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Research Article

Approaches to Development of Analytical Method for Combination Products Containing Fluconazole

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ABSTRACT

Fluconazole is a commonly used antifungal agent. It is marketed as solid oral dosage forms, intravenous preparations as well as combination products along with other antimicrobial agents. Pharmacopoeial analytical methods are available for fluconazole tablets and capsules. However, there are no official methods for analysis of fluconazole in combination products. As a result, in house analytical method has to be developed and validated for such products. The main objective of this article is to identify possible approaches towards the analytical method development for combination products containing fluconazole. For this research papers from various journals were compared with respect to its objectives, methods parameters and results. For fluconazole combination products, an analytical method based on Ultra violet (UV) spectrometry may not be as well suited as a method based on high pressure liquid chromatography (HPLC). In areverse phase (RP) HPLC based method, a mobile phase consisting of water and an organic solvent like methanol and acetonitrile in 60:40 v/v or 80:20 v/v could be used. For a non ionic compound like fluconazole a neutral pH buffer should be preferred to enhance resolution. pH of the buffer should only be changed to increase retention time in favour of another drug which eludes faster. A mobile phase of methanol: neutral buffer in 50:50 v/v may help to produce the lowest retention time. These approaches can lead to a validated and accurate analytical method for fluconazole containing combination products.

Keywords: Fluconazole, Combination products, HPLC, analytical method development.

INTRODUCTION

A combination product is a formulation containing two or more active pharmaceutical ingredients (API). Combination products have been used for both single disease conditions like infections (Tuberculosis, AIDS) and multiple conditions like cardiovascular disease and hypertension[1][2]. Some of the benefits of such combinations include higher patient compliance, reduced cost, reduced pill burden and an improved ability to manage and treat the condition. The success of recent combination products in achieve market growth along with better health outcomes signals an upcoming era of more combination products.Such drug products achieve marketing approval but analytical methods for its evaluation are commonly not official in most pharmacopoeias. Analysis of these combination products is equally crucial as part of quality control.

Quality control is also needed throughout the lifecycle of the product as the degradation pathways of the combination products may vary. It is also possible that the stabilizing strategy for one product may result to destabilization of another product. Thus the quantity and quality of all components in the drug product should be checked. For this an analyst needs to develop an inhouse analytical method which could separate, identify and quantify the individual components of the drug product.Amongst all known analytical methods the most widely used are Reverse phase – high pressure liquid chromatography (RP-HPLC) and ultraviolet (UV) spectrophotometry.

Fluconazole is chemically 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol, a synthetic triazole derivative antifungal agent[3]. It is known to be used for superficial and systemic fungal infections. It interacts with 14-α demethylase, which is needed to convert lanostreol to ergosterol. Ergosterol is an essential component of fungal cell membrane. Inhibiting the synthesis of this component results in leaky fungal cell. This results in leakage of cellular contents. Fluconazole is thus a fungi static agent[4]. It is found in oral and intravenous preparations. The oral administrations majorly include tablets[5]. For many years, fluconazole as an active pharmaceutical ingredient (API) was official and not its pharmaceutical preparations. Recently fluconazole tablets and capsules have been made

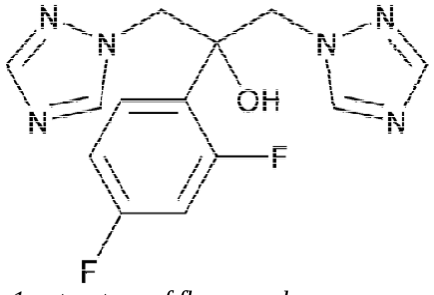

Figure 1.: structure of fluconazole

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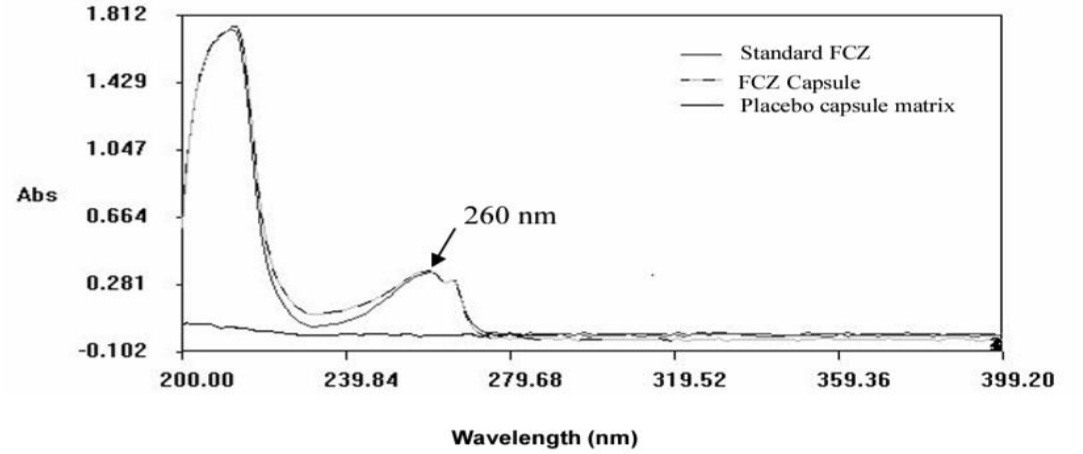

figure2.: UV scan of fluconazole

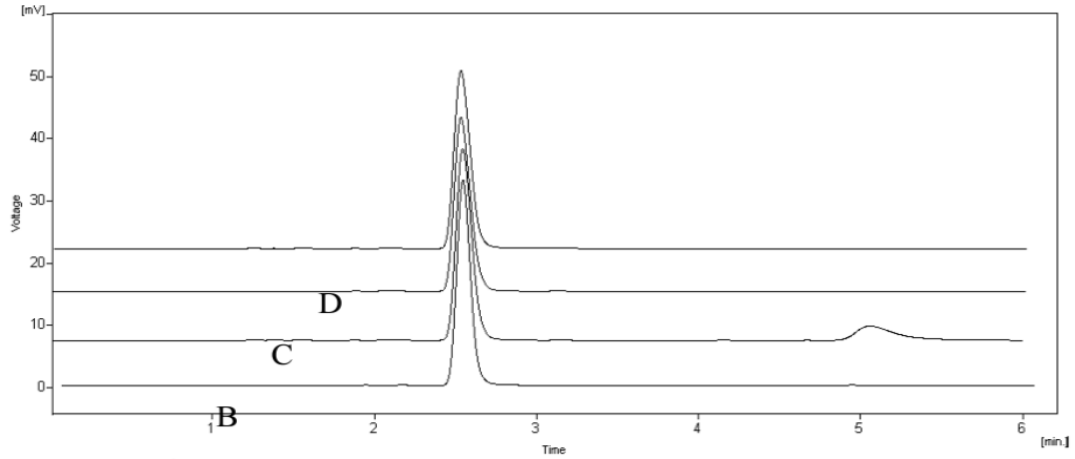

figure 3.: RP-HPLC chromatograms of fluconazole reference standard 40mg/ml (A), fluconazole dispersible tablet (B), uncoated tablet (C), and capsule (cap A) (D)

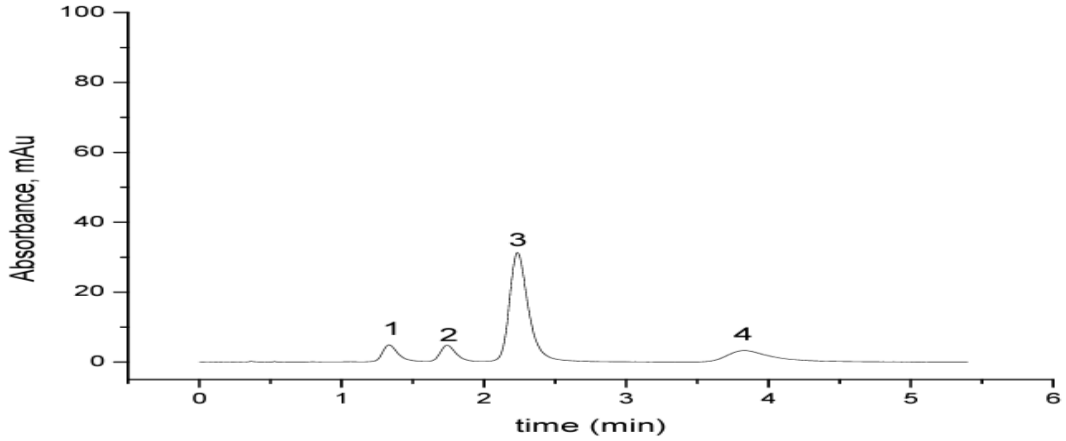

figure 4.: chromatogram of fluconazole and related compounds using mobile phase of water: methanol in ration 60:40 v/v

official under Indian Pharmacopoeia 2010 and other pharmacopoeias[6]. Many combination therapies are tried with this drug for treatment of various fungal infections. It is combined with amphotericin B[7]. It is also combined with tinidazole, ketoconazole to name a few.

Many analytical methods have been reported for detection of impurities of fluconazole, for its identification in pharmaceuticals dosage forms as well as in various biological samples like urine and plasma. But there are no official analytical method for combination drug product containing fluconazole. This article focuses on identifying and contrasting various analytical methods for fluconazole. This assists in finding a suitableapproach towards the analytical method development of drug products containing fluconazole. It can be further extended as part of bioanalysis in case of combination therapy.

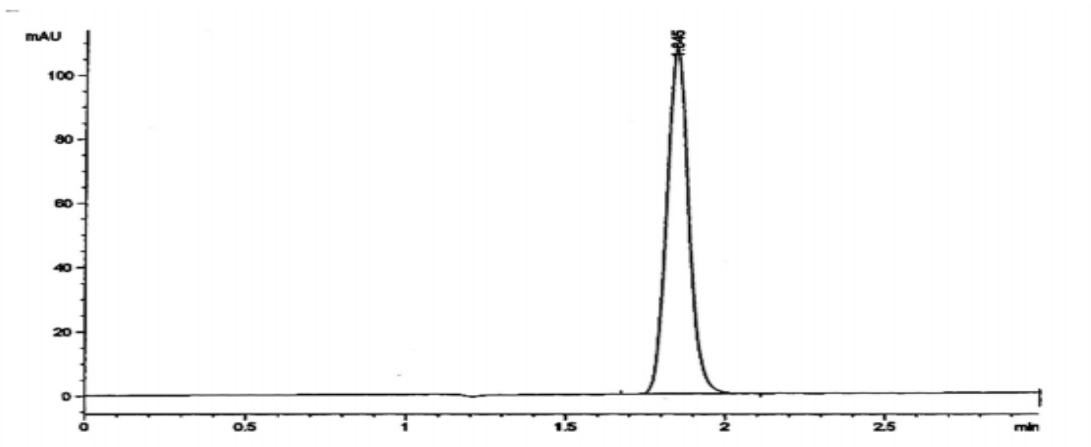

figure 5.:chromatogram of fluconazole using mobile phase methanol:10 mM pH7.0 phosphate buffer 50:50

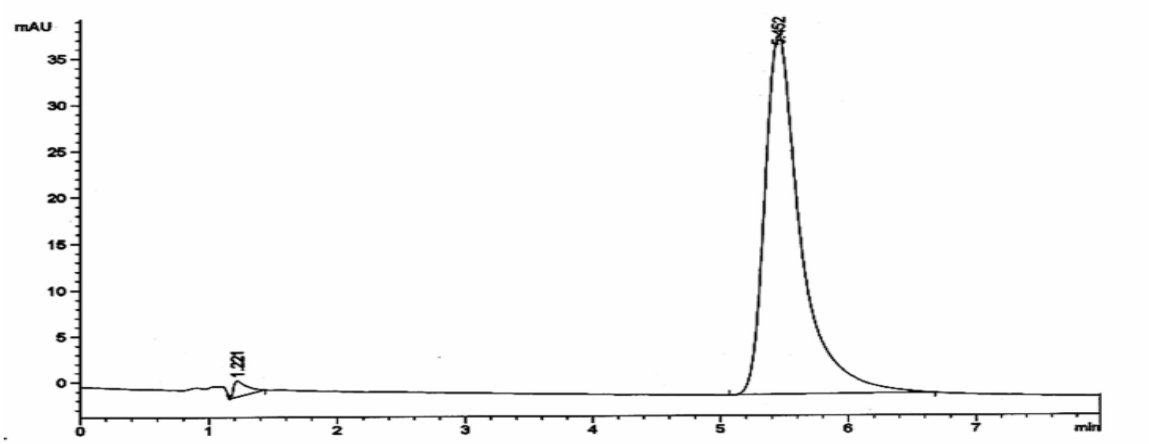

figure 6.: chromatogram of fluconazole using mobile phase methanol: 10 mM pH 5.0 acetate buffer 30:70

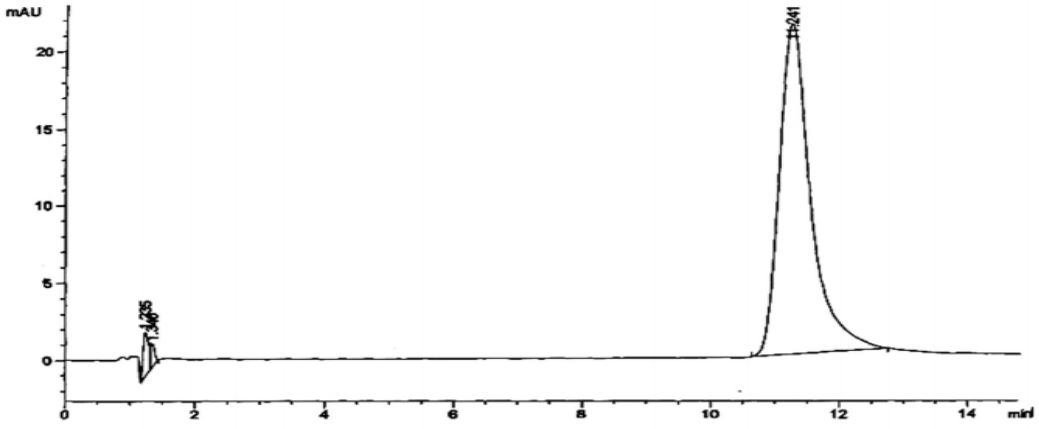

figure 7.: chromatogram of fluconazole using mobile phase of methanol: 10 mM pH 5.0 acetate buffer : acetonitrile in ration 20:70:10

OBJECTIVE

The objective of this article is to compare and identify a good analyticalmethod for separation and identification of fluconazole when present in pharmaceutical preparations containing more than one API.

METHOD

To achieve this objective an initial literature study was done to understand the approach towards analytical method development. Research papers from various journals focusing on analysis of fluconazole were identified. They were then compared with respect to their objectives, method specifications and results.

OBSERVATIONS

Fluconazole is a neutral compound by nature and hence approaches are made considering it as a simple normal compound[8] (Figure 1). Due to its neutral nature normal phase chromatography is not recommended and reverse phase chromatography serves as an easy approach. UV spectroscopy is also preferred as it is an easy and less time consuming technique.

Pothana Sadasivudu et al studied fluconazole assay using a simple UV spectroscopy. They found good results in analysis of fluconazole capsules[9]. Fluconazole capsules were emptied and drug equivalent to 100 mg of fluconazole was transferred and volume was made upto 0.1 M HCl. The drug was then serially diluted to 200ug/ml concentration and its UV absorbance was measured. Fluconazole shows maximum UV absorption at 260nm (figure 2) and hence absorbance of capsule sample was also taken at 260nm. The method was validated and was found most suitable for analysis of fluconazole capsules, as no interference was observed at 260 nm in placebo capsule matrix when compared with standard fluconazole solution. However, the study shows that this method was is not useful with pharmaceutical preparations like tablets due to interferences of the excipients present in it[9].

The problem of excipient interferences can be best overcome by separating the analyte from the interferents. Separation is achieved using chromatographic techniques. RP-HPLC is the most suitable approach due to its specificity and accuracy. This shows there is a need to develop an HPLC based analytical method for various preparation available in market. The official IP/USP method for fluconazole, fluconazole tablets and fluconazole capsules describes various conditions for RP-HPLC. It uses water: acentonitrile in the ration 80:20 v/v (IP) with a C18 column (5um, 150 mm * 4.6mm) and a flow rate of 0.5ml/min[ip].Pothana Sadasivudu et al used mobile phase of water : acetonitrile in 65:35 v/v with a flow rate of 1ml/min. keeping rest conditions same they achieved a good peak with a retention time of 2.47 mins[9] (figure 3).

Methanol can be substituted for acentonitrile as there lies an easy availability of methanol as compared to acetonitrile and also the cost of methanol is less as compared to acetonitrile[8]. Keeping all chromatographic conditions as mentioned in pharmacopoeia and changing the mobile phase to methanol: water 40:60 v/v is also reported to show good results with a lesser runtime by F. Al-Rimawi (figure 4).This mobile phase composition was found to be optimum with a flow rate of 1.5 ml/min[8].

Silvia Regina Cavani Jorge Santos et al used mobile phase composition of water: acetonitrile 60:40 v/v for bioanalytical methods and proved to show satisfactory results. Thus a mobile phase of water: methanol 60:40 v/v can also be used for separation and analysis of fluconazole in boianalytical samples[10].Buffers are added to the mobile phase to avoid ionization of components. Fluconazole being a neutral compound addition of buffer is not essential but when added it shows different results with respect to peak shape and retention time[11]. Duangsamron Limpiti et al used mobile phase composition of methanol: 10mM phosphate buffer adjusted to pH 7 (50:50 v/v)and observed that it resulted in lowest retention time for fluconazole identification (figure 5). It showed a fluconazole peak at 1.8 mins unlike others which have retention time of more than 2 mins. This mobile phase with a flow rate 1ml/min and a C18 column gives a satisfactory peak of fluconazole at a retention time less than 2 mins[11].

Duangsamron Limpiti et al also reported that when the ratio and pH of buffer are changed in the mobile phase it can change the retention time. Methanol: 10mM acetate buffer adjusted to pH 5 in the ration 30:70 v/v gives a peak with satisfactory shape but a retention time of 5.4 mins[11](figure 6). This is not desirable as in case of a mixture the run time will be long.

Apart from two component mobile phase a three component approach is also reported by them. Mobile phase composing of methanol: 10mM pH 5 acetate buffer: acetonitrile in ratio 20: 70: 10 also results in a satisfactory peak for fluconazole (figure 7). The retention time in this case is 11.2 mins[11]. We can use this mobile phase system in cases of mixtures where other component is a faster eluting substance.

CONCLUSION

On the basis of these possible reported analytical methods for fluconazole a simple and precise method can be developed for analysing fluconazole in various pharmaceutical preparations.In combination products containing fluconazole it is essential to identify and quantify all contents. For fluconazole combination products, an analytical method based on UV spectrometry may not be as well suited as a method based on HPLC. Most of the official assays are also on chromatographic methods and hence analysis of combinations are also developed on HPLC. In analysis of combination preparations that is a mixture, it is beneficial to elute one of the drug fast and hence a mobile phase eluting fluconazole at a low retention time is suitable. This will lead to separation within a reasonable run time. Gradient elution should also be considered while developing method for analysis.In an HPLC based method, a neutral pH buffer should be preferred. pH of the buffer should only be changed to increase retention time in favour of another drug which eludes faster. A mobile phase of 50:50 v/v methanol may help in producing the lowest retention time. The comparison of various mobile phase and chromatographic parameters can lead to precise and accurate analytical method development for combination products containing fluconazole.

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