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A VALIDATED HPTLC METHOD FOR THE ESTIMATION OF CYCLOBENZAPRINE HCL IN BULK AND ITS TABLET DOSAGE FORM

POKHARKAR D[1*], TYAGI CK[1], JADHAV V[2] AND KADAM V[2]

1: Sri Satya Sai University of Technology and Medical Sciences, Sehore (Madhya Pradesh)

2: Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai -400614 (Maharashtra)

***Corresponding Author: Deepak Pokharkar: E Mail: deepak.pokharkar@ncrdsip.com**

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ABSTRACT

A simple, rapid, reliable and accurate HPTLC method has been developed for the quantitative determination of Cyclobenzaprine HCl in bulk and tablets. Various aliquots of the sample solution were spotted automatically by means of camag ATS 4 applicator on precoated silica gel 60 F_{254} on aluminium sheet as stationary phase pre washed with methanol using n-Hexane: Ethyl acetate: Methanol: Glacial acetic acid (7:2:2.5:0.5) v/v/v/v as mobile phase. The spots were scanned at 292 nm. The R_f value of Cyclobenzaprine HCl was 0.10 ± 0.02. Calibration curves were linear in the range of 200-1000 ng/band. The limit of detection and limit of quantification were found to be 160 ng/band and 486 ng/band respectively. The suitability of this method for the quantitative determination of compound was proved by validation in accordance with requirements of pharmaceutical regulatory standards.

Keywords: Cyclobenzaprine HCl, HPTLC, Validation, Chromatography and Recovery

1. INTRODUCTION

Cyclobenzaprine hydrochloride [3-(5H-dibenzo [a, d] cyclohepten5-ylidene)-N-N-dimethyl-1-propanamine hydrochloride; (CB) is a centrally acting muscle relaxant and related to tricyclic antidepressant **(Figure 1)**. It is used in the treatment of the muscloskeletal condition which associated with painful muscle spasms **[1]**. It is an FDA approved drug, 10mg dose was approved in 1977 and 5 mg dose was approved in 2003. It is a white coloured, crystalline in nature tricyclic amine salt. The empirical formula is $C_{20}H_{21}N.HCl$ having molecular weight of 311.9 gm with melting point 217^{0}C and pka value 8.47 **[2, 3]**. It is freely soluble in water, alcohols like methanol, ethanol, sparingly soluble in isopropyl alcohol, but insoluble in hydrocarbon solvents such as n-hexane **[4-6]**. The exact mechanism of cyclobenzaprine is not been fully determined, but this drug seems to primarily act at the brain stem to reduce tonic somatic motor activity, influencing both gamma and alpha motor neurons leading to a reduction in muscle spasms. It is entirely absorbed during first pass through GI tract. Cyclobenzaprine is metabolized in the liver by oxidative and glucuronide conjugative pathways. It is primarily excreted in urine as conjugate metabolites (38-51%) and also in feces (14-15%). Metabolites eliminated in urine are likely water-soluble glucuronide conjugates **[7]**.

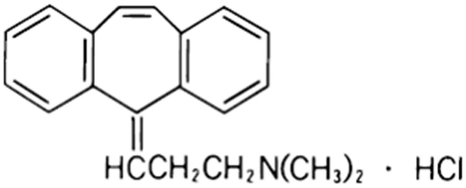

Figure 1: Structure of Cyclobenzaprine hydrochloride

In pharmaceutical preparations, multiple analytical procedures have been reported for the analysis of glimepiride when it is used as a single active principle or in combined dosage forms, UV and Spectrofluorimetric method **[8-11]**, High-performance liquid chromatography **[12-14]** and High performance thin layer chromatography **[15, 16]**. The aim of present study is to develop a HPTLC method for the estimation of Cyclobenzaprine HCl in bulk and in tablets.

2. METHODOLOGY

Materials

An analytically pure gift sample of Cyclobenzaprine HCl from Unichem Laboratories Ltd, GOA, India was used as working standard. Methanol and Chloroform of HPLC grade (Merck Chem.) were used to prepare the mobile phase.

Instrumentation

Camag (Switzerland) ATS 4 applicator, a Camag Twin trough TLC Chamber. Camag TLC scanner 3, Camag Wincats Software. Hamilton (Reno, Nevada, USA) syringe (100 µL). HPTLC conditions are given in **Table 1**.

Table 1: HPTLC Conditions

Stationary Phase	**TLC aluminium sheets Silica gel 60 F_{254}**
Sample Solvent type	**Methanol**
Mobile Phase	**n- Hexane: Ethyl acetate: Methanol: Glacial acetic acid (7:2:2.5:0.5) v/v/v/v**
Migration distance	**70 mm**
Slit Dimensions	**4.00 x 0.45 mm, micro**
Wavelength scanning	**292 nm**
R_f value of Cyclobenzaprine HCl	**0.10 ± 0.02**

Standard preparation

Standard stock solution containing 1 mg/ml of Cyclobenzaprine HCl was prepared by dissolving 10 mg standard drug in 10 mL chloroform and used as working standard solution.

Sample preparation

Twenty tablets were weighed and powdered. An amount of powder equivalent to 5mg of Cyclobenzaprine HCl transferred to 25 mL calibrated volumetric flask. 10mL of Methanol is added and sonicated for 10 min, the solution was made up of volume with the same solvent and filtered. A sample solution was spotted for the assay of Cyclobenzaprine HCl.

Chromatography

Chromatography was performed on 10 cm x 20 cm aluminium packed silica gel 60 F_{254} HPTLC plates. Before use, the plates were washed with methanol and dried in an oven at 50°C for 5 min. Samples were applied as 6 mm bands by spraying at a rate of 15 µL S^{-1} by means of a Camag Linomat ATS 4 applicator equipped with a 100 µL syringe, the distance between the bands was 10.5 mm. Ascending development of the plate, migration distance 70 mm, was performed at 25 ± 2°C with n- Hexane: Ethyl acetate: Methanol: Glacial acetic acid (7:2:2.5:0.5) v/v/v/v as mobile phase in a Camag twin-trough chamber previously saturated for 20 min. The average development time was 20 min. Densitometric scanning was performed with Camag TLC scanner 4 equipped with Wincats software at λ_{max} 292 nm using Deutrium light source, the slit dimensions were 4.00 x 0.45 mm, micro.

3. Validation of the HPTLC method

Linearity

Amount of standard solutions equivalent to 200-1000 ng/band. was spotted on the prewashed HPTLC plates. The plates were developed, dried and scanned as described above. The calibration curve was constructed by plotting the peak areas against the corresponding concentrations in ng. The linearity response for Cyclobenzaprine HCL assessed in the concentration range is 200-1000 ng/band. The statistical analysis of data correlation coefficient and coefficient of variation are found to be 0.9998 and 0.39% respectively, over the concentration range studied with six replicate readings of each concentration. The chromatogram of Cyclobenzaprine HCL Standard, Sample and Calibration plotare showed in **Figure 2 , 3 and 4** respectively.

SENSITIVITY

The sensitivity of proposed method is estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated by the use of equation LOD = 3 X N/B and LOQ = 10 X N/B, where N is the standard deviation of peak areas of the drug taken as a measure of noise, and B is the slope of the corresponding calibration curve. The limit of detection and limit of quantification for Cyclobenzaprine HCl were found to be 160 ng/ band and 486ng / band respectively **(Figure 5)**.

SPECIFICITY

Specificity of the method was determined by analyzing standard drug and sample. The specificity of the method was ascertained by analyzing Cyclobenzaprine HCl. The ability of the method to separate the drug from tablet excipients indicates the specificity of the method. There was no interference or Co elution from excipients at Rf value of drug, The band of Glimepiride was confirmed by comparing the Rf of the sample with that of standard, indicating absence of interference of mobile phase, diluent and excipients **(Figure 6)**.

PRECISION

Precision was determined by demonstrating interday variation. 6 replicates of standard solution of 8µl were applied on two plates on two different days (Inter- day precision) and the coefficient of variation was found to be 1.54% and 1.49% respectively.

DATA OF REPEATABILITY (Table 2)

INTERDAY PRECISION DATA (Table 3, 4)

RECOVERY

Recovery was carried out to determine accuracy of the method. Recovery was determined at 3 concentration level at 80% (4µl), 100% (5µl) and 120% (6µl). Recovery percentage was found to be 98.0084%. Sample (5mg/25ml) and Standard (0.1mg/ml) were diluted in methanol **(Table 5)**.

Analysis of Marketed formulation

Twenty tablets were weighed, their mean weight was determined and was finely powdered Powder equivalent to 1 tablet of Cyclobenzaprine HCL was weighed. Sample preparation (1mg/5ml) was made and Drug content per tablet was determined by performing assay. The percent content was found to be 96.42%

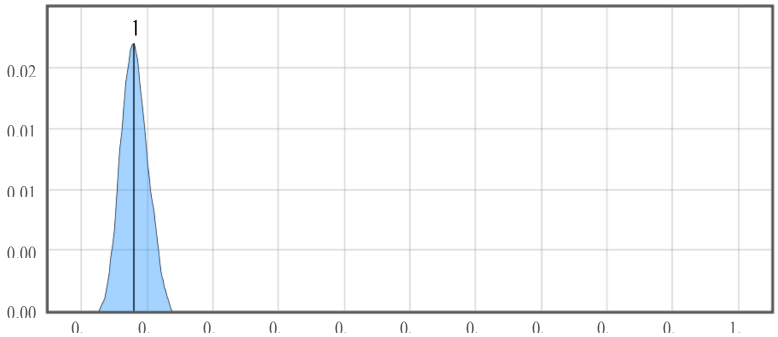

Figure 2: Chromatogram of Standard Cyclobenzaprine HCL

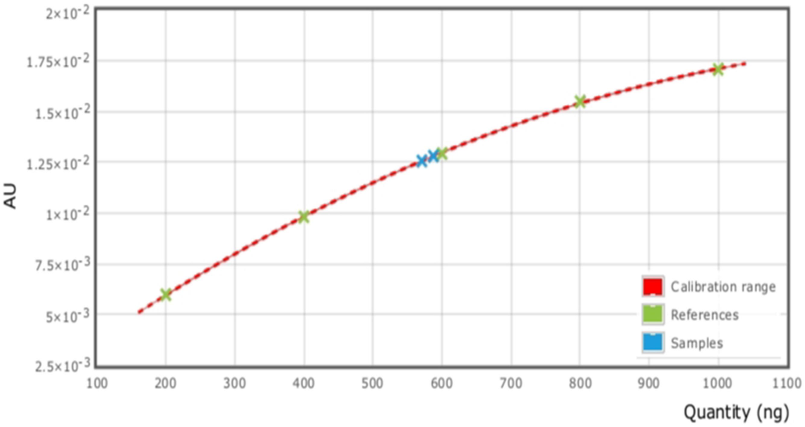

Figure 3: Chromatogram of Sample Cyclobenzaprine HCL

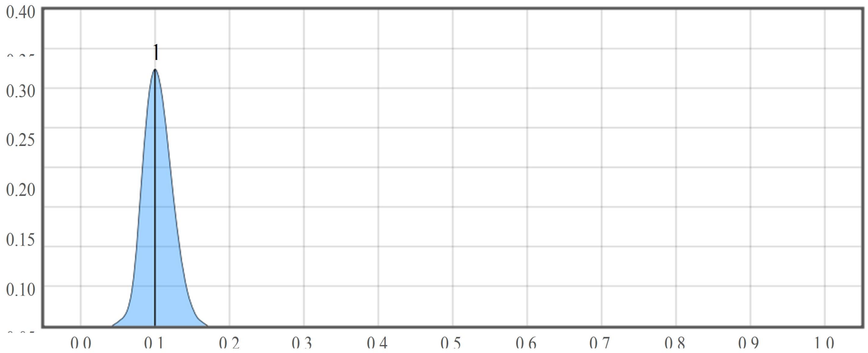

Figure 4: Calibration curve of Cyclobenzaprine HCl

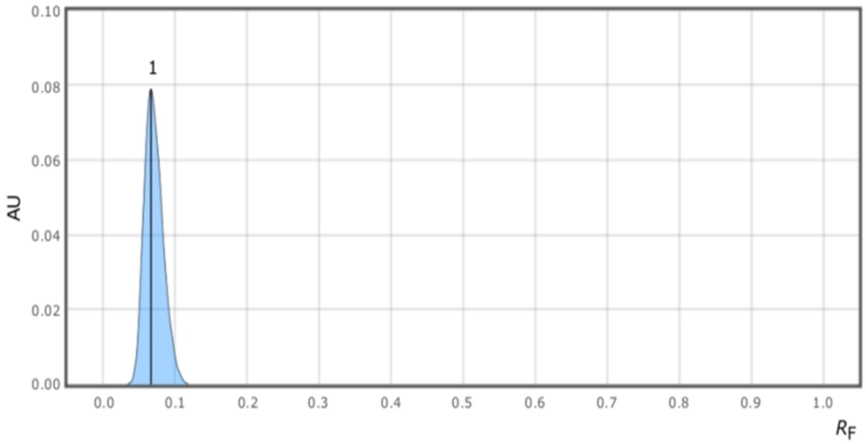

Figure 5: Chromatogram of Standard Cyclobenzaprine HCl

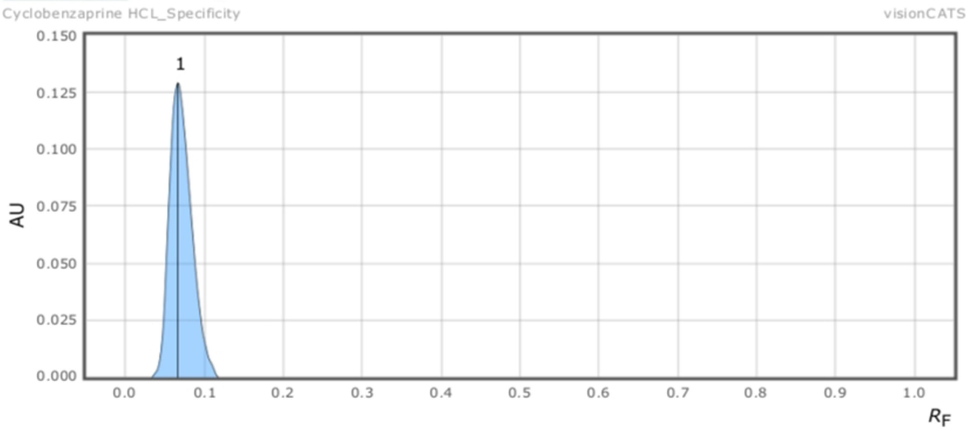

Figure 6: Chromatogram of Sample Cyclobenzaprine HCl

Table 2: Data of Repeatability (n=6)

	Conc. (8µl)	Avg = 0.016	CV=1.54%
Sr. No.	**Rf**	**Peak area**	**Deviation**
1	0.099	0.01536	-1.98 %
2	0.100	0.01556	-0.65 %
3	0.100	0.01561	-0.33 %
4	0.099	0.01567	0.03 %
5	0.100	0.01570	0.23 %
6	0.100	0.01609	2.70 %

Table 3: Day 1

	Conc. (8µl)	Avg = 0.016	CV=1.54%
Sr. No.	**Rf**	**Peak area**	**Deviation**
1	0.099	0.01536	2.85%
2	0.100	0.01556	1.09%
3	0.100	0.01561	-1.02%
4	0.099	0.01567	-1.88%
5	0.100	0.01570	-1.66%
6	0.100	0.01609	0.61%

Table 4: Day 2

	Conc (8µl)	Avg = 0.011	CV=1.49%
Sr.No	**Rf**	**Peak area**	**Deviation**
1	0.088	0.01063	-2.34 %
2	0.088	0.01089	0.00 %
3	0.086	0.01111	2.01 %
4	0.088	0.01099	0.96 %
5	0.087	0.01091	0.15 %
6	0.087	0.01080	-0.79 %

Table 5: Data of Recovery

Percent Amount spiked	Area x 106	Average Area	Area Obtained	Excepted Area	Percentage	Mean Percentage
Sample (100%)	**0.0010946**					
0.4µl	**0.0011698**	**0.0011322**				
Standard (80%)	**0.0006835**					
0.4µl	**0.0005770**	**0.0006302**	**0.003279**	**0.00176**	**97.991**	
Standard (100%)	**0.0007518**					
0.5µl	**0.0007544**	**0.0003316**	**0.003788**	**0.00188**	**97.562**	**98.0084**
Standard (120%)	**0.0009151**					
0.6µl	**0.0008661**	**0.0008906**	**0.004251**	**0.00202**	**98.471**	

4. RESULTS AND DISCUSSION

HPTLC method was validated as per ICH guidelines. The developed method was found to be linear within the range of 200-1000 ng with correlation coefficient and coefficient of variation are found to be 0.9998 and 0.39% respectively for Cyclobenzaprine HCL. The accuracy of method was determined at 80%, 100%, 120% level. The % recovery was found to be 98.0084%. The LOD for Glimepiride was found to be 160 ng/band and the LOQ Cyclobenzaprine HCL was found to be 486 ng/band indicating the sensitivity of the method. The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. Summary of the results of validation parameters is shown in **Table 6**.

Table 6: Summary Of Validation Parameters

PARAMETERS	Cycolbenzaprine HCL
Linearity [ng/band]	**200-1000**
LOD [ng/band]	**160**
LOQ [ng/band]	**486**
Precision (%RSD) Intra-day (n=6)	**1.5346**
Precision (%RSD) Inter-day (n=6)	**1.5062**
Specificity	**Specific**
% Recovery	**98.0084%.**

5. CONCLUSION

The developed HPTLC technique is precise, specific and accurate. The advantages lie in the simplicity of sample preparation and the low cost of reagents used. Statistical analysis proves that the method is suitable for the analysis of Cyclobenzaprine HCL as bulk drug and in Pharmaceutical formulation without any interference from the excipient. Hence this HPTLC method can be used for routine drug analysis.

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REFERENCES

[1] Wisher D. Martindale: The complete drug reference. Journal of the Medical Library Association. 2012 Jan 1; 100(1): 75-7.

[2] Toth PE, Urtis J. Commonly used muscle relaxant therapies for acute low back pain: a review of carisoprodol, cyclobenzaprine hydrochloride, and metaxalone. Clinical therapeutics. 2004 Sep 1; 26(9): 1355-67.

[3] https://www.accessdata.fda.gov/drugsatfda_docs/label/2003/017821s045lbl.pdf

[4] Philip TRH, David AY, Arthur BS, Marvin CM. J Liq Chrom & Rel Technol, 1993; 16(5): 1163-1171.

[5] Spell JC, Stewart JT. J Pharm Biomed Anal, 1998; 18 (3): 453-460.

[6] Constanzer M, Chavez C, Matuszewski B. J Chrom B: Biomed Sci and Appl, 1995; 666(1): 117-126.

[7] https://pubchem.ncbi.nlm.nih.gov/compound/Cyclobenzaprine

[8] ICH Q2B (1996) Validation of Analytical procedure: Methodology International Conference on Harmonization, Geneva, March, 71.

[9] Ramadan NK, Mohamed TA, Fouad RM, Moustafa AA, Development and Validation of Spectrophotometric and Spectrofluorimetric Methods for the Determination of Cyclobenzaprine HCl, Pharm Anal Acta, 2018, 9:1.

[10] Naguib IA, Abdelaleem EA, Abdallah FF, Ali NW. Development and validation of three spectrophotometric methods for determination of Cyclobenzaprine HCl in the presence of its two major degradation products. Analytical Chemistry Letters. 2016 Jan 2; 6(1): 24-34.

[11] Gaikwad AS, Madibone MN, Rajguru JR, Jadhav PB. Development and Validation of RP-HPLC and UV-spectrophotometric absorptivity method for simultaneous estimation of cyclobenzaprine hydrochloride and Aceclofenac in Pharmaceutical dosage form. 2020 5 (4): 320 – 329.

[12] Roshni D Patel, Usmangani K Chhalotiya, Falgun A Mehta, Dimal A Shah, and Kashyap K Bhatt, Liquid Chromatographic Estimation of Cyclobenzaprine Hydrochloride and Aceclofenac in Pharmaceutical Formulation, Research and review in pharmacy and pharmaceutical sciences, July – September 2014, 3(3): 37-44.

[13] Ramadan NK, Mohamed TA, Fouad RM, Moustafa AA. Stability-indicating high-performance liquid chromatography and thin-layer chromatography methods for the determination of cyclobenzaprine hydrochloride and asenapine maleate. JPC–Journal of Planar Chromatography–Modern TLC. 2017 Aug; 30: 313-22.

[14] Baru RT, Bitla P. Cyclobenzaprine drug assay and Cyclobenzaprine-excipient interaction study by chromatography, thermal and spectral analysis. J Pharm Chem Chem Sci. 2017; 1 (1): 1-9.

[15] Sethi PD. HPTLC: high performance thin-layer chromatography; quantitative analysis of pharmaceutical formulations. CBS publishers & distributors; 1996.

[16] Harde MT, Wankhede SB, Chaudhari PD. A validated inherent stability indicating HPTLC method for estimation of cyclobenzaprine hydrochloride in tablets and use of MS–QTOF in characterization of its alkaline stress degradation product. Bulletin of Faculty of Pharmacy, Cairo University. 2016 Dec 1; 54(2): 145-56.