A Review on Spectrophotometric Techniques used for estimation of Terbinafine Hydrochloride and Salicylic acid

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Abstract

Terbinafine is active against broad range of fungi so acts as an antifungal drug. Terbinafine hydrochloride includes into allylamine classification of drugs. Along with this, when salicylic acid used with the aid of cyclodextrin, it indicates improved keratolytic, bacteriostatic, photoprotective and fungicidal properties as well. Moreover, method development is the process of proving that an analytical technique is acceptable for use to determine concentration of an API in particular compound dosage form. The validation of analytical method is essential for its development, while it is extensively applied for testing specificity, linearity, accuracy, precision, range, LOD, LOQ, robustness and ruggedness respectively. There has been different analytical methods established and documented to determine terbinafine and salicylic acid simultaneously. Whereas, the current review highlights the process of drug development and method of analysis, including the chromatographic and spectroscopic techniques which have been applied for several pharmaceuticals.

Keywords: *Terbinafine hydrochloride, salicylic acid, HPLC, UV, spectral techniques.*

1. Introduction

1.1.Terbinafine:

The allylamine derivative terbinafine can be used orally or topically. Terbinafine presents an advancement in antifungal therapy in that it has a novel mode of action; it has fungicidal activity, it is active when given orally as well as topically, and it has a better efficacy and safety profile than other currently used antifungal agents. It is active against a broad range of fungi including filamentous fungi and to lesser extent, yeast like fungi. Terbinafine acts by inhibiting the synthesis of fungal ergosterol at the level of squalene oxidase, leading to depletion of ergosterol and accumulation of toxic squalenes in the fungal cell membrane [1]. Biotransformation is mainly takes place through N-demethylation and oxidation of the tertiary butyl group [2].

Terbinafine hydrochloride: Terbinafine hydrochloride (TH) is a new potent antifungal agent of the allylamine class that selectively inhibits fungal squalene epoxidase.
This drug is indicated for both oral and topical treatment of mycoses. Chemically it is (E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalenemethanamine hydrochloride and is not yet official in any pharmacopoeia. Previously, the drug has been determined in biological fluids by HPLC and in tablets by UV-spectrophotometric methods. However, an HPLC method to determine TH in dosage forms has not been reported. Because of their selectivity, sensitivity and overall versatility, the development of reliable and validated HPLC methods has received considerable attention in the quality control of drugs [3]. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost [4].
Because of its therapeutical importance, quantitative determination of terbinafine in pharmaceuticals and human physiological fluids is of considerable significance in both quality control of preparations and chemical diagnosis. In the last approximately 25 years, several methods have been reported for the determination of terbinafine in pharmaceuticals and biological materials including body fluids [5].

Pharmacopoeial Method: In the method described by United States Pharmacopoeia, TFH assay was done by using high performance liquid chromatography (HPLC) in which C18 (150 mm × 3.0 mm; 5µm) column was used as stationary phase and the mobile phase was composed of buffer (0.2% triethylamine in water, pH is adjusted to 7.5 with dilute acetic acid), acetonitrile and methanol with a gradient elution. Column temperature was set at 40°C and the flow rate at 0.8 mL/min. Column effluent was monitored at 280nm.

Spectrofluorimetric Method: Based on the measurement of native fluorescence of TFH in water at 376 nm after excitation at 275 nm, a rapid and highly sensitive spectrofluorimetric method has been reported for TFH in pharmaceuticals. The fluorescence-concentration plot was linear over the concentration range of 0.02 - 0.05 µg/mL (r = 0.9998) with LOD and LOQ values of 0.0031 and 0.0094µg/mL, respectively. The method was applied to the

determination of TFH in commercial tablets, cream, gel and spray formulations with a relative error of <2% [5].

Liquid Chromatography-Mass Spectrometric Method: Numerous HPLC methods have been reported so far for the determination of terbinafine in pharmaceutical preparations as well as in biological matrices. Furthermore, the latest methods have used liquid chromatography coupled to a mass spectrometry detector for the determination of terbinafine in human hair or tandem mass spectrometry detector in human and minipig plasma. Only one LC-MS/MS method in human plasma, applied to a bioequivalence study, has been reported [6].

1.2.Salicylic acid:

Salicylic acid is the most widely consumed analgesic, antipyretic, and anti-inflammatory agent in the world. It is a natural product found in the bark of a willow tree and has been used for centuries to relieve fever and pain. Salicylic acid is a precursor to acetylsalicylic acid, better known as aspirin. Salicylic acid is used topically for its keratolytic, bacteriostatic, fungicidal, and photoprotective properties. Topical application has been shown to reduce the rate of keratinocyte proliferation [7].

The primary goal of delivery systems for salicylic acid has been to diminish its rapid penetration into stratum corneum. By this approach, irritation potential of the acid is greatly reduced, and a reservoir of the material is created for sustained topical activity. One conclusion that emerges from a review of such systems is that the topical bioavailability of salicylic acid in the stratum corneum varies substantially among formulations employing different systems. These delivery technologies can be categorized in:

- Polymeric complexation
- Liposomes systems etc.

Polymers such as polyolprepolymers and cyclodextrins are examples of polymers that have the capacity to complex salicylic acid [8].

Polyolprepolymers: Polyolprepolymers (Polyurethane type polymers) are the mixture of oligomers ranging in molecular weight from about 525 to 5,000 Daltons. When applied to the skin, a gradient of such oligomers is formed within the stratum corneum. Polyolprepolymers form a liquid matrix of oligomers that associate with the stratum corneum.

Cyclodextrins: These are a class of molecular cavity containing compounds (oligosaccharides). These possess the ability to form special types of complexes known as molecular-inclusion complexes. These complexes form with variety of chemicals, including salicylic acid. The physical and chemical properties of the “guest” molecules, such as salicylic acid, change due to complex formation with cyclodextrin “host”.

While the solubility of salicylic acid in aqueous solution is low because of its lipophilicity, its complex with a cyclodextrin molecules make this acid significantly more water soluble. Thus, some potentially irritating reactions that result from use of the free acid form can be prevented because of better homogeneity provided by the cyclodextrin. It is claimed that cyclodextrin complexes enhance the disinfectant, bacteriostatic and keratolytic properties of

salicylic acid [8]. Moreover, if cyclodextrins are used along with Terbinafine then it demonstrates the potential of cyclodextrin as a transfungal permeation enhancer. In the case of Itraconazole and levodopa when they combine with HP-β-CD and β-CD respectively. They show greater cutaneous drug deposition and also stability of levodopa enhanced when complexed with β-CD in transdermal patches [9].

2. Comparative studies

Comparative effectiveness research aimed evaluating and comparing the implications and outcomes of two or more health care strategies to address a particular medical condition. Some of the comparative studies including terbinafine with other respective drugs are given as followed:-

- The safety and efficacy of terbinafine 250 mg/day and itraconazole 200 mg/day given for 12 weeks for toenail onychomycosis have been compared in a double-blind, randomized study in 372 patients. Adverse events were reported in 39% of the terbinafine-treated patients and in 35% of the itraconazole-treated patients. The mean values of biochemical parameters of liver and kidney function did not change significantly. Terbinafine produced higher rates of clinical cure (76 versus 58%) and mycological cure (73 versus 46%) than itraconazole.
- A double-blind, randomized, prospective evaluation of 50 patients with a clinical and mycological diagnosis of tinea capitis was performed. One group received 4 weeks of terbinafine followed by 4 weeks of placebo. The other group received 8 weeks of Griseofulvin. The evaluation was done with 5 clinical parameters. Mycologic examinations were performed at baseline and at the end of weeks 8 and 12. Patients' ages ranged from 1 to 14 years. Fifty-four percent were girls and 46% were boys. Mycologic examinations disclosed Trichophyton tonsurans in 74% of patients and Microsporum canis in 26%. At week 8, the griseofulvin-treated group showed a cure rate of 76%, and the terbinafine-treated group 72%. At week 12, the efficacy of griseofulvin decreased to 44%, whereas the efficacy of terbinafine was 76%.Here, Terbinafine constitutes an alternative for the treatment of tinea capitis. Recurrences were less frequent. No significant side effects were reported [10].
- Ketoconazole is largely fungistatic and has limited efficacy in systemic mycoses in immunocompromised hosts. The drug penetrates the blood-brain barrier poorly and is not recommended in fungal meningitis.
- With the introduction of the newer antifungal agents such as terbinafine, itraconazole, fluconazole and amorolfine, the therapy for superficial and deep mycoses is likely to change significantly. For many indications the antifungal agents discussed will be suspended by the new generation of drugs [11].

3. Method development and validation

- The development of a pharmaceutical product requires a broad spectrum of scientific expertise to lead it through a complex pathway from discovery through characterization of quality, efficacy, and safety, which are the hallmarks of a successful drug product. A company must be highly proactive in setting targets for appraising and selecting a compound that has the highest probability of success [12].

- Validation is one of the key elements to fulfill the requirement of current good manufacturing specifications (CGMP) and good laboratory specifications (GLP).
- In every pharmaceutical industry, the testing of raw materials, in-process materials, final containers and excipients should be effectively performed. Analytical method validation is considered as the essential requirement for the testing of such pharmaceutical materials. For the testing of API, excipients and final product, an analytical procedure should be developed. Such well-developed procedure should essential assure that it will consistently produce the intended and precise result with high degree of accuracy. To gain such specific result, analytical method should be validated [13].

3.1. Objective of analytical method validation

- When there are changes in the formulation or if changes are done in the concentration, further validation is not required if and only if the method validation of the analytical method is performed.
- It decreases risk of regulatory noncompliance.
- Critical parameters of the process can be fully understood due to analytical method.
- Minimization of interference on accuracy and precision.
- It is used in authorization of product and marketing license for new products which are non-pharmacopoeia [13].

4. Spectroscopy

Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism. Later the concept was expanded greatly to include any interaction as a function of its wavelength or frequency. Spectroscopic data is often represented by an emission spectrum, a plot of the response of interest as a function of wavelength or frequency. Spectroscopy and spectrography are the terms used to refer, to the measurement of radiation intensity as a function of wavelength and are often used to describe experimental spectroscopic methods. Spectral measurement devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analyzers [14].

Spectrophotometric analytical techniques

4.1. Near-Infrared Spectroscopy:

The near-infrared (NIR) wavelength region (780 – 2500 nm) is situated between the red band of the visible light and the mid-infrared (mid-IR) region. The NIR signal (spectrum) is a consequence of the absorbance of light due to molecular vibrations (overtones and combinations of fundamental vibrations) of hydrogen bonds like C-H, N-H, and O-H. It is a very fast technique with the most modern instruments employing Fourier transform (FT-NIR), a spectrum can be recorded within a few seconds. The most interesting advantage of this technique is the non-destructive character of analysis: a sample can be analyzed without (or with only minimal) preparation procedures, thus avoiding important steps responsible for error sources.

4.2.Nuclear Magnetic Resonance:

Spectroscopy NMR spectroscopy can be used for the identification of a drug substance, the identification and quantification of impurities arising from the synthesis pathway, degradation, or the determination residual solvents in the assay. The NMR signal intensity I is directly proportional to the number N of nuclei evoking the signal. Hence, the intensities of NMR signals (defined as areas under the specific signals) can be taken for quantitative analysis. The linear relationship between signal intensity I and the number of nuclei (in case of single pulse excitation) is given by the formula:

$$I = Cs \times N$$

Where, the proportionality factor Cs depends on the parameters of spectrometer ("spectrometer constant") and properties of the sample. The NMR spectroscopy is widely used in pharmaceutical analysis to identify drugs and the accompanying impurities.

4.3.Fluorescence spectroscopy:

It is a rapid, sensitive method for characterizing molecular environments and events samples. Fluorimetry is chosen for its extraordinary sensitivity, high specificity, simplicity and low cost as compared to other analytical techniques. It is widely accepted and powerful technique that is used for a variety of environmental, industrial, medical diagnostics, DNA sequencing, forensics, genetic analysis and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis [15].

4.4.UV Spectrophotometry:

The absorption of light in the UV-Vis wavelength (between ~180-800 nm) occurs frequently from many molecules such as a wide number of organic molecules. The main energy changes happen at electronic level but can also happen at vibrational quantum levels. $n \rightarrow \sigma^*$ and $\sigma \rightarrow \sigma^*$ transitions need more energy and are related with absorption in the UV region and $\pi \rightarrow \pi^*$ transition with absorption in the UV–Vis region. The area of the molecule where the electronic transitions happen is called chromophore [16].

The development of a new UV Spectrophotometric method was confirmed after selecting the suitable solvent combination and identifying the wavelengths in the literature. Different solvents were used to assess the solubility of the samples, including methanol, acetone, chloroform, dimethyl sulfoxide, 1N NaOH, and 0.1N HCL. Methanol and water were selected as solvents after considering solubility conditions. Using methanol and water (1:1) as a solvent sample were scanned in the UV region between 200 and 400 nm [17].

5. Chromatography

The term "chromatography" is derived from the Greek words "chroma" meaning "colour" and "graphien" meaning "to write". The technique was originally developed by the Russian Botanist Mikhail Tswett in 1903. It is an analytical technique utilized for the separation, purification and identification of constituents from the mixture. It works on the principle of differential interaction of solutes with two different phases, viz., the stationary phase and the mobile phase. Many modifications were made to the techniques of chromatography to overcome the shortcomings like analysis time and the range of compounds that could be

detected. Application of pressure was practiced by use of pumps to reduce the time of run. Technologies like spectroscopy and electrochemical methods were added to enhance detection. With these developments and modifications, the functional efficiency of chromatographic techniques improved to a great extent and also the range and type of substances that could be analyzed [18].

5.1. High performance liquid chromatography:

HPLC is a widely used method of separation with high precision and accuracy. It allows the separation between the drug and excipients, as well as degradation products, which is useful as an indicative stability method [19].

HPLC is a superior form of liquid chromatography and is one of the most used analytical techniques. High pressure should be applied to have an eluent flow through the column due to the physical properties of HPLC columns. Because of this earlier the method was termed as high pressure chromatography.

5.2. HPLC-UV (High performance liquid chromatography-Ultraviolet):

High performance liquid chromatography is used in combination with various spectroscopy techniques to get appropriate results. For an instance, HPLC combined with mass spectrometric detection (MS/MS) has been widely used due to its high sensitivity. Nevertheless, this technique involves sophisticated and high-priced instrumentation. Additionally, the samples which can be analyzed using this method are limited and some problems may occur because of ion suppression. Accordingly, it would be beneficial to couple a UV detector with HPLC as it has been reported to show simplicity of the instrumentation process and excellent response stability [20].

6. Literature review

Following are some literature reviews given by several authors for the current drugs.

Bodiwala KB *et al.*, (2021) established a method to assay 3 active components in pharmacy compounded terbinafine ointment simultaneously. Methods High performance liquid chromatography (HPLC) equipped with the ZORBAX SB-C8 (4.6 mm×250 mm, 5 μm) was used for the assay. The mobile phase was methanol-0.1% phosphoric acid (70∶30). The flow rate was 1.0 ml/min with the 248 nm detection wavelength, 10 μl injection volume and 30˚C column temperature. Results A good linear relationship was observed in the range of 20.4-204.0 μg/ml for terbinafine hydrochloride (R^2=0.9997), 40.4-404.0 μg/ml for mupirocin (R^2=0.999 8), 2.02-20.20 μg/ml for mometasonefuroate (R^2=0.999 7). The average recovery of each detected component in terbinafine ointment was 99.39%, 99.21%, 99.97% with the RSD 0.82%, 0.59%, 0.81%(n=9) [21].

Shivaranjani K *et al.*, (2021) developed and validated simple, fast, economical and eco-friendly RP-HPLC method for the estimation of Itraconazole and Terbinafine in bulk and tablet dosage form according to ICH guidelines. Method: This method achieved by Shimadzu LC-20A instrument with isocratic elution with the mobile phase of methanol and water in the ratio of (9.5:0.5v/v) on Zodiac C18 (250mm x 4.6mm, 5μm) with a flow rate of 1mL/min. at

a wave length of 257nm with UV detector. Tablets were allowed to undergo different stress conditions like acid, base, oxidation, thermal degradation studies. Results: Retention time of Itraconazole and Terbinafine was found to be 4.288 and 2.551 respectively. The linearity of proposed method investigated in the range of 10-50µg/mL for both Itraconazole and Terbinafine. The Limit of Detection for Itraconzole and Terbinafine 1.25µg/mL and 8.00µg/mL respectively. The Limit of Quantification of Itraconazole and Terbinafine are 3.79µg/mL and 24.00µg/mL respectively [22].

Yildrim *et al.*, (2021) developed and validated a novel and fast method combining dilute and shoot approach and high-performance liquid chromatography coupled with photodiode array detection for the determination of terbinafine in human urine. The separation of terbinafine and naproxen (internal standard) was achieved within 3 min using a C18 core-shell column (Raptor ARC-18, 100 x 4.6 mm, 2.7 μm) under isocratic conditions. Samples were eluted from the column at the flow rate of 1.4 mL/min using a mobile phase containing 0.2% triethylamine in water (pH 3.4 with formic acid): acetonitrile (45:55 v/v). The presented technique was linear in the range of 25-2000 ng/mL. Intra- and inter-day reproducibility at four quality control levels (25, 200, 750 and 1500 ng/mL) were less than 7%, with relative errors ranging from -5.40% to 5.91%. The limit of detection was 12.60 ng/mL [23].

Thakur *et al.*, (2020) developed and validated a new, simple, precise, accurate, and reproducible method for the simultaneous estimation of terbinafine hydrochloride (TH) and fluconazole (FLZ) in pure form by the ultraviolet (UV) spectrophotometry method. The method was based on the measurement of absorbance at two wavelengths 222 nm and 239 nm of terbinafine hydrochloride and fluconazole in 0.1N HCl respectively. Calibration curves terbinafine hydrochloride and fluconazole were found to be linear in the concentration ranges of 0.5-3.0 μg/ml and 80- 400 μg/ml, respectively, with their correlation coefficient values (R^2) 0.999 and 0.998. LOD and LOQ of TH were found to be 0.067, 0.203 at 222 nm and 0.175, 0.531 at 239 nm, similarly for FLZ; 31.089, 94.210 at 222 nm and 94.380, 286.00 at 239 nm respectively. In the precision study, the % RSD value was found within limits (%) [24].

Das B *et al.*, (2020) developed a high performance liquid chromatographic strategy for the assessment of Terbinafine HCl from formulation was created. Terbinafine HCl was chromatographed on a BDS Hypersil C18 column 150 cm long and having an inner measurement of 4.6 mm. Mobile phase involving Mobile phase A - Buffer of K_2HPO_4, Mobile phase B of Methanol, Mobile phase C of Acetonitrile within the volume proportion of 15:35:50. The pH of the buffer adjusted to 7.5. The detection was carried out using an ultraviolet detector set at a wavelength of 223 nm. The technique was extended for the stability studies of TerbinafineHCl [25].

Belov VY *et al.*, (2018) developed an HPLC-UV method for determination of acetylsalicylic acid and its main metabolite, salicylic acid, in a model solution and in rabbit blood plasma. Plasma samples were prepared by salting out. Chromatographic analysis was performed in isocratic mode over a Hypersil BDS C18 column using mobile phase MeCN—H2O (pH 2.5,

30:70) with detection at 230 nm. The limit of quantitation for acetylsalicylic and salicylic acids in the model solution was 0.05 μg/mL; in blood plasma, 0.2 μg/mL. The developed method was applied to the development of new acetylsalicylic-acid dosage forms based on biocompatible polymer carriers, including pharmacokinetic studies after i.m. implantation [26].

Chen *et al.*, (2018) developed and validated an RP-HPLC method for salicylic acid. The high pressure liquid chromatography method for determining the content of salicylic acid in organic amine curing agent was established. The optimized HPLC conditions were as follows:C18 column(4.6 mm × 250 mm,5 μm),injection volume 20 μL,214 nm UV detection wavelength, gradient elution by acetonitrile (mobile phase A)-triethylamine phosphate buffer(mobile phase B, pH=6.35). The results showed that the UV absorption intensity had a good linear relation with the concentration of salicylic acid solution in the test range. The linear regression coefficient was 0.999 7 and the recoveries of standard addition met the regular standard of 85%~115% [27].

Kamal AH *et al.*, (2020) applied Reversed-phase high performance liquid chromatographic method (RP-HPLC) for simultaneous determination of aspirin (ASP) and omeprazole (OMP) in presence of salicylic acid (SA) as the most common degradation product of ASP. The drugs were exposed to different stress conditions including acid, alkali, neutral hydrolysis, oxidation, and photodegradation. The separation was carried out on the Thermo Scientific Hypersil ODS column (250 × 4.6 mm, 5 μm) column. Mobile phase composed of acetonitrile: 0.05 M sodium phosphate monobasic buffer with 0.1% TEA (23:77, v/v). The pH of the buffer was adjusted to pH 3.5 with o-phosphoric acid. The flow rate was 1.2 mL/min. The UV detector was set at 230 nm. The retention time of SA, ASP and OMP were 3.51 ± 0.19 min, 4.77 ± 0.23 min, and 16.65 ± 0.34 min, respectively. Linearity was established for in the range of 1–150 μg/mL for ASP, 2–80 μg/mL for OMP and 1–25 μg/mL for SA [28].

Roshdy *et al.*, (2021) employed a 2^3 full factorial design model was used for the development of a new high performance liquid chromatography method with UV detection to estimate three antifungal drugs simultaneously (fluconazole, itraconazole and terbinafine). They have been determined using MOS-1Hypersil C18 column and an isocratic eluent; methanol 95% and phosphate buffer 5% with 0.001% triethylamine. The pH was adjusted to 7, and the flow rate was 0.7 ml min^{-1}. The three drugs were separated within less than 7 min at 210 nm. The developed method gave a linear response over 5–80 μg ml^{-1}, 5–50 μg ml^{-1} and 1–50 μg ml^{-1} for FLU, ITR and TRH, respectively. It showed detection limits of 0.88, 0.29 and 0.20 μg ml^{-1} and quantification limits of 2.66, 0.88 and 0.60 μg ml^{-1} for the three drugs, respectively [29].

Sharma *et al.*, (2020) developed and validated an RP-HPLC method for the combination of imiquimod and salicylic acid. The method was carried out on Nucleodur C18 (250 mm × 4.6 mm I.D., 5m) using low-pressure gradient elution mode. The mobile phase was used as 30M potassium dihydrogen phosphate and acetonitrile (45:55) pH 6.5 adjusted using ortho-phosphoric acid. The concentration of solvents was 1-20 μg/ml and the volume of injection

was 20 ml with the flow rate of 1.0 ml/min. The absorption maxima of salicylic acid and imiquimod were found 234 nm and 226 nm, respectively. The method was validated and showed the linearity greater than 0.99% and with precision (RSD% <1). The limit of detection (LOD) and limit of quantification (LOQ) of salicylic acid was found to be 0.09756µg/ml and 0.2956µg/ml, respectively, and imiquimod was found to be 0.044031µg/ml and 0.13334µg/ml, respectively [30].

Chowk *et al.*, (2020) performed pre-formulation studies of the obtained drug sample by various analytical techniques such as IR spectroscopy, UV spectroscopy, melting point and partition coefficient for novel drug delivery system formulation. Terbinafine was found white in color, odourless and crystalline in form. The melting point of Terbinafine was found to be in range 197.667°C. The calibration curve for drug was obtained by using the 4 to 36µg/ml solution in methanol and the absorbance was measured at 223nm & 282nm. The calibration curve of drug indicated the regression equation Y=0.279x-0.011 & Y =0.028x+0.013 and R^2 value 0.999, shows good linearity. Drug was freely soluble in dichloromethane, methanol & ethanol. The partition coefficient of Terbinafine in n- Octanol: Water was found to be 3.312±0.005 shows lipophilic nature of drug. The FTIR observed peaks confirmed the purity and authenticity of the Terbinafine [31].

Thakur B *et al.*, (2020) developed a new, simple, precise, accurate, and reproducible method or simultaneous equation method was developed and validated for the simultaneous estimation of terbinafine hydrochloride (TH) and fluconazole (FLZ) in pure form. Simultaneous estimation of terbinafine hydrochloride and fluconazole was estimated by the ultraviolet (UV) spectrophotometry method. The method was based on the measurement of absorbance at two wavelengths 222 nm and 239 nm, of terbinafine hydrochloride and fluconazole in 0.1N HCl respectively. Calibration curves terbinafine hydrochloride and fluconazole were found to be linear in the concentration ranges of 0.5-3.0 µg/ml and 80-400 µg/ml, respectively, with their correlation coefficient values (R^2) 0.999 and 0.998 [32].

Elmansi H *et al.*, (2020) carried out the determination of terbinafine and itraconazole down to biological concentration level. The determination is based on increasing the selectivity of the spectrofluorimetric technique by combining both derivative and synchronous spectrofluorometric approaches, which permits successful estimation of terbinafine at 257 nm and itraconazole at 319 nm in the presence of each other at Δλ of 60 nm. As the detection limits were down to 0.013 and 0.1 µg ml−1 and quantitation limits were 0.04 and 0.032 µg ml−1 for terbinafine and itraconazole, respectively; the in vitro determination of terbinafine and itraconazole in spiked plasma samples was applicable. The percentage recoveries in biological samples were 97.17 ± 4.54 and 98.75 ± 2.25 for terbinafine and itraconazole, respectively [33].

Saeed AM *et al.*, (2018) developed easy and precise methods for estimation of aspirin (ASP), impurities from such as salicylic acid (SAL) and heavy metal ions (HMI) in ASP tablets that using High-performance liquid chromatography (HPLC), UV–VIS spectrophotometry and atomic absorption spectrophotometric (AAS). HPLC separation was carried out using C18 as

stationary phase and acetonitrile (ACN): water in the ratio of (10: 90 v/v) as a mobile phase for HPLC method and as a solvent for UV-VIS spectrophotometric for quantitative ASP and SAL at 254 nm for HPLC, 226 and 296 nm for UV measurements. AAS was used for HMI determination. ASP and SAL gave absorbance maxima at 226 and 296 nm in ACN: H2O solvent. The Beer's law was obeyed in the range of 0.05-20 for ASP and 0.02-8 µg/ml for SAL. Correlation coefficients (R^2) were 0.9996 and 0.9992 for ASP and SAL respectively, for HPLC and LOD value was 0.006 for ASP and 0.004µg/ml for SAL [34].

Tandel F *et al.*, (2018) developed stability indicating HPLC method for simultaneous estimation of Nicotinamide and Salicylic acid and to validate developed method in accordance with ICH guidelines. The separation was achieved using Chromatopak C-18 (250mm x 4.6 mm, 5 µm) column, mobile phase containing Methanol and Water (0.1% TEA and 0.15 gm Hexane sulphonic acid and pH 3.0 adjusted with Glacial acetic acid) in the ratio of (40:60 v/v), at a flow rate of 1.0 ml/min. The retention time for Nicotinamide and Salicylic acid was 4.343 min and 17.673 min respectively. The linearity was observed in the concentration range of 225-315 µg/ml for Nicotinamide and 75-105 µg/ml for Salicylic acid. The correlation coefficient was found to be 0.9971 and 0.9985 for Nicotinamide and Salicylic acid respectively. Degradation in all conditions wasfoundto be 10 -20% [35].

7. Conclusion

HPLC is a technique used to separate molecules according to their size and surface charge but when used HPLC-UV in combination with each other, UV allows the concentration of meolecules to be detected which are subjected to determine separation. HPLC-UV is preferred since it offers excellent linearity and rapid quantitative analysis. Furthermore, Terbinafine hydrochloride and Salicylic acid when used collectively, shows more enhanced physicochemical stability, solubility, dissolution rate and bioavailability as well. Hence, indicates extensively high fungicidal effect when used topically or orally.

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