 0.5 ml for each component), mobile phase volume varied ± 2%, scanning wavelength ± 1 nm and R_f value and peak areas were measured after development of plate and% RSD was calculated. A concentration level of 500 ng spot^{-1} was employed. Summary of validation parameters are shown in (Table 2). The stability of standard solutions was tested after 0, 6, 12, 24, 48 and 72 h of storage. The stability of the solutions was determined by comparing peak area percentage and peak purity at 500 ng spot^{-1}. The specificity of the method was ascertained by analyzing standard drugs and sample. The spots of Trifluoperazine Hydrochloride in samples were confirmed by comparing the R_f and spectra of the spots with that of standard. The peak purity of Trifluoperazine Hydrochloride was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

RESULTS AND DISCUSSION

The method was validated as per ICH guidelines [18] in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application (Table 1). This method utilizes the active analogue principle that lies at the HPTLC method [10-17].

Table 1: Method validation parameters

Parameters	Results
Linearity range	200 – 600 ng/spot
Correlation coefficient	0.9994
Limit of detection (LOD)	40 ng/spot
Limit of quantification (LOQ)	88 ng/spot
Accuracy/Recovery	101.25%
Precision (%CV)	
Repeatability of application (n=7)	0.71
Repeatability of measurement (n=7)	0.49
Inter day (n=3)	1.14-1.88
Intra day (n=3)	0.732-1.36
Specificity	Specific

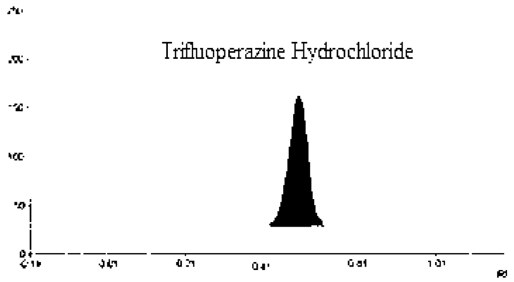

Fig.1: Chromatogram of Trifluoperazine Hydrochloride

Aliquots of working standard (10 µg/ml) solution (1, 2, 3, 4, 5µl) of Trifluoperazine Hydrochloride were spotted as sharp bands on the precoated TLC plate. The plate was developed under chromatographic conditions mentioned above. The plate was removed from the chamber and dried in hot air dryer. Densitometric measurements were performed at 345 nm in absorbance mode. Data peak height and peak area of each band were recorded. The calibration curve was prepared by plotting peak area vs. concentration corresponding to each spot. The method was found to be linear in the range of 200-600ng/spot, (Y=2.5439 X + 0.5648), (r = 0.9994) in six replicates. The signal to noise ratios of 6 and 16 were considered as LOD and LOQ respectively. LOD and LOQ of found to be 40 and 88 ng/spot. The intraday precision was determined by analyzing standard of Trifluoperazine Hydrochloride solution in the concentration range of 200 ng/spot and 600 ng/spot for three times on same day while interday precision was determined by analyzing corresponding standards daily for six days over a period of one week. The percentile RSD of intraday and interday precision of Trifluoperazine Hydrochloride the range of 1.14 – 1.87. The value indicates the method is precise. Repeatability sample application was assessed by spotting 5µl of drug solution six times on TLC plate followed by development of plate and recording the peak area of spots. The percentile RSD peak area values of Trifluoperazine Hydrochloride were found to be 0.42. Repeatability measurement of peak area was determined by spotting 5 µl Trifluoperazine Hydrochloride solution TLC plate and developed. The separated spot was scanned six times without changing the position of plate and the percentile RSD measurement of peak area value of Trifluoperazine Hydrochloride was found to be 0.33. To confirm the specificity of proposed method the solution of formulation was spotted on TLC plate which was then developed and scanned. It was observed that the excipients present in the formulation did not interfere with peak of Trifluoperazine Hydrochloride.The average of percent recoveries at three different levels was found to be 99.97%. The content of Trifluoperazine Hydrochloride estimated in the sample extracts of dosage forms, by the proposed method was found to be in 99.96% and 101.25%, respectively. Proposed method for the quantification of Trifluoperazine Hydrochloride from dosage forms was found to be simple, specific, sensitive, accurate and precise and may be used for routine quality control.

CONCLUSION

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of Trifluoperazine Hydrochloride in pharmaceutical formulations. The amounts found in formulations well agreed with label claim. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of Trifluoperazine Hydrochloride from bulk drug and formulations.

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REFERENCES

1. Tripathi, K.D, 2003. Essentials of Medical Pharmacology, fifth edition, Jaypee brothers, Medical Publishers (P) Ltd, pp: 391-392.
2. Saudagar, R.B., S. Swarnlata and S. Saraf, 2007. Spectrophotometric determination of chlordiazepoxide and trifluoperazine hydrochloride from combined dosage form. Indian. J. Pharm. Sci., 69(1): 149.

3. Trivedi, P. and A. Sachan, 1999. Simultaneous spectrophotometric estimation of benhexol HCl and trifluperazine HCl in tablet formulation. East Pharm., 42(493): 117-120.
4. Basavaiah, K. and G. Krishnamurthy, 1998. Spectrophotometric assay of some antipsychotropic and anticholinergic phenothiazine drugs using ammonium molybdate. J. Analyt. lett., 31(6): 1037-46.
5. Laassis, B., M. Maafi, J.J. Aaron and M.C. Mahedero, 1997. Fluorimetric and photochemically induced fluorimetric determination of ethopropazine, levomepromazine, thioproperazine and trifluoperazine. J. Analyt. lett., 30(8): 1541-54.
6. Midha, K.K., R.M.H. Roscoe, K. Hall and E.M. Hawes, 1982. A gas chromatographic mass spectrometric assay for plasma trifluoperazine concentrations following single doses. Biol. Mass. Spectr., 9(5): 186-190.
7. EL-Gindy, A., B. EL-Zeany, T. Awad and M.M. Shabana, 2002. Derivative spectrophotometric, thin layer chromatographic-densitometric and high performance liquid chromatographic determination of trifluoperazine hydrochloride in presence of its hydrogen peroxide induced-degradation product. J. Pharm. Biomed. Anal., 27: 9.
8. Maslanka, A. and J. Krzek, 2005. Densitometric High Performance thin-layer chromatography identification and quantitative analysis of psychotropic drugs. J. AOAC Intl., 88: 70.
9. Bhongade, S.L. and A.V. Kasture, 1993. Spectrophotometric determinations of phenothiazine derivatives. J. Analyt. Chem., 55(4): 151-4.
10. Sharma, M.C., S.Sharma, D.V. Kohli and A.D. Sharma, 2010. A validated HPTLC method for determination of simultaneous estimation Rosuvastatin Calcium and Ezetimibe in pharmaceutical solid dosage form. Arch. Appl. Sci. Rese., 2(1): 1-7.
11. Sharma, M.C., S. Sharma and D.V. Kohli, 2010. HPTLC method development and validation for the estimation of Atorvastatin Calcium and Pioglitazone Hydrochloride in pharmaceutical combined tablet dosage form. Ann. Biol. Res., 1(1): 124-129.
12. Sharma, S. and M.C. Sharma, 2010. Development and Validation of An HPTLC Method for Determination of Oseltamivir phosphate in pharmaceutical dosage form. Ind. Drugs., 47(11): 68-72.
13. Sharma, M.C., S. Sharma, D.V. Kohli and A.D. Sharma, 2010. Validated TLC Densitometric method for the quantification of Torsemide and Spironolactone in bulk drug and in tablet dosage form. Der. Pharma. Chemica., 2(1): 121-126.
14. Sharma, M.C., S. Sharma., D.V. Kohli and A.D. Sharma, 2010. Development and Validation of TLC-Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine Dihydrochloride Pharmaceutical Solid dosage form. Der. Pharmacia. Lette., 2(1): 489-494.
15. Sharma, M.C. and S. Sharma, 2011. Development and Validation of Densitometric Method for Metronidazole and Tetracycline hydrochloride in capsule Dosage form. Int. J. Pharm. Tech. Resea., 3(2): 1169-1173.
16. Sharma, M.C. and S. Sharma, 2011. Validated Densitometric Method for the Quantification of Lamotrigine in Dosage Form. Int. J. Pharm. Tech. Resea., 3(2): 1174-1178.
17. Sharma, M.C. and S. Sharma, 2011. Development and Validation of TLC Densitometric Method for Gatifloxacin in Pharmaceutical Formulations. Int. J. Pharm. Tech. Resea., 3(2): 1179-1185.
18. ICH Q2B, 1996. Validation of analytical procedure: Methodology. International Conference on Harmonization, Geneva.