Development and Validation of an HPTLC Densitometric Method for Assay of Griseofulvin in Tablets

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ABSTRACT

A new HPTLC densitometric method for the assay of griseofulvin tablets was developed and validated according to ICH guidelines and USP specifications. The method was developed using a mobile phase prepared with an environmentally friendly solvent system, diethyl ether: toluene (4:1) on pre-coated TLC silica gel $60F_{254}$ glass plates with a saturation time of 25 min. Development time of 10 min was required for a migration distance of 70 mm. The best detection wavelength was 299 nm. The R_f value was 0.22 and there were no interferences from the excipients or solvents. With regard to repeatability and intermediate precision, the RSD values were 1.43 and 1.58, respectively. Linearity testing gave the polynomial R^2 values, calculated on three consecutive days, of 0.9910, 0.9801 and 0.9840, respectively. The accuracy values tested at 80 %, 100 % and 120 % concentrations were between 98.11-102.66 %. The developed HPTLC densitometric method for the assay of griseofulvin tablets is simple, selective, accurate, reproducible, high throughput, cost-effective and environmentally friendly.

Key Words: High Performance Thin Layer Chromatography (HPTLC), griseofulvin, densitometry, validation

INTRODUCTION

Griseofulvin (7-chloro-2', 4, 6-trimethoxy-6'-methyl-gris-2'-en-3, 4'-dione), is an orally active antifungal antibiotic derived from the mold *Penicillium griseofulvum* that is primarily used to treat dermatophyte infections in humans and animals [1]. The drug acts by binding to tubulin, hence interfering with the microtubule functioning and inhibiting mitosis. It binds to keratin within the keratin precursor cells and makes them resistant to fungal infections. The drug reaches its site of action by replacement of hair or skin with a keratin-griseofulvin complex and then enters the dermatophyte through energy dependent transport where it binds to microtubules altering mitosis as well as deposition of fungal cell walls [2–4].

Analysis of the Active Pharmaceutical Ingredient (API) as well as Finished Pharmaceutical Products (FPP) is of vital importance to ensure that good quality products are manufactured and supplied to the end users. High performance thin layer chromatography (HPTLC) is a simple, high throughput, less time-consuming and cost-effective technique of analysis which suits the developing countries towards successful performance of product quality assessments, including field applications [5–8].

Literature survey reveals that various analytical methods which include TLC, HPLC, GLC and LC-MS have been developed for detection of griseofulvin in human plasma and pharmaceutical formulations [9–11]. An HPTLC method for determination of griseofulvin in rat plasma has also been reported [12]. However, there is no validated HPTLC method for quantitative and qualitative analysis of griseofulvin tablets to the best of our knowledge. The aim of this work was to develop an HPTLC method for qualitative and quantitative analysis of griseofulvin tablets.

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EXPERIMENTAL

Solvents

Analytical grade solvents which included ethyl acetate (Carlo Erba reagents group, German), diethyl ether and toluene (Scharlau Chemie SA, EU) were used during method development and validation. Sodium carboxylmethylcellulose and polyvinlypyrrolidone cross-linked (Associate Co. Ltd,, Shenzhen, China), magnesium stearate (Shandong Liaocheng Ehua Medicine Co. Ltd, China) and microcrystalline cellulose (FMC Biopolymer, Philadelphia, USA) were used for simulation in determination of specificity.

Reference standards and sample tablets

Griseofulvin reference standards were obtained from Chifeng Pharmaceutical Co. Ltd, Chifeng, China while griseofulvin sample tablets were obtained from Elys Chemical Industries Ltd, Nairobi, Kenya.

Equipment

Instrumentation employed in method development consisted of a densitometer with TLC scanner 3 operated with Wincats (version 1.4.3) planar chromatography software as data manager and integrator. A Linomat 5 semi-automatic sample applicator with a 100 µl Hamilton syringe was used for sample application. The TLC plates employed were either 5 × 10 cm or 20 × 10 cm in size, pre-coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany) The plates were developed in a Camag rectangular flat-bottomed developing tank (CAMAG, Muttenz, Switzerland).

Method development

Preparation of standard stock and working solutions: Griseofulvin powder (3.5 mg) was weighed and transferred into a 10 ml volumetric flask followed by addition of 5 ml of ethyl acetate. The solution was sonicated for 5 minutes and filled to the mark with ethyl acetate to obtain 0.35 mg/ml of griseofulvin standard stock solution. A 5.0 ml aliquot of this stock solution was pipetted into a 10 ml volumetric flask, filled to the mark with ethyl acetate and shaken thoroughly to obtain 0.175 mg/ml of griseofulvin working standard solution.

Preparation of sample stock and working solutions: Griseofulvin tablets were powdered and powder equivalent to 17.5 mg was weighed and transferred into in a 50 ml volumetric flask. About 30 ml of ethyl acetate was added into the flask. The mixture was sonicated for 30 minutes and filled to the mark with ethyl acetate. The solution was then filtered to obtain a concentration of 0.35 mg/ml sample stock solution. A 5.0 ml aliquot of this solution was pipetted into a 10 ml volumetric flask, filled to the mark with ethyl acetate and shaken for 1 minute to obtain 0.175 mg/ml of working sample solution.

Chromatography: Pre-activated TLC plates were freshly removed from their packing only when in use. The plates were labelled and the solvent front marked at 70 mm from the bottom before spotting. Using a Linomat 5 applicator, 5 µl of the solution was applied 8 mm from the bottom of the plate as an 8 mm band. Subsequently, the plate was dried in open air before development. To aid saturation, a filter paper was placed on one side of the developing tank and the mobile phase was poured into the tank, in the process wetting the filter paper. The tank was closed for 25 minutes to allow for saturation. The solvent was allowed to run to the 70 mm mark. The developed plate was scanned using a TLC scanner 3 in the reflectance absorbance mode, equipped with Wincats (Version 1.4.3) planar chromatography software for data acquisition.

Selection of suitable mobile phase: Choice of mobile phase was done by observation and consideration of the polarity indices of different reagents in literature. Toluene, ethyl acetate, methanol, 25% ammonia solution, glacial acetic acid and diethyl ether were chosen due to their polarity indices, cost, availability as well as relative safety to the user and the environment. A number of trials were run involving different ratios of two or three of these solvents, aiming for the best resolution of the griseofulvin peak.

Method validation.

The method was validated for robustness, specificity and selectivity, linearity, precision and accuracy using standard samples of griseofulvin tablets. A validation protocol was prepared and applied based on the ICH Q2R1 guidelines and USP guidance [13–15].

RESULTS AND DISCUSSION.

Selection of a suitable mobile phase.

Choice of a suitable mobile phase was done by combinations of different solvents by considering the polarity of griseofulvin. Combinations of solvents used during method development included ethyl acetate: toluene (2:1 v/v), ethyl acetate: methanol (5:1 v/v); ethyl acetate: toluene: methanol (5:2:1 v/v); ethyl acetate: 25% ammonia solution (30: (0.1-0.6) v/v) and diethyl ether: toluene (3:2 v/v). The mobile phase containing diethyl ether: toluene (3:2) was selected for optimization since it showed the best resolution and Rf value. During optimization, the proportions of diethyl ether: toluene tested included 1:1 v/v, 2:3 v/v, 4:1 v/v, 2:1 v/v and 3:2 v/v. The 4:1 v/v diethyl ether: toluene solvent system gave the best peak resolution at 0.175 mg/ml (100% solution) and was selected as the most suitable solvent system (**Figures 1 and 2**). The optimum saturation time was determined to be 25 min. A development time of 10 min resulted in the desired migration distance of 70 mm at room temperature (23 to 28°C) and a relative humidity of between 36 and 54 %. The optimum wavelength was determined to be 299 nm after scanning the developed plate between 200 nm and 800 nm using the deuterium and tungsten lamps.

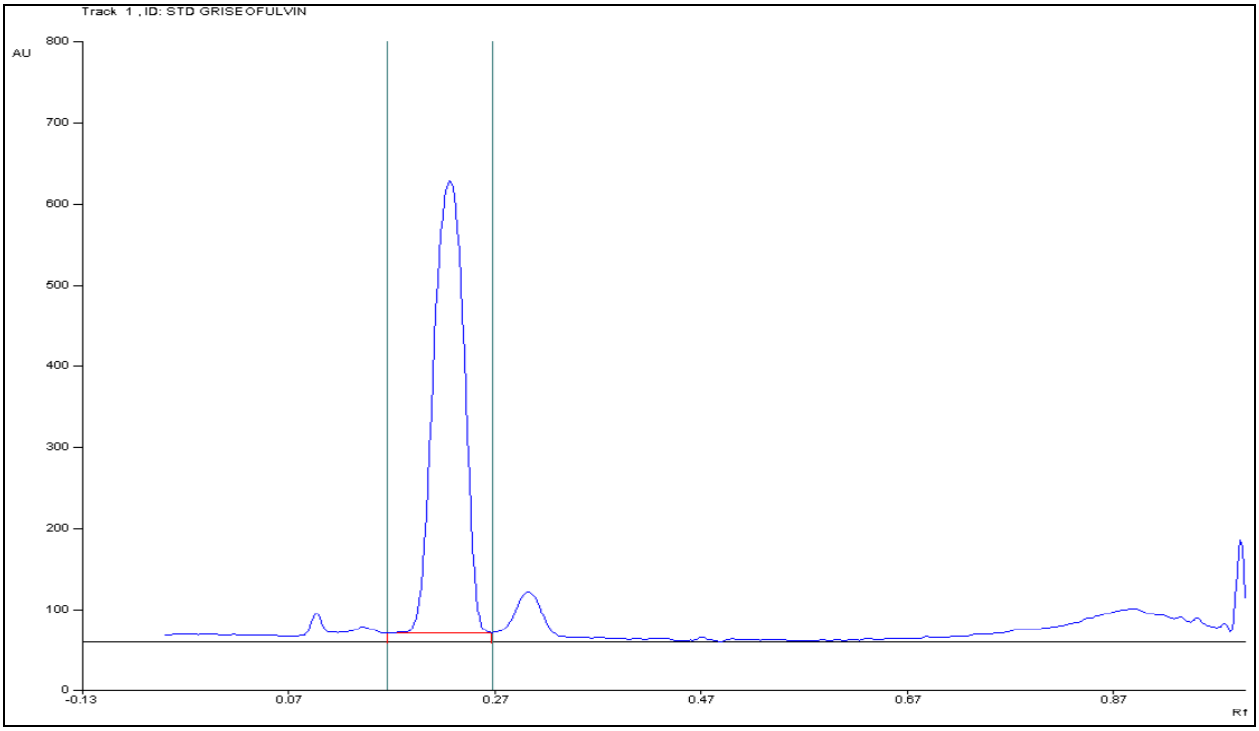

Figure 1: Chromatogram of griseofulvin obtained using diethyl ether: toluene (4:1)

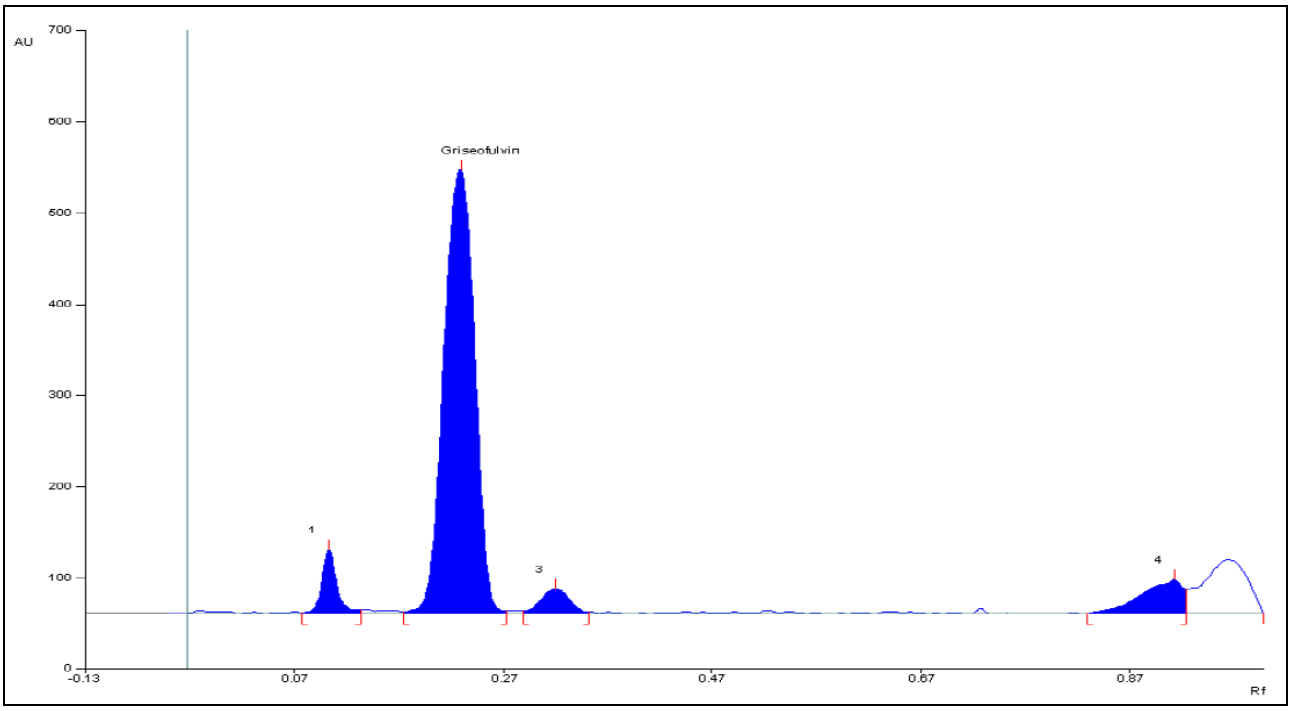

Figure 2: Peak display for system suitability

Method validation.

The assay method was validated for specificity/selectivity, linearity, precision, accuracy and robustness.

Specificity and selectivity: The common excipients used for preparation of griseofulvin tablets without active substance (placebo) were prepared and spotted to examine possible interference with the analyte peaks during the chromatographic run. The method was to be accepted if no interference was observed between analytes, placebo or solvent densitograms [13]. The obtained densitograms showed that the method was selective for griseofulvin, the active pharmaceutical ingredient, since there were no interferences with the simulated excipients or solvents (**Figure 3**). Hence the method is acceptable for routine assay of the active pharmaceutical ingredient in griseofulvin tablets.

Linearity of the calibration curve: Evaluation of linearity of the griseofulvin assay was demonstrated by preparing five standard concentrations ranging from 50% to 120% (437.5 to 1050 ng/spot) using serial dilutions from the stock solution of 0.35 mg/ml. Spots were applied on the plate for each concentration starting with the lowest concentration to avoid a carryover effect. The procedure was repeated for three days. The results were analyzed using peak area of the developed spot.

The regression coefficient (r^2) for a polynomial function was found to be between 0.9801 and 0.9915 which meets the acceptance criteria ($r^2 >$ 0.98) (**Figure 4**) and (**Table 1**).

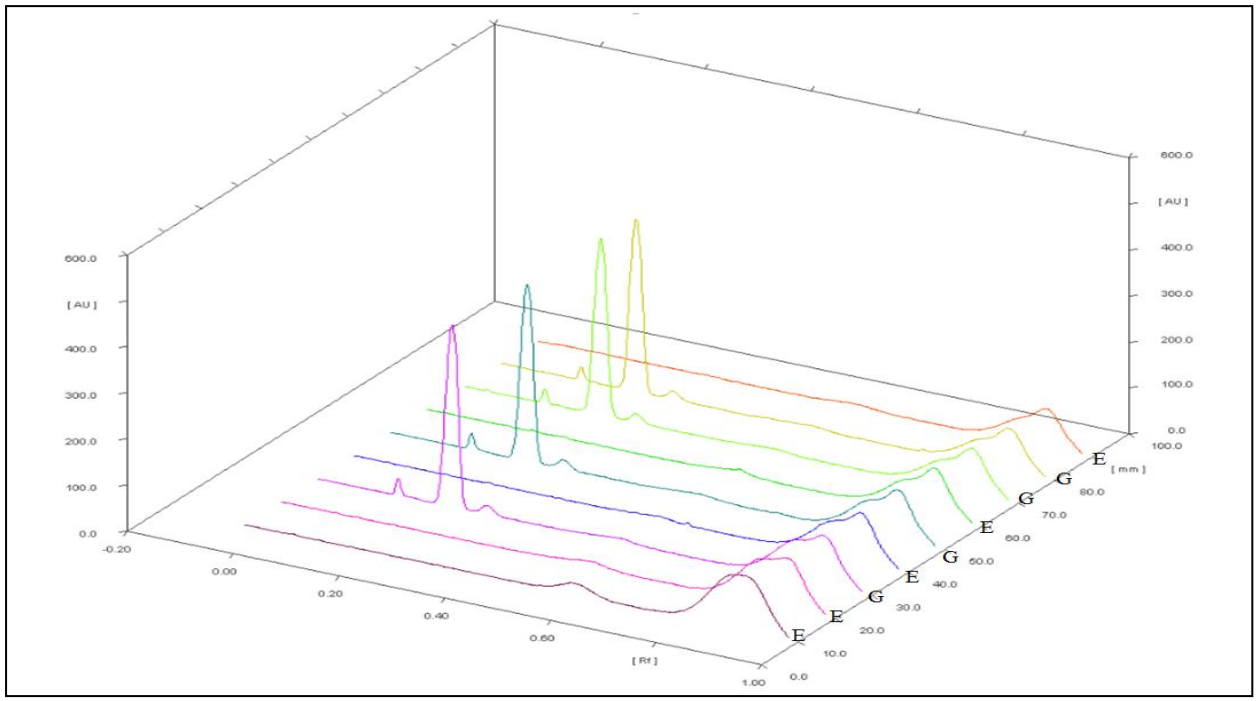

Figure 3: Method selectivity test showing densitograms for mixture of excipients (E) and griseofulvin peaks (G)

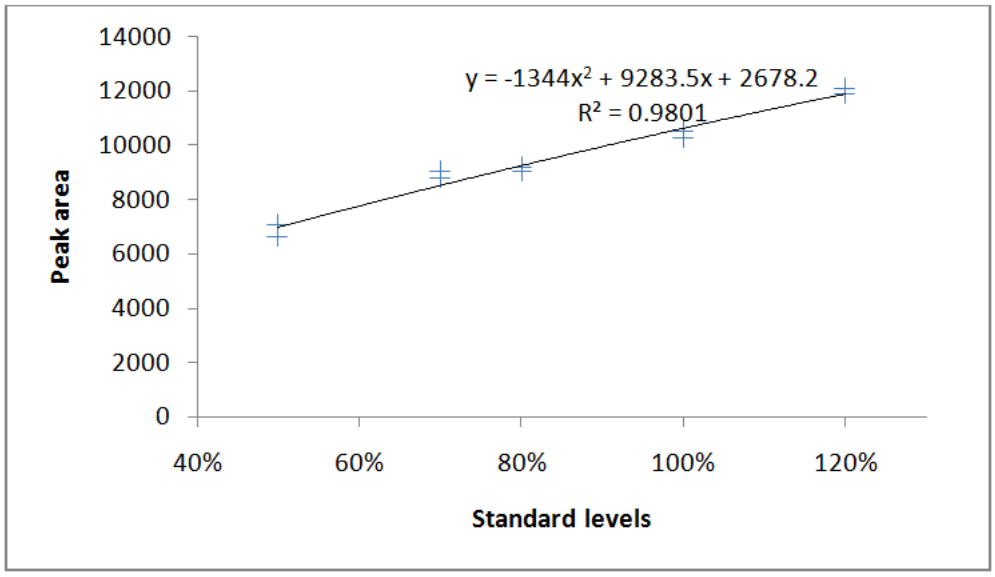

Figure 4: Graph of coefficient of polynomial regression for linearity test.

Table 1: Linear and polynomial regression equations obtained from the HPTLC densitometric method for griseofulvin tablets at concentration levels of 50% to 120%.

	Day1	Day 2	Day 3
X^2 coefficient	-1871.3	-1344	-2292.4
X coefficient	8691.9	9283.5	9882.2
Y intercept	2368.8	2678.2	3023.6
R^2(>0.98)	0.9915	0.9801	0.9842

Precision: The repeatability and intermediate precision for griseofulvin were assessed for the assay method using sample tablets with six replicates of sample solutions equivalent to 100% (0.175 mg/ml) level of assay concentration prepared independently. Intermediate precision was assessed on different days by two different analysts. The calculated percentage relative standard deviations (% rsd) using peak areas were 1.43 and 1.58 for repeatability and intermediate precision, respectively, as shown in Tables 2 and 3. These were in compliance with the ICH guidelines.

Table2: Repeatability values from the HPTLC densitometric method for griseofulvin tablets.

Parameter	Value
Mean of peak areas	10260.43
Standard deviation	147.06
%Relative SD	1.43 (<2)

Table 3: Repeatability values and intermediate precision from the HPTLC densitometric method for griseofulvin tablets

	Analyst 1, day 1	Analyst 2, day 2	Analysts 1 and 2
Mean peak areas	10260.43	10198.18	10229.30
Standard deviation	147.06	183.21	161.69
%Relative SD	1.43	1.79	1.58 **(<2)**

Accuracy: Separate control solutions were prepared corresponding to the 80%, 100% and 120% level by independently weighing the standard griseofulvin in triplicate. Standard solutions were made and spotted in triplicate on the same plate. The percentage recovery (accuracy) ranged between 99.98% and 102.94% at 80% concentration, between 98.11% and 100.56% at 100% concentration and between 100.10% and 102.66% at the 120% level (**Table 4**). The accuracy of the analytical method was within limits as stated by the USP (90% to 115%) [15].

Table 4: Accuracy values from the HPTLC densitometric method for griseofulvin tablets.

Level	Run test	Expected amount of std conc (ng)	Sample conc corrected (ng)	% Accuracy
80%	1	700.0	699.93	99.98
	2	700.0	720.59	102.94
	3	700.0	720.50	102.92
100%	1	875.0	867.44	99.14
	2	875.0	879.87	100.56
	3	875.0	858.43	98.11
120%	1	1050.0	1057.18	100.68
	2	1050.0	1051.01	100.10
	3	1050.0	1078.00	102.66

Method robustness: Robustness was tested by varying the established mobile phase composition (diethyl ether: toluene) by ±5% and the established saturation time by ±5 min while observing the relative changes in the R_f value. The observed changes in the R_f value were 0.22 ± 0.02, showing that the method was robust. Hence the method can withstand changes in mobile phase composition and saturation time within the stated limits.

CONCLUSION

A simple, reproducible, accurate, cost-effective and environmentally friendly method was developed. The method was validated according to the ICH guidelines and USP guidance and is suitable for use in qualitative and quantitative analysis as well as screening of griseofulvin tablets. The method involves the use of a mobile phase composed of diethyl ether: toluene (4:1) v/v on pre-coated TLC silica gel $60F_{254}$ glass plates with a saturation time of 25 min, development time of 10 min/ 70 mm and a densitometer detection wavelength of 299 nm. Moreover, the R_f value was 0.22 with no interference from the excipients or solvents.

With regard to repeatability and intermediate precision, the RSD values were 1.43 and 1.58, respectively. The polynomial regression coefficient (r^2) was found to lie between 0.9801 and 0.9915. The accuracy values tested at 80%, 100% and 120% concentration levels ranged between 98.11% and 102.66%.

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REFERENCES.

[1] British National Formulary- BNF, Volume 61, Pharmaceutical Press, London, 2011, p 321–372

[2] C. Grover, P. Arora and V. Manchanda. Int. J. Dermatol., 51(4), 2012, 455–8.

[3] A. Shemer, I.B. Plotnik, B. Davidovici, M.H. Grunwald, R. Magun and B.J. Amichai. Dtsch. Dermatol. Ges., 11(8), 2013, 737–42.

[4] S.K. Pitman, R.H. Drew and J.R. Perfect. Exp. Opin. Emerg. Drugs., 16(3), 2011, 559–86.

[5] E. Kaale and B. Nyaweru. Pharm. Anal. Acta., 04(01), 2013, 1–4.

[6] E. Kaale, P. Risha and T. Layloff. J. Chromatogr. A, 1218(19), 2011, 2732–6.

[7] D.H. Shewiyo, E. Kaale, P.G Risha, B. Dejaegher, J. Smeyers-Verbeke and Y.V. Heyden. J. Pharm. and Biomed.Anal., 66, 2012, 11–23.

[8] D.H. Shewiyo, E. Kaale, P.G. Risha, B. Dejaegher, J. Smeyers-Verbeke and Y.V. Heyden. J. Chromatogr. A, 1216(42), 2009, 7102–7.

[9] E. Townley and P. Roden. J. Pharm. Sci., 69(5),1980, 523–6.

[10] H.N. Mistri, A.G. Jangid, M. Sanyal and P. Shrivastav. J. Chromatogr. B Anal. Technol. Biomed. Life Sci., 850(1-2), 2007, 318–26.

[11] G. Kahsay, A.O. Adegoke, A. Van Schepdael and E. Adams. J. Pharm. Biomed. Anal., 80, 2013, 9–17.

[12] B. Wei, D. Liang and T.R. Bates. Anal. Chem. Insights, 3, 2008, 103–9.

[13] ICH. Validation of analytical Procedures : text and methodology Q2 (R1). Guidance. 2005, 17.

[14] USP–NF. [cited 2015 Jul 19]. Available from: http://www.usp.org/usp-nf

[15] WHO Pharmaceuticals Newsletter 2000, No. 01. [cited 2015 Jul 19]. Available from: http://apps.who.int/medicinedocs/en/d/Jwhozip01e/