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Review Article

Analysis of Tolnaftate - Review

Sonali Gire*, P.A. Datar, R.V. Shete, Vrushali Harnaskar

Department of Quality Assurance Technique, Rajgad Dnyaanpeeth College of Pharmacy, Bhor, Dist-Pune- 412206, Maharashtra, India.

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****Corresponding author E-mail address:*** *giresonali01@gmail.com*

ABSTRACT
Tolnaftate has been widely used as a kind of topical antifungal in the treatment of cutaneous disease such as Jock itch, athlete's foot and other skin infections due to, Epidermophyton, Microsporum, Trichophyton species, and Malassezia furfur. The aim of this review to focus on a comprehensive update of chromatography determination of Tolnaftate in bulk and in pharmaceutical preparations, tolnaftate preparations are available in powder, cream, ointment, gel, emulgel, Solution and spray. In which has been described using RP-HPLC, LC, HPTLC, UV methods, Indirect Spectrophotometric method, Isocratic Supercritical Fluid Chromatography, Indirect spectroflurometric method. Pharmacopeial methods and Titrimetric methods also studied in this review. This review provides detailed information on separation conditions for Tolnaftate alone, combinations with other drugs and in the presence of its degradation products.

KEYWORDS
Tolnaftate, Chromatography, RP-HPLC, LC, HPTLC.

1. INTRODUCTION

Tolfanate is an antifungal compound which has activity against such species as Epidermophyton, Microsporum, Trichophyton species, and Malassezia furfur. Chemical name of tolnaftate is methyl (3-methylphenyl) carbamothioic acid*O*-2-naphthalenyl ester or m,*N*-dimethyl thiocarbanilic acid *O*-2-naphthyl ester. (fig.1) Molecular Formula of tolnaftate is C19H17NOS, having Molecular Weight- 307.4. It is soluble in chloroform (50 mg/ml), also soluble in acetone and sparingly soluble in ethanol and methanol yielding a clear, colorless to very faint yellow solution. Melting Point of tolnaftate is 110.5-111.5 °C. [1,2]Storage Temperature is 2-8 °C Its proposed mechanism of action includes the inhibition of squalene epoxidase.[3]The use of tolfanate to inhibit squalene epoxidase activity in Candida albicans (500 μM)[4]and in Trichophyton rubrum (IC50 = 51.5 nM) has been studied.[5]The activities of various antifungal compounds, including tolfanate, against dermatophytes from different species have been investigated.[6]An *in vitro* investigation of tolfanate penetration into the human nail plate in the presence of N-acetyl-L-cysteine or 2-mercaptoethanol has been described.[7]The effect of tolfanate on the biosynthesis of lipids in Aspergillus nigerprotoplasts has been studied.[8]

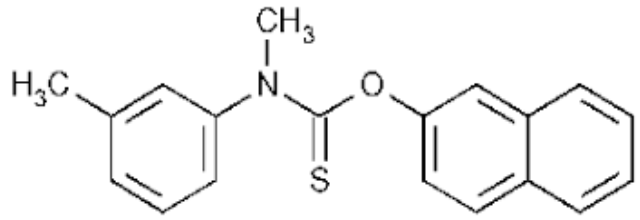

Fig. 1: Structure of Tolnaftate

1.1. Mechanism of Action

Tolnaftate is a synthetic antifungal agent.Tolnaftate is a thiocarbamate derivative with either fungicidal or fungistatic property. Tolnaftate is a selective, reversible and non-competitive inhibitor of membrane-bound squalene-2, 3- epoxidase, an enzyme involved in the biosynthesis of ergosterol. Inhibition leads to the accumulation of squalene and a deficiency in ergosterol, an essential component of fungal cell walls, thereby increasing membrane permeability, disrupting cellular organization and causing cell death. In addition, this agent may also distort the hyphae and stunts mycelial growth in susceptible fungi.[9]

1.2. Pharmacological Properties

Pharmacodynamic Properties

Tolnaftate is a topically used antifungal agent. Tolnaftate is effective on dermatophytes. Tolnaftate has specific and significant fungicidal effect on Tricophytone, Microsporum and Epidermophytone. It also exerts fungicidal effect against T.mentagrophytes at a concentration of 0.08 g/ml. While it relieves discomfort such as itching and burning derived from tinea pedis, tinea cruris and tinea corporis, it also effectively eliminates fungal symptoms. Thus physical properties such as odorless, colorless and easy solubility in water and organic solvents enable this product to be applied by topical route easily.[21]

Pharmacokinetic Properties

Tolnaftate is applied topically. There are no data available on the pharmacokinetics of tolnaftate. It is unknown if tolnaftate is absorbed through the epidermis or if detectable systemic concentrations of tolnaftate are achieved in the blood. Tolnaftate is an antifungal which inhibits growth of dermatophytes. Onset of action is 24-72 hours.[22]

1.3. Analytical Methods for Determination of Tolnaftate-

HPLC Method-

Safeena Sheikh et al. develop a stability indicating high performance liquid chromatography (HPLC) method for the quantification of salicylic acid (SA) and Tolnaftate (TF) in combined pharmaceutical ointment base formulations. The separation was performed on a Merck" C-18 column with the mobile phase consisting of Acetonitrile**:** Methanol: Water (50:20:30v/v) at flow rate 1.5ml/min. Both the drugs were resolved successfully with retention time 1.318 and 8.805minute when detection was carried out at UV 245nm. The overall retention time of analytes were 10.0minutes.The method was validated with respect to linearity, precision, accuracy and recovery. The relative standard deviation for six replicate measurements of SA and TF were 0.259% and 0.240% respectively. Total recoveries of analytes were 100.56, 100.63, 100.58% and 100.23, 100.73, 100.22% of SA and TF respectively when examine over the range of 80, 100, and 120% of added drugs in placebo. No chromatographic interference from the formulation excipient was found. The linearity of SA and TF were found in the range of 256-384µg/ml and 32.0 to 48.0µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operator proven that the method is robust and rugged.[10]

Another study of Kezutyte T. et al. optimize and validate a simple, rapid, accurate and reproducible High performance liquid chromatography (HPLC) method with UV detection was used to validate tolnaftate assay for linearity, specificity, accuracy, precision, limit of quantitation, limit of detection, drug extraction recovery and stability in skin extracts. In vitro tolnaftate penetration studies were carried out using flow-through diffusion cells, mounted with human skin. Epidermis and dermis, separated by heat-separation method, were extracted using ultrasonication in methanol. Linear range of the analytical procedure was within 0.6-100 pg/mL. The assay was specific, accurate (within-day and between-day recovery values were 98.2-104.2% and 98.7-101.4%, respectively) and precise (within-day and between-day imprecision was = 3.8%). Mean extraction recoveries of tolnaftate from epidermis and dermis were satisfactory and reaching 90%. In vitro skin penetration studies revealed that after application of 1% (w/w) tolnaftate solution in polyethylene glycol 400 for 24 hours, the mean amount of tolnaftate penetrating into the epidermis and dermis was 2.60 +/- 0.28 microg/cm2 and 0.92 +/- 0.12 microg/cm2, respectively. A validated reliable HPLC method could be recommended for biopharmaceutical evaluation of tolnaftate preparations and studies of pharmacokinetics in human skin after in vitro penetration studies.[11]

Another study of Chandra Nath Saha et al. developed a new RP-HPLC method for selective and simultaneous determination of betamethasone dipropionate and tolnaftate in combined semisolid formulation containing other components. The proposed method was validated for linearity, precision (system precision, method precision, intermediate or inter-day precision), accuracy,

stability in analytical solution, robustness or system suitability and ruggedness. Chromatographic conditions consisted of a gradient HPLC (Shimadzu LC2010CHT) with serial dual plunger pump; analytical column: Inertsil ODS, 150 × 4.6 mm, with particle size 5 µm; The flow rate is 1.2 ml/min The column temperature is ambient and detection with UV at 240 nm data processing software: LC solution Ver. 1.2and a mobile phase composed of Acetonitrile, methanol and water in the ratio of 60:15:25 was employed in the study. Betamethasone dipropionate demonstrate linearity in the range of 17.92 µg to 33.28 µg with r2 value 0.9992 and tolnaftate demonstrate linearity in the range of 280 µg to 520 µg with r2 value 0.9970.In system precision study, the % RSD for betamethasone dipropionate and tolnaftate were found to be 0.58 and 0.10,In method precision study, the mean % drug content for betamethasone dipropionate and tolnaftate were found to be 103.56 % and 103.13 % and intermediate or inter-day precision study, the mean % drug content for betamethasone dipropionate and tolnaftate were found to be 103.59 and 102.75 respectively. Accuracy of the method considered acceptable as it was well within 98 to 102 %.In robustness or system suitability study, there was no significant impact on the % RSD and tailing factor. In ruggedness study, the % RSD for analyst-I was 0.1088% for betamethasone dipropionate and 0.1078% for tolnaftate and for analyst-II was 0.3208% for betamethasone dipropionate and 0.7329% for tolnaftate, respectively. [(12)]

Mayank Bapna et al. developed a simple, accurate and precise RP-HPLC method was developed for simultaneous estimation of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin. Eclipse C18 (250mm×4.6mm) 5µ (particle size) was used as stationary phase. The mobile phase used was KH2PO4 buffer of pH 6.3: Acetonitrile 30:70 V/V. The mobile phase was delivered at flow rate 1.5 ml/min. UV detection was set at 254nm. The retention time of Betamethasone dipropionate, tolnaftate and Iodochlorhydroxyquin was found to be 5.2 minutes, 9.8 and 7.2 minutes respectively. Linearity was observed over the concentration range of 3.9-9.1µg/ml, 60-140 µg/ml and 60-140 µg/ml for Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin respectively. The LOD was found to be 0.44 µg/ml, 6.49 µg/ml and 4.64µg/ml for Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin respectively. Whereas LOQ was found to be 1.34 µg/ml,19.67 µg/ml and 14.08 µg/ml for Betamethasone dipropionate ,Tolnaftate and Iodochlorhydroxyquin respectively Moreover, the % RSD for repeatability, inter and intraday precision was found to be less than 2%, which reveals that the method is precise. The % recovery was found to be 100.84% for betamethasone dipropionate, 101.11% for tolnaftate and for 100.53% Iodochlorhydroxyquin. However, the change in flow rate and mobile phase ratio also did not show any significant variance. Assay of the combined dosage form finalized the applicability of this method for simultaneous estimation of Betamethasone dipropionate, Tolnaftate and Iodochlorhydroxyquin in combined dosage form.[(13)]

Małgorzata Sznitowska et al. reported that, quantitative determination of TOL was performed by reverse phase high performance liquid chromatography (HPLC). Reverse phase C18 5 µm column was used and TOL was detected at 258 nm. Methanol 80% (v/v) was used as a mobile phase at a flow rate 1 ml/min. The injected volume was 20 µl. Standard curve (Figure 3.) was obtained within concentration range 1 to 10 µg/ml. The retention time (tR) of TOL was approximately 9.6 min as shown in Figure 2.[(14)]

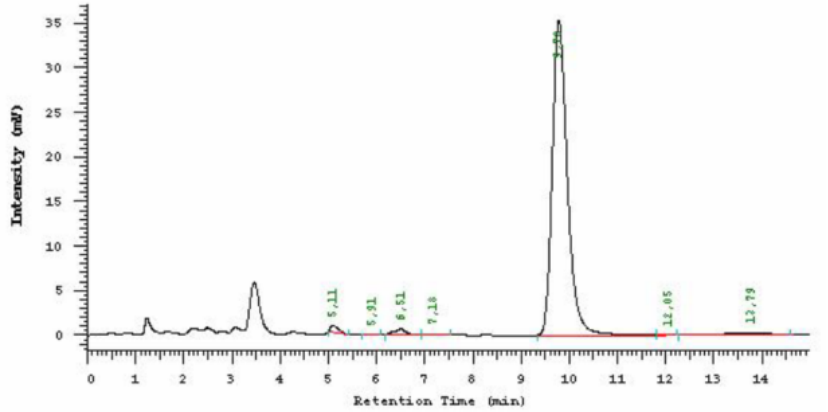

Fig. 2: HPLC chromatogram of 10 µg/ml standard TOL solution in methanol.

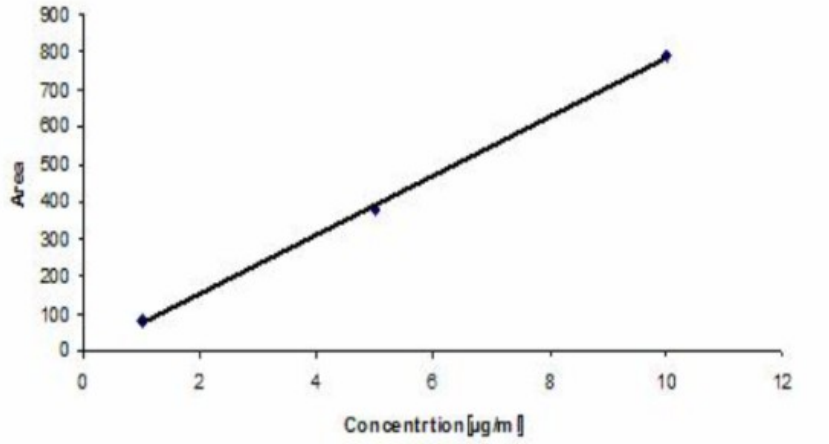

Fig. 3: HPLC calibration curve of TOL in methanol.

Concentrations [µg/ml] after HPLC analyses using this calibration (Fig. 3) curve were calculated from area (A) using following equation:
C = (A + 5, 3769) / 79,184
R2 = 0.9993

LC Method
Thompson, R. D. et al. Developed a liquid chromatographic (LC) method for the determination of the antifungal agent tolnaftate was developed. Isolation of the analyte was achieved by direct extraction or dilution with acetonitrile-water (80 + 20) followed by reverse-phase liquid chromatography using a C18 column. The mobile phase was acetonitrile-water (80 + 20) acidified with phosphoric acid. Detection was by UV absorption at a wavelength of 257 nm. The proposed procedure was applied to 20 consumer products comprising 6 formulation types, including solutions, powders, liquid and power aerosols, creams, and gels. The precision (RSD) for the products ranged from 0.23 to 1.16% (n = 5), and recoveries via fortification ranged from

98.1 to 103.0%. Six different brands of C18 columns were evaluated for use with the method. The overall simplicity and versatility of the method suggest possible adaptations to both regulatory and quality-control situations.[15]

Alekha K. Dash et al. Developed a simple LC method and validated for the analysis of tolnaftate in various pharmaceutical formulations. This method did not require any complex sample extraction procedure. The chromatographic separation was achieved on a reversed-phase, C18 column with UV detection at 258 nm. This isocratic system was operated at ambient temperature and required 9 min of chromatographic time. The mobile phase consisted of methanol-aqueous potassium dihydrogen phosphate solution (80:20, v/v) at a flow rate of 1.5 ml min-1. Standard curves were linear over the concentration range of 1.0-51.0 mug ml-1. Within-day and between-day relative standard deviation values ranged from 0.7 to 2.9% and from 1.3 to 3.4%, respectively. This method was used to quantify tolnaftate in microcapsule, microsphere, cream, powder, liquid, liquid aerosol and powder aerosol formulations. This method was also used to study the stability of tolnaftate in solution during its extraction from microcapsule formulations. [16]

UV Methods

Małgorzata Sznitowska et al. reported an UV-Vis Spectrophotometry was used in preliminary experiments for quantitative determination of TOL in solutions: ethanol, diluted dissolution media (10 times with ethanol) and dissolution samples. The calibration curve was made in range of 1 to 10 µg/ml. The absorbance spectrum (Fig. 4) was measured between 200 and 310 nm. Two peaks with maximum at 222 and 258 nm were registered. Dissolution samples were diluted 10 times with ethanol and placed in a 1 cm glass cell for analyses. PEG400 and ethanol 95% mixture (5:95) was used as a reference. [14]

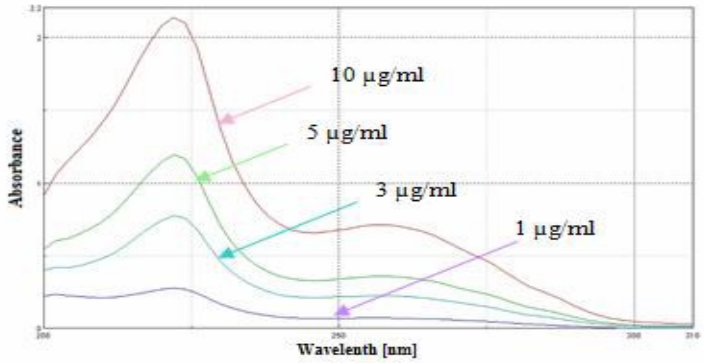

Fig. 4: Spectrum of TOL solutions in ethanol (concentration range 1-10µg/ml)

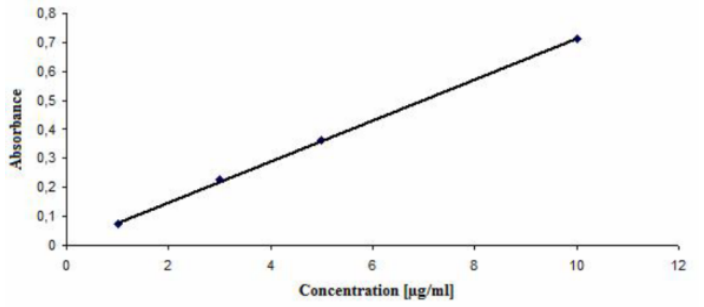

Fig.5: UV-Vis calibration curve of TOL in ethanol.

Concentrations [µg/ml] after UV-Vis analyses using this calibration curve (Fig. 5) were calculated from absorbance (A) using following equation:

C = (A – 0.0082) / 0.0706

R2 = 0.9996

Another study of Naina Bhoyar et al. reported the Tolnaftate (O-2-Naphthyl m, N-dimethylthiocarbanilate) is a topically used antifungal agent, most commonly employed in the treatment of tinea pedis at 1% (w/w) concentration. Analysis of this thionocarbamate fungicide which is also antidermatophytic, antitrichophytic and antimycotic agent primarily inhibits the ergosterol biosynthesis in the fungus. The spectra of Tolnaftate in methanol showed maximum wave length at 257nm and obeyed Beer's law in the concentration range of 1-5 ug/ml. Standard curve depict line equation y =0.148x-0.024 with correlation coefficient of 0.990. The developed method was validated with respect to linearity. The objective of present work is to develop simple, precise and accurate UV Spectrophotometric method for estimation of Tolnaftate in organic solvent. The organic solvent methanol is used was HPLC grade. This method can also be used for Tolnaftate determination in its marketed formulations.[17]

HPTLC Method

Dhananjay B. Meshram et al. developed a simple, rapid, reproducible, and economical HPTLC method has been established for analysis of tolnaftate in a topical solution. Silicagel 60 GF254 HPTLC plates were used with toluene–chloroform4:1 (v/v) as mobile phase; the retention factor was 0.59. UV detection was performed at 248 nm. Linear regression analysis showed response was a linear function of amount of tolnaftate in the range100–400 ng per band. The amount of drug in the commercial formulation was 99.77 ± 0.86 and 99.54 ± 0.50% by peak height and peak area, respectively. Recovery of the drug, determined by the standard addition method, was 100.2 ± 1.0 and 99.59 ± 0.18%, again by peak height and peak area, respectively. The method was validated for accuracy, precision, and specificity.[18]

Indirect Spectrophotometric Method

Nief Rahman Ahmed et al. developed a highly, sensitive, indirect spectrophotometric method for the determination of tolnaftate in pharmaceutical preparations. The method is based on the oxidation of tolnaftate with Fe(III) in acidic medium. Fe (III) subsequently reduces to Fe(II),which is coupled with potassium ferricyanide after heating for 10 minutes at 70 0C to form prussian blue and the absorbance of the product was measured at 785 nm against a reagent blank. Beer's law was obeyed in the range of 0.02-0.16 ppm with molar absorptivity of 1.7 x106 L.mol-1.cm-1, relative standard deviation of the method was less than 2.5% and accuracy (average recovery %) was 100.3%. The effect of various factors such as temperature, heating time, concentration of reagents, and interferences were investigated to optimize the procedure. The proposed method has been applied for the determination of tolnaftate in pharmaceutical preparations (quadrim cream and topical solution). A statistical comparison of these results with those of official method shows good agreement using "t" and "F" test at 95% confidence level. The results indicated that there is no systematic error and the present method has good validity.[19]

Isocratic Supercritical Fluid Chromatography Method

Suvarna T. Patil et al. reported that the Tolnaftate, is an antifungal drug (TF) and related impurities arising from synthesis, viz., N-methyl-m-toluidine (NMmT) and β-naphthol-1-chlorothio carbamate (β-NCTC) can be determined by supercritical fluid chromatography. Even though it was possible to elute TF completely with neat SCF CO_2, the peaks of the impurities were found to merge. The chromatographic figures of merit of the three analytes such as retention time (t_R), capacity factor ($k^{|}$), selectivity factor (α), no. of theoretical plates (N), were optimized. The three compounds can be resolved in 5 min on a Hypersil (250 × 4.0 mm) 5 μ, C_{18} °C. Detection was carried out at 220 nm. The data as evaluated by the linear regression least squares fit method gave linearity ranges from 0.2 to 10.0 μg/mL for TF and NMmT and 0.3 to 10.0 μg/mL for β-NCTC with correlation coefficients column with supercritical carbon dioxide, modified with 1.96% methanol as the mobile phase at 9.81 MPa and at 40 > 0.99. The method was successfully employed to estimate levels of 0.01% for NMmT and 0.02% for β-NCTC with respect to TF.[20]

Spectroflurometric Methods

Tang Bo et al. describes an indirect spectrofluorimetric method with high sensitivity and selectivity was developed for the determination of antifungal drug tolnaftate (TNF), depending on the supramolecular multi-recognition interaction among the anionic surfactant sodium lauryl sulfate (SLS), beta-cyclodextrin (beta-CD) and beta-naphthol (ROH). The mechanism of the inclusion was studied and discussed by means of fluorescence spectrum, infra-red spectrograms and (1) HNMR spectroscopy. Results showed that the naphthalene ring of ROH and the hydrophobic hydrocarbon chain of SLS were included into the beta-CD's cavity to form a ROH:SLS:beta-CD ternary inclusion complex with stoichiometry of 1:1:1 at room temperature, which provided effective protection for the excited state of ROH. At lambda (ex)/lambda (em) = 273/360 nm, the fluorescence intensity was linear over a tolnaftate concentration range of 2.46 x 10 (-9) to 2.10 x 10(-6) mol L (-1). The detection limit and relative standard deviation was 7.50 x 10(-10) mol L(-1) and 1.4%, respectively. The interference of 31 foreign substances was slight. The proposed method had been successfully applied to the determination of tolnaftate in artificial mixed samples with almost quantitative recovery.[23]

Khashaba P.Y. et al describes spectrophotometric and spectroflurometric methods are suggested for the determination of antifungal drugs; clotrimazole, econazole nitrate, ketoconazole, miconazole and tolnaftate. Spectrophotometric one depends on the interaction between imidazole antifungal drugs as n-electron donor with the pi acceptor 2, 3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol or with p-chloranilic acid (p-CA) in acetonitrile. The produced chromogens obey Beer's law at lambda(max) 460, and 520 nm in the concentration range 22.5-200 and 7.9-280 microg ml(-1) for DDQ, and p-CA, respectively. Spectroflurometric method is based on the measurement of the native fluorescence of ketoconazole at 375 nm with excitation at 288 nm and or the induced fluorescence after alkaline hydrolysis of tolnaftate with 5 M NaOH solution at 420 nm with excitation at 344 nm. Fluorescence intensity versus concentration is linear for ketoconazole at 49.7-800 ng ml(-1) while for tolnaftate, it is in the range of 20.4-400 ng ml(-1). The proposed methods were applied successfully for the determination of all the studied drugs in their pharmaceutical formulations.[24]

Pharmacopeial Methods

Tolnaftate is official in Indian Pharmacopoeia, European Pharmacopoeia, and United State Pharmacopoeia. In USP Monograph contains tolnaftate topical preparation contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{17}$ NOS. In USP describe the chromatographic methods like, The liquid chromatograph is equipped with a 254-nm detector and a 4-mm x 30-cm column that contains 10 µm packing L1.The flow rate is about 1 ml per minute. The relative retention times are about 0.7 for progesterone and 1.0 for tolnaftate. Calculate the quantity in mg of C19H17NOS in the portion of powder taken by the formula:

$62.5C (R_U / R_S)$

In which C is the concentration, in mg per ml of USP Tolnaftate RS in the standard preparation and R_U and R_S are the peak response ratios obtained from assay preparation and the standard preparation respectively.[25]

Titrimetric Methods

Weight accurately about 0.4 g of Tolnaftate, dissolve in 50 ml of anhydrous glacial acetic acid. Previously neutralized with 0.1 M Perchloric acid. Using Crystal Violet as indicator and add 20 ml mercuric acetate solution (Previously neutralized with 0.1 M Perchloric acid. Using Crystal Violet as indicator) Titrate with 0.1 M Perchloric acid upto green colour changed. Each ml of 0.1 M Perchloric acid is equivalent to 0.030741g of Tolnaftate. [26]

2. CONCLUSION

The review of the analysis of Tolnaftate showed that the various chromatographic methods are used for the determination of tolnaftate in alone or in combination with other drugs in which mainly RP-HPLC, LC, UV Methods are used. Also the indirect Spectrophotometric Method, Isocratic Supercritical Fluid Chromatography Method, Spectroflurometric Methods are used for determination of tolnaftate in pharmaceutical dosage form. The review article also provides the detail information about Pharmacopeial method and titrimetric method for analysis o tolnaftate.

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