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A novel HPTLC method for simultanoeus determination of alprazolam and methyl paraben in tablet dosage form

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ABSTRACT

A simple, sensitive, validated high performance thin-layer chromatographic method has been developed for simultaneous determination of alprazolam and methyl paraben in tablets. Chromatography was performed on aluminum-backed silica gel 60 F_{254} TLC plates using chloroform:ethylacetate:methanol:ammonia (4:5:1:0.1, v/v/v) as mobile phase. The TLC scanner set at 254 nm was used for direct evaluation of the chromatogram in reflectance absorbance mode. The Rf value for alprazolam and methyl paraben was found to be 0.26 ± 0.03 and 0.75 ± 0.05, respectively. The calibration range was found to be 800–2000 ng per band (r^2 = 0.9935) and 20-200 ng/band (r^2= 0.9990) for alprazolam and methyl paraben, respectively. The average percent labeled amount was found to be 100.42 % and 99.45 % for alprazolam and methyl paraben, respectively. The mean percent recovery for both alprazolam and methyl paraben was around 100 percent with standard deviation well below 2 indicating accuracy of the method. The suitability of method for quantitative determination of these compounds was proved by validation in accordance with the requirements of ICH guidelines.

Keywords: Alprazolam, Methylparaben, HPTLC, Validation

INTRODUCTION

Alprazolam (ALP), chemically is 8-chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzo-diazepine[1]. It is a short-acting drug of the benzodiazepine class primarily used to treat moderate to severe anxiety disorders (e.g., social anxiety disorder) and panic attacks, and is used as an adjunctive treatement for anxiety associated with moderate dperession. Methlyparaben (MEP), chemically is methyl p-hydroxybenzoate[2]. It is widely used as preservative in pharmaceutical formulations.

Preservatives such as methylparaben and propylparaben have been used for many years to ensure the quality of drug product throughout the shelf life of the product. Formulator must be fully aware of the procedure for preservative systems in a product need to be analysed to establish their effectiveness throughout the shelf life of the product. Actual concentration of preservative(s) in a formulation is vital in establishing the shelf life of the product. Besides, an analytical test result of preservative(s) is required by regulatory agencies.

Literature survey revealed, UV-Spectrophotometric[3], derivative spectrophotometric[4-5], spectrofluorimetric[6], RP-HPLC[7-11] and HPTLC[12] methods for determination of alprazolam alone or in combination with other drugs. Methylparaben is reported to be estimated by HPLC[13-16], UPLC[17], microextraction-ion mobility

spectrometry[18] and micellar electrokinetic chromatography[19]. Although analysis of alprazolam-containing pharmaceuticals has been described in numerous papers, no methods for simultaneous analysis of alprazolam and preservatives are available in the literature. This prompted us to develop and evaluate a HPTLC method for simultaneous analysis of alprazolam and commonly used preservatives, i.e. methylparaben, in tablet dosage form.

MATERIALS AND METHODS

Reagents and Chemicals

Alprazolam and methylparaben were provided as gift sample by Litaka Pharmaceuticals Ltd., Pimpri, Pune. A tablet formulation containing alprazolam (0.5 mg) and methylparaben (0.06 mg) was purchased from local market. AR grade solvents were used throughout the analysis.

Instrumentation and optimum chromatographic conditions:

Chromatography was performed on aluminum-backed silica gel 60 F_{254} TLC plates (10 cm × 10 cm with 200 µm thickness, HPTLC; Merck, Germany). Equal volume (10 µL) of standard and sample solutions were applied on a TLC plate in the form of band (band size: 5 mm) by means of CAMAG Linomat V automatic sample applicator (CAMAG, Muttenz, Switzerland) fitted with a 100 µL syringe (Linomat syringe 659.004, Hamilton-Bonaduz Schweiz, CAMAG). The mobile phase consisted of chloroform:ethylacetate:methanol:ammonia (4:5:1:0.1, v/v/v) and 10 mL of mobile phase was used in each chromatographic run. Linear ascending development technique was performed in a twin trough glass chamber (10 cm × 10 cm, CAMAG). The optimum time for the saturation of the chamber with mobile phase vapor was 10 min at room temperature (25 ± 2°C). The development distance was 80 mm. The bands were scanned using the TLC Scanner III (CAMAG) in the reflectance absorbance mode, with a slit dimension of 4 × 0.30 mm, at 254 nm. All measurements were operated by winCATS version 1.4.0 software (CAMAG). The concentration of alprazolam and methylparaben was determined from the intensity of reflected light, and peak areas of standard and sample bands were used for comparison.

Preparation of Standard Solution

Accurately weighed quantity of alprazolam (25 mg) was transferred to 25.0 mL volumetric flask, added 3.0 ml of 1 mg/mL solution of methylparaben in methanol, and diluted to the mark with methanol to get final concentration of 100 µg/mL of ALP and 12 µg/mL of MEP.

Preparation of Sample Solution

To determine the content of ALP and MEP in tablets, 20 tablets were weighed; their mean weight was calculated and crushed to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 1 mg of alprazolam was transferred to a 10.0 mL volumetric flask, added to 6 mL of methanol, sonicated for 10 min, and diluted to the mark with methanol. The resulting solution was mixed and filtered through Whatmann filter paper No. 42. The filtrate obtained was used as such for analysis.

METHOD VALIDATION

The method was validated in compliance with ICH guidelines. The following parameters were used for the validation of developed method.

Linearity

Calibration standards with concentration range of 80- 200 µg/mL of ALP and 2-20 µg/mL of MEP were prepared in in methanol. Equal volume (10 µL) of calibration solutions were applied to the TLC plate (concentration: 800-2000 ng per band ALP and 20-200 ng/band MEP, respectively) and chromatographed under optimum chromatographic conditions. Each solution was applied and chromatographed in triplicate fashion. The linear relationship between peak area and concentration was evaluated over the range of concentrations expressed in ng per band.

Accuracy/Recovery Studies

Recovery studies were performed in triplicate by standard addition method at three levels (80%, 100%, and 120%). Hence, 0.8, 1, and 1.2 mg ALP pure drug and .096, 0.12 and 0.144 MEP pure (in solution form), were added to pre-analyzed tablet powder equivalent to about 1 mg of ALP. The samples, after suitable dilution, were subjected to analysis by proposed method.

Precision
Precision of the developed method was studied by considering intra-day and inter-day precision. For intra-day and inter-day precision, tablet samples were analyzed by developed method three times on the same day and on three different days, respectively.

Limits of Detection and Quantification
The limit of detection (LOD) and quantification (LOQ) of the developed method were calculated using 3.3a/S and 10a/S phenomena, respectively, where a is the standard deviation of the y intercepts and S is slope of calibration curve.

Robustness
The change in composition of the mobile phase, volume of mobile phase, and chamber saturation time was involved in this study. The composition and volume of the mobile phase were varied in the range of ± 0.1 mL and ± 10%, respectively, of the used optimized conditions. Time variations were varied from the optimized times in the range of ± 2 min. The effects of these changes on the Rf values were evaluated by calculating the relative standard deviations (RSD).

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions
Several trials were made by using different solvent systems containing non-polar solvents and relatively polar solvents. Different proportions of chloroform, ethyl acetate, methanol, and ammonia were tried while selection of mobile phase. Among the mobile phase combinations tested, chloroform:ethylacetate:methanol:ammonia (4:5:1:0.1, v/v/v) was finalized as mobile phase. The bands developed were dense and compact, and the peaks obtained for alprazolam and methylparaben was sharp and is shown in Figure 1. The peak was symmetrical in nature and no tailing was observed when plates were scanned at 254 nm.

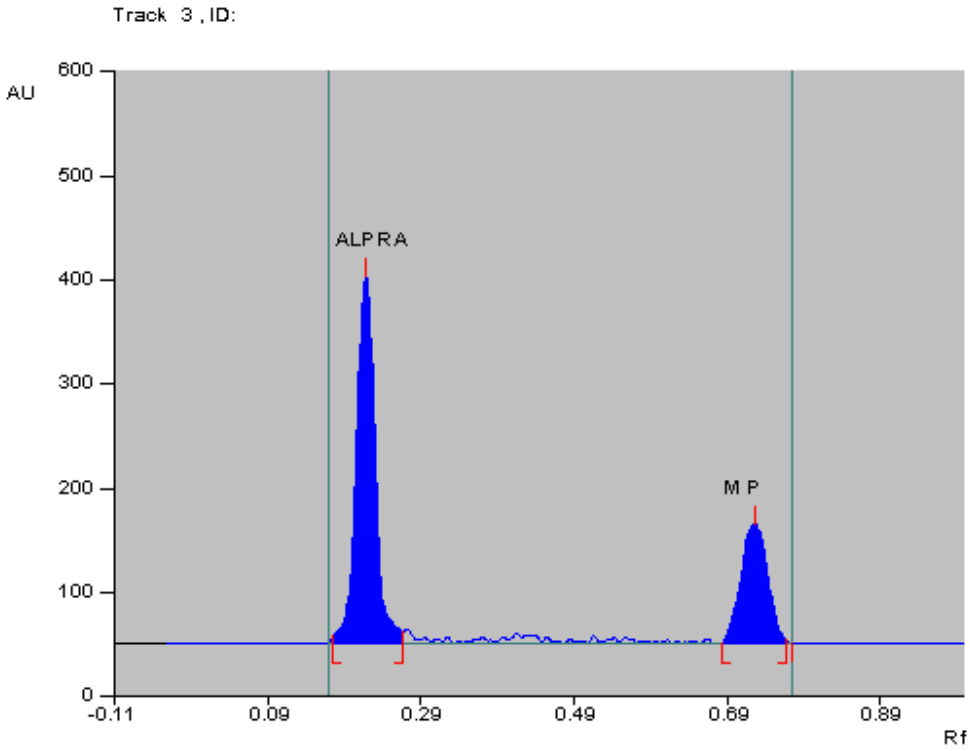

Fig. No. 1: Typical Densitogram of Alprazolam and Methylparaben

Analysis of Marketed Formulation
On TLC plate two bands of standard and four bands of sample solution, 10 µL each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the peaks obtained for

standard and sample bands were integrated. Amount of the drug/preservative present in applied volume of sample solution was obtained by comparison between peak area of standard and sample bands. Six samples were prepared and analyzed in similar manner. Results of analysis of tablet formulation are shown in Table No. 1

Table 1: Results of Analysis of Tablet Formultion

Component	Label Claim (mg/tablet)	Percent Label Claim*	S.D
ALP	0.5	100.42	± 0.72
MEP	0.06	99.45	± 1.27

**mean of six determinations*

Method Validation

Linearity

Peak areas were found to have good linear relationship with the concentration than the peak heights. Calibration graphs were constructed in the concentration range of 800–2000 ng per band for ALP and 20-200 ng/band for MEP. The correlation coefficient was found to be 0.9935 and 0.9990 for ALP and MEP calibration curves, respectively.

Accuracy/Recovery Studies

To ascertain the accuracy of proposed method, recovery studies were performed in triplicate by standard addition method at three levels (80%, 100%, and 120%). Results of recovery studies are expressed in terms of percent recovery (Table 2). Percent recovery was found to be around 100 %, indicating the accuracy of proposed method.

Table 2: Results of Recovery Studies

Level of Recovery	Percent Recovery*	
	ALP	MEP
80 %	99.88	99.92
100 %	100.22	100.46
120 %	100.44	100.03
Mean Percent Recovery, SD**	100.61, ± 0.82	100.56, ± 1.25

**mean of three determinations, **mean of nine determinations*

Precision Studies

Repeatability and intermediate precision of the developed method were expressed in terms of % RSD. The % RSD for both intra-day and inter-day precision of the method was found to be less than 2%.

Limit of Detection and Quantification

The LOD and LOQ values were calculated from standard deviation of y-intercept and slope of calibration curve. LOD & LOQ values for ALP and MEP were found to be 24 & 75 ng per band and 5 & 16 ng/band, respectively.

Robustness

The percent relative standard deviations of Rf values were calculated for the aforementioned three parameters (variation in composition of the mobile phase, volume of the mobile phase, and chamber saturation time). There was no significant change in the Rf value of drug peak. The method was found to be unaffected by small changes in the method parameters as the % RSD for all the parameters was less than 2%, indicating the robustness of method.

Specificity

The specificity of the method was determined by analyzing standard drug/preservative and test samples. The band for ALP and MEP in the samples was confirmed by comparing the Rf value and spectrum of the sample band with that of a standard.

CONCLUSION

The HPLTC method was developed successfully for simultaneous determination of alprazolam and methylparaben in tablet dosage form. The method has been validated; the results obtained were accurate and precise and the limit of detection were sufficiently low. It has also utilized the merit of applying several samples on TLC plate which can be advantageous for regulatory quality control laboratories. Since, actual content of preservative can be estimatied by the proposed method, it can be utilized to establish the effectiveness of preservative used i.e., methylparaben and

hence the stability of pharmaceutical formulation can be studied using proposed HPTLC method. The method can be used for routine analysis of compounds in pharmaceutical products containing the active compound alprazolam and preservative methylparaben.

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REFERENCES

[1] Indian Pharmacopoeia
[2] Indian Pharmacopoeia
[3] A. K. Kumar, A. Mohanakrishna, M. Sudheer, K.Sai Rajesh and P. Ramalingam, *International Journal of ChemTech Research*, **2011**, 3(1), 161.
[4] S. Sharma and M. C. Sharma, *European Journal of Applied Sciences*, **2011**, 3(3), 81.
[5] R. B. Patel, M. R. Patel, B. S. Madhira and K. K. Bhatt, *Eurasian Jouranal of Analytical Chemistry*, **2009**, 4(1), 76.
[6] N. S. Nudelman and C. G. Cabrera, *Journal of Pharmaceutical and Biomedical Analysis*, **2002**, 30(2), 887.
[7] M. Acikkol, B. Durgun-Barlas and S. Rollas, *Pharmazie*, **1991**, 46, 461.
[8] G.T. Rani , D.G. Shankar, P.Kadgapathi and B. Satyanarayana, *Journal of Pharmacy Research*, **2011**, 4(2), 358.
[9] J. A. Wade, *Analytical Letters*, **1979**, 12(6), 657.
[10] R. R .Prajapati, J.B.Dave, C.N.Patel, *International Journal of Pharmacy and Technology*, **2011**, 3(2), 2510.
[11] P Perez-Lozano, E. Garcia-Montova, A. Orriols, M. Minnarro, J. R. Tico and J. M Sune-Negre, *Journal of Pharmaceutical and Biomedical Analysis*, **2004**, 34(5), 979.
[12] K. Venkateswarlu, R. K. Venisetty, N. R. Yellu, S. Keshetty and M. G. Pai, *Journal of Chromatographic Science*, **2007**, 45(8), 537.
[13] M. X. Kokoletsi, S. Kafkala and M. Tsiaganis, *Journal of Pharmaceutical and Biomedical Analysis*, **2005**, 38(4), 763.
[14] G. A. Shabir, *Indian Journal of Pharmaceutical Sciences*, **2010**, 72(4), 421.
[15] J. Boonleang and C. Tanthana, *Songklanakarin Journal of Science and Technology*, **2010**, 32 (4), 379.
[16] P. Solich, R. Jajkova, M. Pospisilova and J. Sicha, *Chromatogrphia*, **2002**, 56(1), 5181.
[17] R. K. Trivedi, M. C. Patel and S. B. Jadhav, *Scientica Pharmaceutica*, **2011**, 79(3), 525.
[18] J. J. Loknanth and N. H. Snow, *Analytical Chemistry*, **2005**, 77(18), 5938.
[19] J. Safra, M. Pospísilova, *Journal of Pharmaceutical and Biomedical Analysis*, **2008**, 48(2), 452.