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Simultaneous HPLC and derivative spectrophotometry detrmination of tioconazole and benzyl alcohol in bulk and cream with tioconazole forced degradation study

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ABSTRACT

New high performance liquid chromatographic (HPLC) and derivative spectrophotometric methods have been developed and validated for the simultaneous determination of tioconazole and benzyl alcohol in bulk and cream dosage form. The first method involved the use of chromatographic system composed of methanol: water (0.2% *v/v*, aqueous tri ethyl amine at pH 7.5) (85:15, *v/v*) as a mobile phase at a flow rate 1ml min^{-1} at ambient temperature with a Hypersil BDS-C18 (250 mm×4.6 mm, 5µm) column as a stationary phase. Quantitative determination of tioconazole and benzyl alcohol was achieved with UV detection at 210 nm and 220 nm, respectively. Linearity, accuracy and precision were found to be acceptable over the concentration range of (2–64µg ml^{-1}) for tioconazole and (8–192µg ml^{-1}) for benzyl alcohol. This chromatographic system was utilized to study the effect of acid and alkali hydrolysis, oxidation, thermal and photo-degradation on tioconazole stability. The second method involved the use of first order derivative spectrophotometry with the zero-crossing technique. Quantitative determination of tioconazole and benzyl alcohol was achieved by measuring amplitude at 233.4nm and 217.8 nm respectively. Linearity, accuracy and precision were found to be acceptable over the concentration range of (3–9 µg ml^{-1}) for tioconazole and (5–14 µg ml^{-1}) for benzyl alcohol. The optimized methods proved to be specific, precise and accurate for the quality control of the cited drugs in pharmaceutical preparations.

KEYWORDS

Tioconazole;
Benzyl alcohol;
Reversed phase liquid chromatography;
Forced degradation study;
Derivative spectrophotometry.

INTRODUCTION

Tioconazole;(RS)-1-[2-[(2-Chloro-3-thienyl)methoxy]-2-(2,4dichlorophenyl)ethyl]-1H-imidazole (Figure 1a) is an imidazoleantifungal agent with a broad spectrum of activity against dermatophytes, Malassezia furfur, and candida albicans[1]. Imidazoles impair the biosynthesis of ergosterol for the cytoplasmic membrane inhibiting growth of the fungi[2]. Benzyl alcohol; (Figure 1b) is used as antimicrobial preservative. It is bacteriostatic mainly against G+veorganisms and some fungi. It is used in a range of pharmaceutical

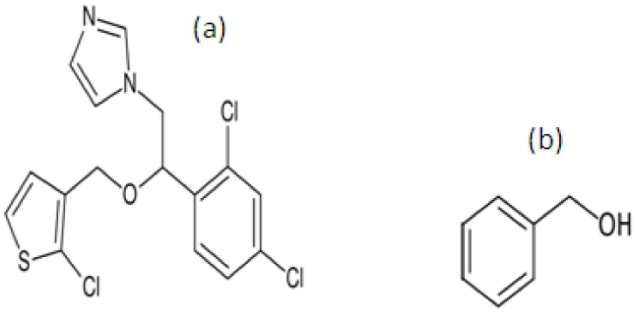

Figure 1 : Chemical structure of (a) Tioconazole and (b) Benzyl alcohol

preparations in concentrations up to 2%. Concentrations of 5% or more are employed when it is used as a solubilizer[1].

Literature survey reveals that several analytical methods have been reported for the determination of tioconazole (TIO) alone or in combinations including HPLC[3-9], GC[10] and spectrophotometry[11]. Besides, several analytical methods have been reported for the determination of benzyl alcohol (BZY) alone or in combination with other drugs including HPLC[12-24] and spectrophotometry[25-27]. No results were obtained for the simultaneous determination of tioconazole and benzyl alcohol. Thus, the aim of this work was to develop simple, selective and validated HPLC and spectrophotometric methods that are suitable for the simultaneous determination of TIO and BZY.

Although, literature review reveals that TIO was determined in presence of its impurities and related substances[6-8], but no forced degradation study has been reported for TIO. So, the chromatographic system used for TIO analysis by HPLC method was utilized to study the effect of acid and alkali hydrolysis, oxidation, thermal and photo-degradation on TIO stability.

EXPERIMENTAL

Instrumentation

For HPLC method

A chromatographic system consisting of Agilent 1200 series; interface equipped with an Agilent quaternary pump G1311A, Agilent UV-visible detector G1314B, an Agilent manual injector G1328B equipped with (20 µl) injector loop, an Agilent degasser G1322A and thermo BDS hypersil C18 column (5µm, 4.6 x 250 mm) was used. An Agilent syringe, LC 50 µl, CA, U.S.A., ultrasonic processor; Soniclean 120T, 220/240v, 50/60Hz, 60W, Thebarton SA, Australia were used.

For derivative spectrophotometric method

A double beam ultraviolet/visible spectrophotometer Shimadzu UV-1601 PC (Tokyo, Japan) connected to an IBM compatible computer and supported with UVPC software version 3.7 was used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1} with quartz cell of 1 cm path length.

Materials and reagents

Pharmaceutical grade TIO was supplied and certified by Pfizer Company (Cairo-Egypt) to contain 99.98%. Pharmaceutical grade BZY was supplied and certified by Hikma pharmaceutical company (6th October-Egypt) to contain 99.71%. Topzol® cream labeled to contain 10 mg TIO and 10 mg BZY for each was supplied by Hikma pharmaceutical company (6th October-Egypt). Methanol HPLC grade (Scharlau, Spain) was used. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, U.K). Membrane filters 0.45 µm from Teknokroma (Barcelona, Spain) were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Standard solution preparation

Standard stock solutions of each of TIO and BZY were prepared by separately dissolving 40 mg of each drug in 100 mL methanol. Further dilutions were made in different sets of 10 mL volumetric flasks with the mobile phase to obtain a concentration range of 0.5–64µgml^{-1} for TIO and 1–192µgml^{-1} for BZY (HPLC method) and completed with the methanol to obtain a concentration range of 3–9 µgml^{-1} for TIO and 5–14µgml^{-1} for BZY (derivative spectrophotometry method).

Sample preparation

Four grams of Topzol® cream were accurately weighed in a conical flask then 80 mL of methanol were added, then the mixture was sonicated with the aid of heat not exceeding 40 °C. The mixture was cooled to room temperature and transferred quantitatively into a 100 mL volumetric flask and completed to volume with the methanol. The mixture was filtered to obtain a sample

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solution of concentration equivalent to 400µgmL^{-1} for TIO and 400µgmL^{-1} for BZY.

Procedure

Linearity and range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in the pharmaceutical product, to give accurate, precise and linear results.

For HPLC method

Accurately measured aliquots of standard stock solutions equivalent to were separately transferred into two series of 10 mL volumetric flasks and completed to volume with mobile phase to give final concentrations of 2–64 µgml^{-1} for TIO and 8–192 µgml^{-1} for BZY. Twenty µL aliquot of each solution was injected in triplicates onto the chromatograph equipped with a Thermo BDS Hypercil C18 column (250 mm x 4.6 mm, 5µm) applying an isocratic elution using methanol: water (0.2% aqueous tri ethyl amine, pH =7.5) (85:15, *v/v*) as a mobile phase at a flow rate 1ml min^{-1}. The pH of the aqueous phase was adjusted to 7.5 using ortho phosphoric acid. Calibration curve was constructed by plotting the peak areas against the corresponding concentrations of each drug in micrograms per milliliter.

For derivative spectrophotometry method

As zero order scan of TIO and BZY shows overlap of both drugs with each other (Figure 2), so first order derivative spectrophotometry is used by zero crossing technique (Figure 3). Accurately measured aliquots of standard stock solutions were transferred

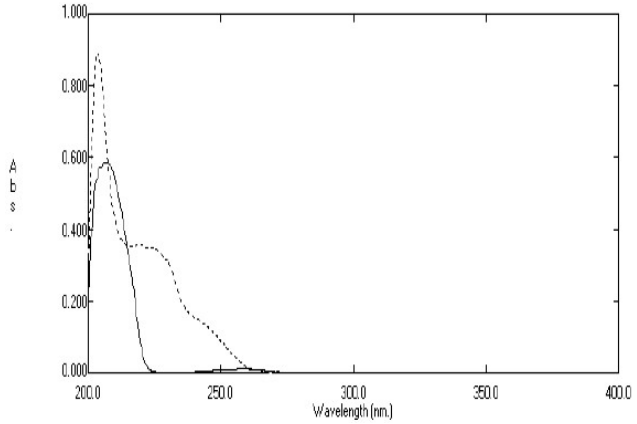

Figure 2 : Zero order scan TIO (.....) & BZY (·) standard solutions (8µg/ml for each compound)

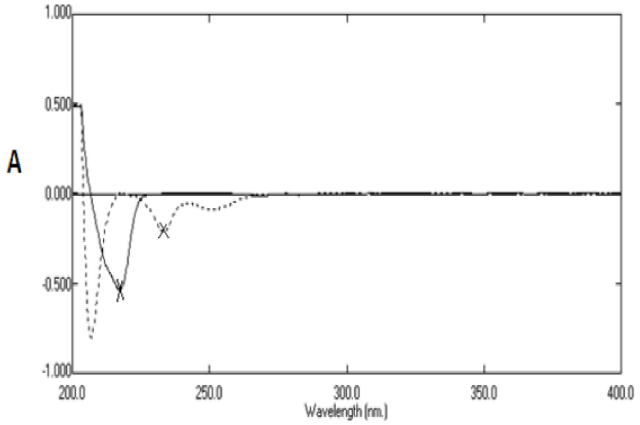

Figure 3 : First order scan of TIO (.....) & BZY (·) standard solution at 233.4 nm and 217.8 nm respectively (8µg/ml for each compound)

into two series of 10 mL volumetric flasks and completed to volume with methanol to give final concentrations of 3–11.5 µg ml^{-1} for TIO and 5–14 µgml^{-1} for BZY. The zero crossing first derivative spectra were recorded for TIO and BZY with trough amplitude measurement at 233.4nm and 217.8nm, respectively (Fig-

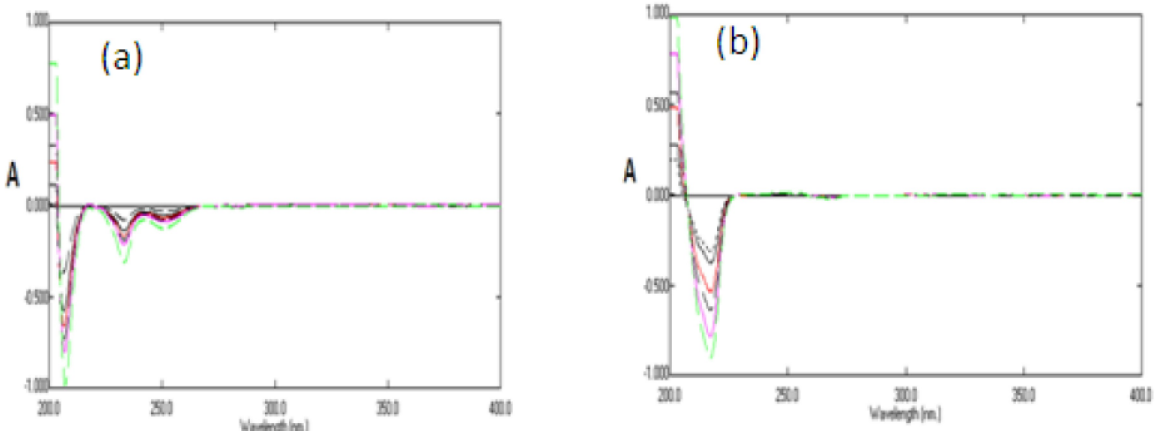

Figure 4 : First order scan calibration curve, (a) TIO calibration curve (3-11.5 µg/ml) (b) BZY calibration curve (5-14 µg/ml)

ure 4a) and (Figure 4b). Calibration curve was constructed by plotting the amplitudes against the corresponding concentrations of each drug in micrograms per milliliter.

Assay of laboratory prepared mixtures and Topzol® cream

Laboratory prepared mixtures

For HPLC method

The procedure mentioned under (2.5.1.1.) was repeated using laboratory prepared mixtures equivalent to 8-20 µg mL^{-1} TIO and 20-128 µg mL^{-1} BZY (Figure 5a)

For derivative spectrophotometry method

The procedure mentioned under (2.5.1.2.) was repeated using laboratory prepared mixtures equivalent to 5.2-7.8 µg mL^{-1} TIO and 5.2-7.8 µg mL^{-1} BZY (Figure 5b).

Assay of Topzol® cream.

For HPLC method

For the determination of the examined drugs in Topzol® cream, the sample solution prepared under (2.4.1.) was diluted with mobile phase to prepare different solutions equivalent to 21-55 µg mL^{-1} TIO and 21-55 µg mL^{-1} BZY and injected in triplicates (Figure 6a).

For derivative spectrophotometry method

For the determination of the examined drugs in Topzol® cream, the sample solution prepared under (2.4.2.) was diluted with methanol to prepare different solutions equivalent to 5.2-8.4 µg mL^{-1} TIO and 5.2-8.4µg mL^{-1} BZY and injected in triplicates (Figure 6b).

Accuracy

Accuracy of the results was calculated by % recovery of laboratory prepared mixtures of 6 different concentrations of the TIO and BZY and also by standard addition technique for Topzol® cream.

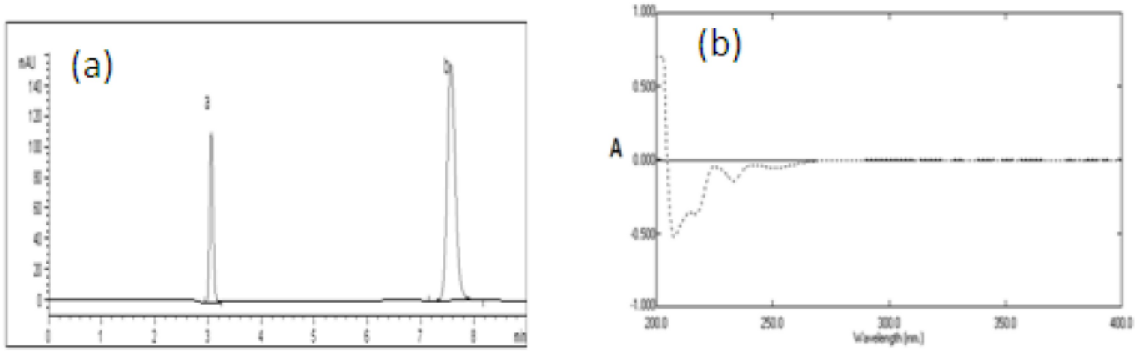

Figure 5 : Lab prepared mixture of TIO&BZY, (a) HPLC chromatogram of TIO and BZY (30µg/ml for each compound) (b) First order derivative spectrophotometry of TIO and BZY (5.5µg/ml for each compound)

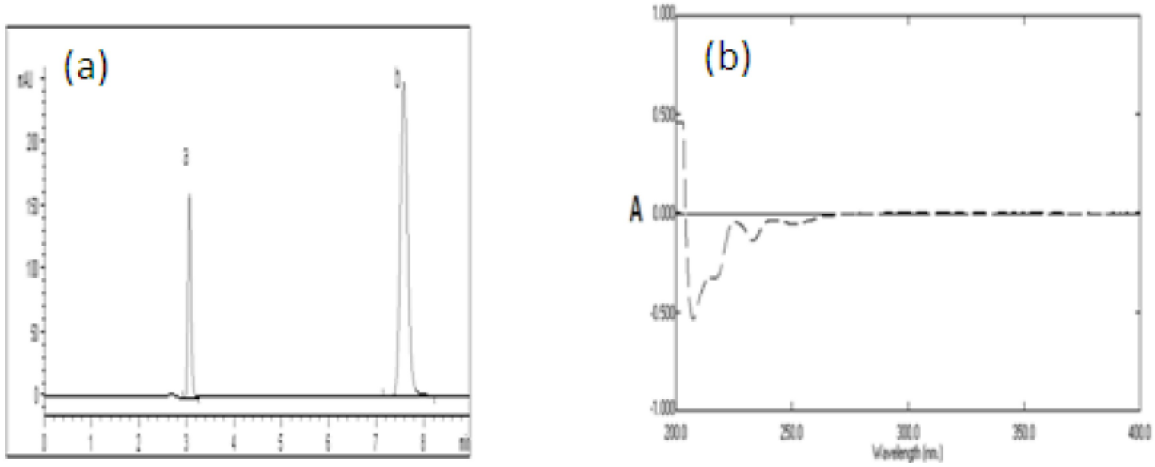

Figure 6 : Dosage form of TIO&BZY [Topzol cream®], (a) HPLC chromatogram of Topzol cream® (49µg/ml for each compound) (b) First order scan derivative spectrophotometry of Topzol cream® (5.2µg/ml for each compound)

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Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. In the present work, the chromatograms and scanned spectra of the samples were checked for the appearance of any extra peaks or any distortion. Besides, recoveries of the mixture in the dosage form were used to investigate the possibility of any interference of the excepients or additives in the cream.

Repeatability, interday and intraday precision

For HPLC method

The repeatability of the method was assessed by analyzing a laboratory prepared mixture containing 32 μg mL^{-1} for TIO and 32 μg mL^{-1} for BZY (n= 6). The precision (R.S.D %) for each was calculated. The values of the repeatability (%R.S.D) and inter-day and intra-day precision using 3 different concentrations (25.6 μg mL^{-1}, 32 μg mL^{-1} and 38.4 μg $mL^{-1}$of each TIO and BZY) in triplicates for three days for both analytes were calculated.

For derivative ratio method

The repeatability of the method was assessed by analyzing a laboratory prepared mixture containing 6.5 μg mL^{-1} for TIO and 6.5 μg $mL^{-}1$ for BZY (n= 6). The precision (R.S.D %) for each was calculated. The values of the repeatability (%R.S.D) and inter-day and intra-day precision using 3 different concentrations (5.2 μg mL^{-1}, 6.5 μg mL^{-1} and 7.8 μg $mL^{-1}$of each TIO and BZY) in triplicates for three days for both analytes were calculated.

Limit of detection and quantification

For HPLC method and derivative ratio spectrophotometry limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10. According to ICH recommendations[29], the approach based on the S.D. of the response and the slope was used for determining the detection and quantitative limits.

Robustness

For HPLC method, robustness is a measure of the method ability to remain unaffected by small variations in the method conditions and is an indication of the method reliability. Robustness was performed by changes in flow rate (changed from 1ml/min to o.8ml/min and 1.1 ml/min), organic strength of mobile phase (methanol percentage changed from 85% to 83% and 87%) and pH of aqueous component of mobile phase (changed from pH 7.5 to pH 7.3 and pH 7.7).

Forced degradation study of tioconazole

Forced degradation studies of TIO included appro-

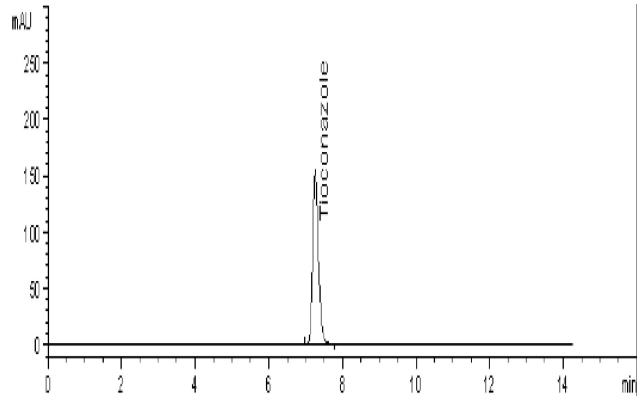

Figure 7 : Intact TIO without degradation (30μg/ml)

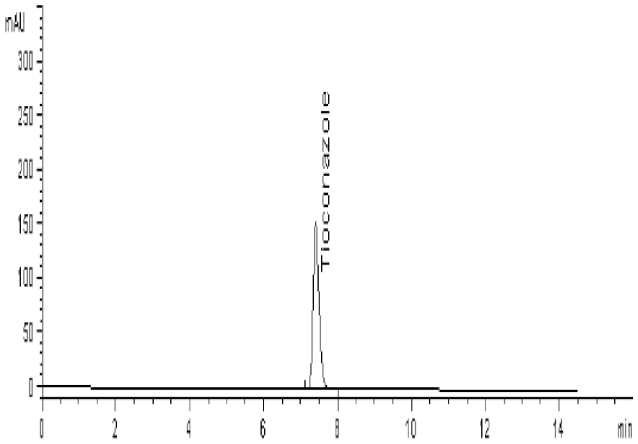

Figure 8 : Effect of heat on TIO powder

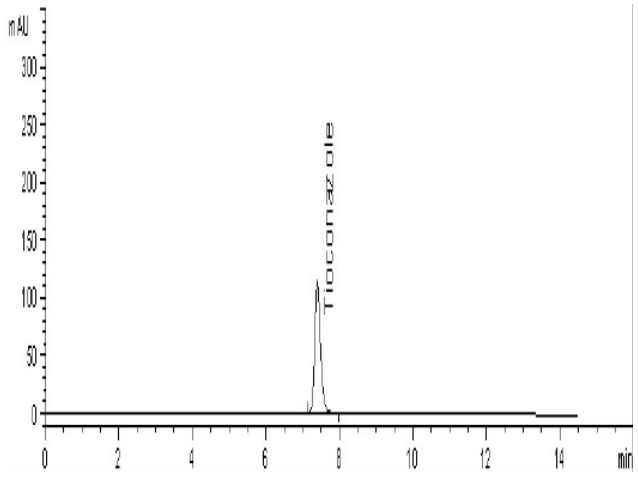

Figure 9 : Effect of UV on TIO powder

priate solid state and solution state stress conditions in accordance with the ICH regulatory guidance[29] was carried out with the HPLC method. The stock solution was used for the forced degradation study to provide an indication of the stability indicating property and specificity of proposed method. Prior to injection, samples were withdrawn at appropriate time, neutralized (in case of acid and alkali hydrolysis) and the solutions were diluted with mobile phase. The Intact drug not exposed to stress condition (Figure 7), was also injected to facilitate recognizing the extent of different stress factors on the drug. The total chromatographic run time was about two times the retention of the drug peak.

Thermal and photolytic degradation

The dry powder of the drug was placed in oven at 55°C for 72 h to study dry heat degradation (Figure 8). The photochemical stability of the drug was also studied by exposing the dry powder to UV light for 24 h. Powder was then dissolved and diluted with methanol to prepare a concentration of 500µg/ml. Then solutions

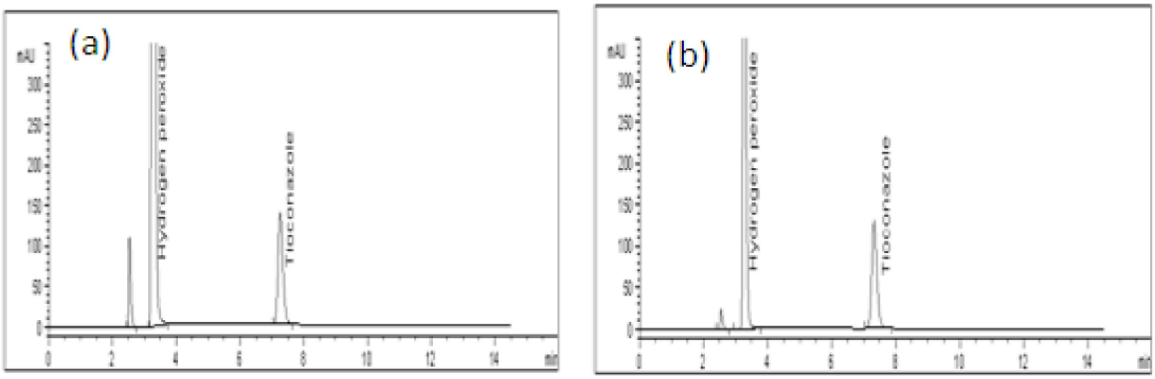

Figure 10 : HPLC chromatogram of effect of H_2O_2 on TIO, (a) 15% H_2O_2, (b) 3% H_2O_2

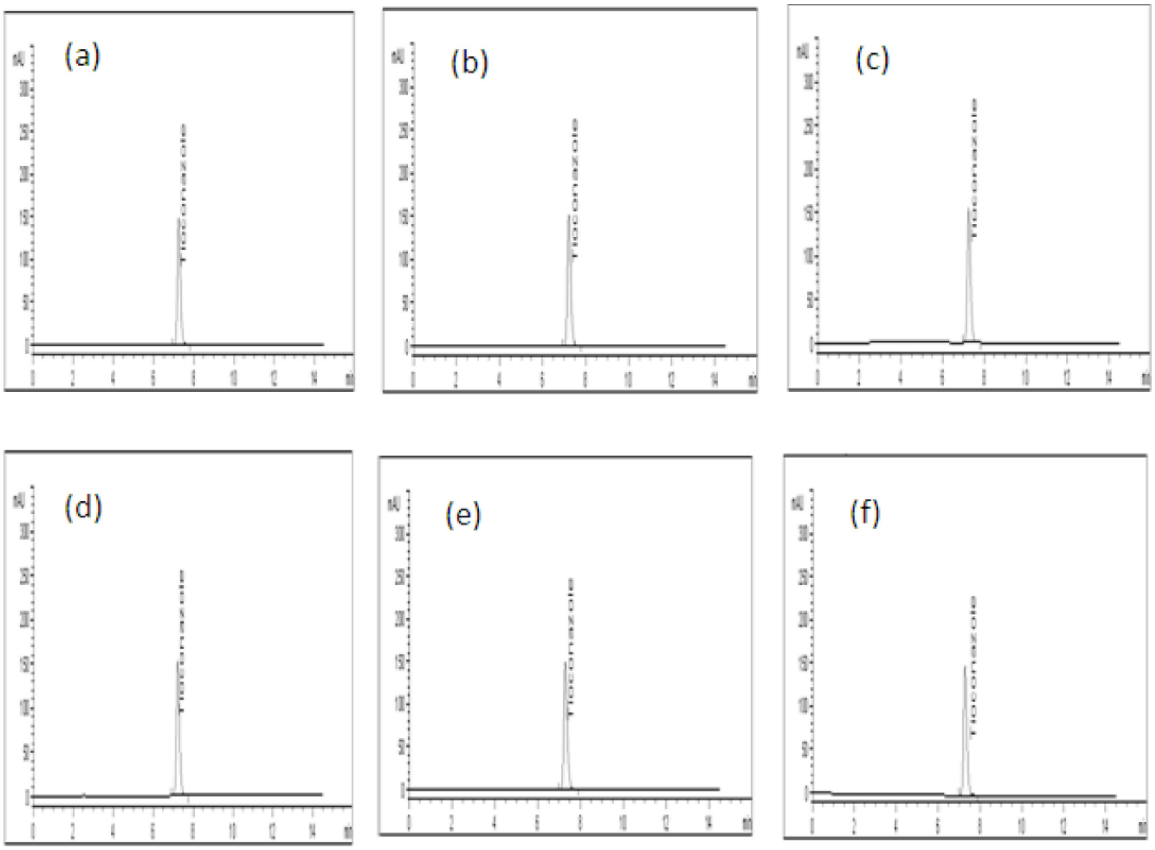

Figure 11 : HPLC chromatogram of effect of acid and alkali on TIO at RT, (a) effect of 1M HcL, (b) effect of 0.5M HcL, (c) effect of 0.1M HcL, (d) effect of 1M NaOH, (e) effect of 0.5M NaOH, (f) effect of 0.1M NaOH

are diluted with mobile phase and twenty micro liters of the resultant solutions were injected onto the chromatograph (Figure 9).

Hydrogen peroxide-induced degradation

To 5 mL of methanolicstock solution, 5 mL of 30% (*v/v*) $H_2O_2$and 6% (*v/v*) H_2O_2 were separately added to reach final concentrations of 15% and 3% (*v/v*) H_2O_2, respectively. The prepared mixtures were kept at room temperature for 5 and 12 h, respectively. Then solutions were diluted with mobile phase and twenty micro liters of the resultant solutions were injected onto the chromatograph (Figure 10a) and (Figure10b).

Acid- and base- induced degradation

To 5 mL of methanolic stock solution, an appropriate volume of 5 M HCL was added and the mixture was diluted with water to 10 mL to reach molarities of 1 M, 0.5 M and 0.1 M HCl, separately. The mixtures were kept at room temperature for 8 h (Figure 11a), (Figure 11b) and (Figure 11c). Alkaline degradation studies were carried out in a similar manner with molarities of 1 M, 0.5 M and 0.1 M NaOH for 8 h (Figure 11d), (Figure 11e) and (Figure 11f). These experiments were repeated at higher temperature of 75 æ%C for 0.5 h. while keeping all other conditions constant. Solutions are neutralized prior to injection. The forced degradation in acidic (Figure 12a), (Figure 12b) and (Figure 12c) and basic media (Figure 12d), (Figure 13e) and (Figure 13f) was performed in the dark in order to exclude the possible degradative effect of light. Twenty micro liters of the resultant neutralized solutions were injected onto the chromatograph.

RESULTS AND DISCUSSION

The aim of this work was to develop simple, selec-

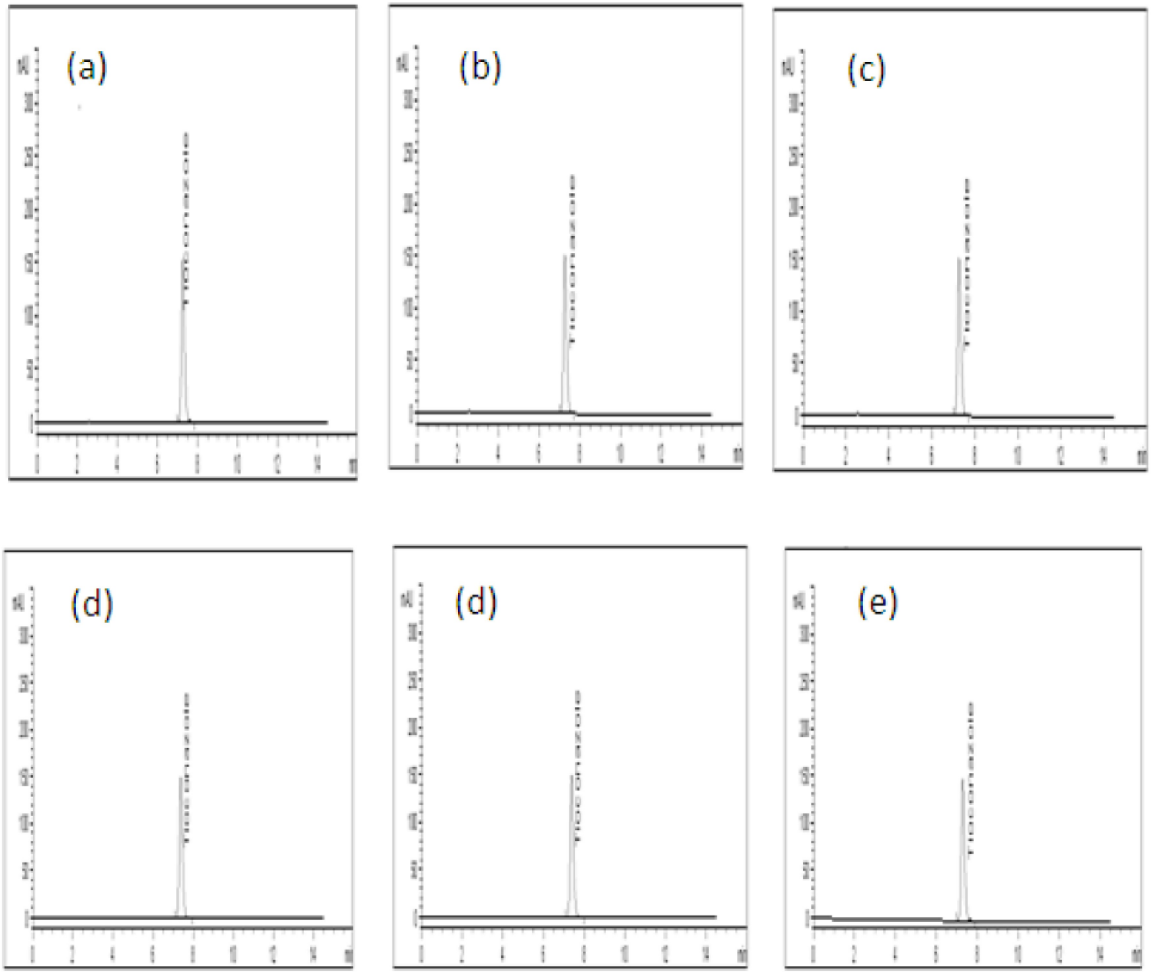

Figure 12 : HPLC chromatogram of effect of acid and alkali on TIO at 75°C, (a) effect of 1M HcL, (b) effect of 0.5M HcL, (c) effect of 0.1M HcL, (d) effect of 1M NaOH, (e) effect of 0.5M NaOH, (f) effect of 0.1M NaOH

tive and validated HPLC and spectrophotometric methods that are suitable for the simultaneous determination of TIO and BZY in bulk solutions and creams. Due to the wide spread and simple use of spectrophotometers, a derivative spectrophotometric method was utilized along with the HPLC method to determine TIO and BZY simultaneously. As BZY is used widely as a preservative in cream formulations, so the proposed methods allowed its determination simultaneously with TIO and proved to be specific, precise and accurate for the quality control of the cited drugs in pharmaceutical preparations.

Method development

HPLC method

The development of a simultaneous and sufficiently selective analytical method to determine TIO and BZY, with quantitation sensitivity for the cited compounds in their binary mixture, was of interest and no analytical method has been reported to analyze this mixture simultaneously. In this work, it was achieved using a simple RP-LC with UV detection. Regarding the relatively different physicochemical properties of the cited molecules, the use of conventional HPLC columns available in most quality control laboratories was aimed. During the optimization cycle, several chromatographic conditions were attempted either by changing the analytical column used for peak separation: C18 column (250 mm×4.6 mm, 5 µm) – Thermo Hypersil,C18column (250 mm×4.6 mm, 5 µm) –Agilent TC-C18(2) and C8 column (250 mm×4.6 mm, 5 µm) – Zorbax SB or by using various mobile phase compositions of buffers, methanol and acetonitrile, in different proportions and pH values. Isocratic and gradient mode were tried and it was found that, an isocratic mode with at least 85% of methanol was needed to elute TIO and BZY with reasonable peak shape and analysis time. At lower methanol concentrations, separations were obtained but with excessive tailing for TIO peak and unreasonable analysis time. Tri ethyl amine was added to aqueous phase of mobile phase (0.2%, *v/v*) to minimize broadening and tailing of TIO peak. Different pH values of aqueous component of mobile phase were tried at pH 3.2, pH 5, pH 6.5 and pH 7.5 and it was found that at pH 7.5, optimum resolution with reasonable retention times was observed but at pH values lower than 7.5, unreasonable resolution and analysis time of TIO and BZY eluted peaks. So, a satisfactory separation of TIO and BZY was obtained using C18 column (250 mm×4.6 mm, 5 µm) – Thermo Hypersil with a mobile phase composed of methanol and water (85:15, *v/v*), adding tri ethyl amine to the aqueous phase (0.2%*v/v*) adjusting pH to7.5byortho-phosphoric acid(Figure 5a).

Derivative spectrophotometry method

The zero order absorption spectra of TIO and BZY show overlapping spectra especially at the maximum of BZY (Figure 2). For the determination of TIO and BZY, the first derivative spectra were calculated using $\Delta\lambda$=4 nm and a scaling factor of 10. The trough amplitudes of the obtained first derivative spectra were measured at 217.8 nm for BZY where TIO showed zero crossing and at 233.4 nm for TIO where BZY showed zero crossing, (Figure 3).

Validation of the methods

Linearity and range

Linearity was studied for TIO and BZY. For HPLC method, a linear relationship between area under the peak (AUP) and analyte concentration (C) was obtained and it was found to be linear in range of 2-64 µg ml^{-1}forTIO and 8-192 µg ml^{-1} for BZY. For derivative specrtrophotometric method, a linear relationship between amplitude (measured at 233.4 and 217.8 for TIO and BZY respectively) and analyte concentration (C) was obtained and it was found to be linear in range of 3-11.5 µg $ml^{-1}$for TIO and 5-14 µg $ml^{-1}$for BZY. The regression equation, correlation coefficient and other analytical data of the calibration curves including standard deviations for the slope and intercept (S_b,S_a) for

TABLE 1: System suitability results of HPLC proposed method

Item	HPLC method	
	BZY	TIO
N	12003	10826
R	----	21.19
T	1.13	1.177
RSD% of 6 injections of		
Peak area	0.702	0.422
Retention time	0.034	0.024

N: Number of theoretical plates; R: resolution factor; T: Tailing factor

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TABLE 2 : Assay parameters and method validation obtained by applying HPLC and derivative spectrophotometry methods for the simultaneous determination of tioconazole and benzyl alcohol in bulk solution and cream.

Item	HPLC method		Derivative spetrophotometry method	
	TIO	BZY	TIO	BZY
Retention time	7.3 min	3.05 min	-----	-----
Wavelength of detection	210 nm	220 nm	233.4 nm	217.8 nm
Linearity				
Range of linearity	2-64 µg ml^{-1}	8-192µg ml^{-1}	3-11.5 µg ml^{-1}	5-14µg ml^{-1}
Regression equation	y = 53.6257x - 3.3977	y = 12.5601x +144.3893	y = 0.0267x + 0.0012	y = 0.0667x - 0.0171
correlation coefficient (r^2)	1.0000	0.9920	0.9987	0.9997
LOD (µg ml^{-1})	0.4859	22.13	0.296	0.206
LOQ(µg ml^{-1})	1.62	73.76	0.986	0.686
S_b	0.235	0.563	0.00049	0.00059
S_a	7.09	55.69	0.0036	0.0057
Confidence limit of the slope	53.6257±0.652	12.5601±1.563	0.0267±0.0014	0.0667±0.0016
Confidence limit of the intercept	3.3977±19.68	144.3893±154.595	0.0012±0.01	0.0171±0.0158
Standard error of the estimation	12.47	91.53	0.0032	0.0046
Precision				
Intra day %R.S.D.	0.423	0.702	0.137	0.164
Inter day %R.S.D.	0.245	0.993	0.76	0.35
Drug in dosage form	100.74±1.324	101.65±1.306	99.124±1.05	99.35±0.67
Accuracy				
Drug in laboratory mix.	100.94±1.183	99.62±1.74	99.25±0.693	98.655±0.395
Drug added	99.71±1.126	100.11±0.809	99.99±1.14	99.81±1.07

S_b: slope standard deviation, S_a: intercept standard deviation, LOD: limit of detection, LOQ: limit of quantification and RSD: relative standard deviation

TIO and BZY are summarized in (TABLE 2).

Accuracy

The recovery percentage attained by analysis of TIO and BZY in laboratory prepared mixtures, dosage form (Topzol® cream) and by standard addition technique was calculated and found to be in range of 98-102% which proved the accuracy of the two proposed methods (TABLE 2).

Precision

Precision was estimated by repeatability and inter and intra-day precision RSD% of repeatability and inter and intra-day precision was found to be less than 1 whish ensured the precision of the two proposed methods (TABLE 2).

Specificity

The chromatograms and scanned spectra of the samples were checked for the appearance of any extra peaks. No interference from any of the excipients was found. In addition, the chromatograms and scanned

TABLE 3 : Influence of flow rate of the mobile phase on resolution of peaks (HPLC method)

Item	0.8mL min^{-1}	1 mL min^{-1}	1.1 mL 1.2 min^{-1}
Resolution factor BZY –TIO	20.63	19.41	18.77

TABLE 4 : Influence of organic strength of the mobile phase on resolution of peaks (HPLC method)

Item	83%	85%	87%
Resolution factor TIO- BZY	22.56	19.36	21.73

TABLE 5 : Influence of pH of the mobile phase on resolution of peaks (HPLC method)

Item	pH 7.3	PH 7.5	PH 7.7
Resolution factor BZY –TIO	20.19	19.29	19.58

TABLE 6 : Statistical analysis of the results obtained by the proposed HPLC, derivative spetrophotometry and the reference methods

Statistical term	HPLC method				Derivative spectrophotometer method			
	TIO		BZY		TIO		BZY	
	HPLC	Reference method**	HPLC	Reference method***	Derivative spetrophotometer	Reference method**	Derivative spetrophotometer	Reference method***
Mean	100.93	99.8	99.62	100.07	99.25	99.8	99.22	100.07
S.D.	1.18	0.5366	1.737	0.868	0.693	0.5366	1.563	0.868
R.S.D.	1.172	0.5377	1.74	0.867	0.698	0.5377	1.575	0.867
N	5	6	5	6	6	6	6	6
Variance	1.3924	0.2879	3.0171	0.7534	0.48	0.2879	2.443	0.7534
t-value	1.988 (2.228)*		0.527 (2.228)*		1.537 (2.228)*		1.165 (2.228)*	0.089 (2.228)*
F-value	4.836 (5.05)*		4.005 (5.05)*		1.667 (5.05)*		3.243 (5.05)*	3.133 (5.05)*

*** Figures in parentheses are the corresponding theoretical t -and F-values at P = 0.05; ** Reference method for TIO according to USP-34 pharmacopeia[28]; *** Reference method for BZY according to British pharmacopeia[30]**

spectra of each analyte in the sample solution were identical to that received by the standard solutions with good recovery percentages of analysed compounds in sample solutions. These results confirmed the specificity of the two proposed method.

Limit of detection and limit of quantification

According to ICH recommendations[29], the approach based on the S.D. of the peak response or measured amplitude and the slope was used for determining the detection and quantitative limits. The theoretical values were assessed practically for two proposed methods (TABLE 2).

Robustness

Robustness was studied only for the HPLC method. The most important parameter to be studied was the resolution factor between the two peaks of TIO and BZY. Although changes made to pH of the aqueous component of mobile phase, mobile phase organic strength and flow rate, no significant change in resolution factor between TIO and BZY peaks indicating good robustness of the proposed HPLC method (TABLE 3-5).

Statistical analysis of the results

A statistical analysis of the results obtained by the proposed methods and the reference methods for each analyte was carried out by "SPSS statistical package version 11". The difference between groups was tested by (t-test) and (F-test) at p=0.05 (TABLE 6). The test ascertained that there was no significant difference among the methods.

CONCLUSION

The proposed HPLC and derivative spectrophotometric methods provide simple, accurate and reproducible quantitative analysis for the simultaneous determination of TIO and BZY in bulk solution and cream. These methods were validated as per ICH guidelines. The proposed methods are suitable for the quality control determination of the cited drugs in ordinary laboratories.

REFERENCES

[1] S.Sweetman; Martindale: The Complete Drug Reference, pharmaceutical press, London, UK, 37th Edition, **(2011)**.

[2] L.Brunton, B.Chabner, B.Knollman; Goodman and Gilman's: The Pharmacological Basis of Therapeutics, McGraw-Hill Professional, New York,USA, 12th Edition, **(2010)**.

[3] L.Gagliardi, D.De-Orsi, P.Chimenti, R.Porra, D.Tonelli; HPLC determination of imidazole antimycotics in antidandruff cosmetic products, Anal-Sci., **19(8)**, 1195-1197 **(2003)**.

[4] P.D.Ferguson, D.M.Goodall, J.S.Loran; Systematic approach to the treatment of enantiomeric separations capillary electrophoresis and liquid chromatography. III. A binding constant-retention factor

relationship and effects of acetonitrile on the chiral separation of tioconazole, J.Chromatogr,-A., **745(1-2)**, 25-35 **(1996)**.

[5] S.G.Penn, D.M.Goodall, J.S.Loran; Differential binding of tioconazole enantiomers to hydroxypropyl-beta-cyclodextrin studied by capillary electrophoresis, J.Chromatogr., **636(1)**, 149-152 **(1993)**.

[6] A.G.Wright, J.C.Berridge, A.F.Fell; Development and optimization of a high-performance liquid-chromatographic assay for tioconazole and its potential impurities.| II.| Selection of detection conditions for potential impurities, The Analyst., **114(1)**, 53-56 **(1989)**.

[7] A.G.Wright, A.F.Fell, J.C.Berridge; Development and optimisation of a high-performance liquid chromatographic assay for tioconazole and its potential impurities. I. Selection of separation conditions, J.Chromatogr., **464(1)**, 27-38 **(1989)**.

[8] J.C.Berridge; Analysis of tioconazole using high-performance liquid chromatography with a porous graphitic-carbon column, J.Chromatogr., **449(1)**, 317-321 **(1988)**.

[9] J.C.Berridge, P.E.Last, R.V.Platt; High-performance liquid-chromatographic determination of tioconazole in pharmaceutical formulations, J-Pharm-Biomed-Anal., **3(4)**, 391-394 **(1985)**.

[10] E.Kublin, T.Kaniewska; Determination of antimycotic substances, derivatives of imidazole, by gas chromatographic method, Chem.Anal. (Warsaw), **41(1)**, 19-26 **(1996)**.

[11] M.M.Abdel-Khalek, M.S.Mahrous, H.G.Daabees, Y.A.Beltagy; Three new sensitive spectrophotometric methods for the assay of non UV-absorbing tioconazole, Anal-Lett., **25(11)**, 2037-2050 **(1992)**.

[12] I.Hewala, H.El Fatatre, E.Emam, M.Mubrouk; Development and application of a validated stability-indicating HPLC method for simultaneous determination of granisetron hydrochloride, benzyl alcohol and their main degradation products in parenteral dosage forms, Talanta, **82(1)**, 184-195 **(2010)**.

[13] H.W.Xi, Q.Ma, Q.Li, H.M.Lei, H.Bai, C.Wang; Simultaneous determination of 17 allergens in cosmetics by high performance liquid chromatography, Journal of instrumental analysis, **29(1)**, 46-50 **(2010)**.

[14] G.A.Shabir; Method development and validation of preservatives determination (benzyl alcohol, ethylene glycol mono phenyl ether, methyl hydroxy benzoate, ethyl hydroxy benzoate, propyl hydroxy benzoate, and butyl hydroxybenzoate) using HPLC, Journal of Liquid Chromatography & Related Technologies, **30(13-16)**, 1951-1962 **(2007)**.

[15] P.Perez-Lozano, E.Garcia-Montoya, A.Orriols, M.Minarro, J.R.Tico, J.M.Sune-Negre; A new validated method for the simultaneous determination of benzocaine, propylparaben and benzyl alcohol in a bioadhesive gel by HPLC, J-Pharm-Biomed-Anal., **39(5)**, 920-927 **(2005)**.

[16] O.A.Čudina, M.I.Čomor, I.A.Janković; Simultaneous Determination of Bifonazole and Benzyl Alcohol in Pharmaceutical Formulations by Reverse-Phase HPLC, Chromatographia, **61(7-8)**, 415-418 **(2005)**.

[17] G.C.Wood, C.Dass, M.R.Iyer, A.M.Fleischner, B.B.Sheth; Stability of deslorelin injection, PDA J.Pharm.Sci.Technol., **51(5)**, 176-80 **(1997)**.

[18] B.J.Stanley, C.R.Foster, G.Guiochon; On the reproducibility of column performance in liquid chromatography and the role of the packing density, J.Chromatogr, A., **761(1-2)**, 41-51 **(1997)**.

[19] S.R.Bakalyar, C.Phipps; Using large sample volumes to maximize sensitivity with microbore HPLC columns, LC-GC., **14(12)**, 1024, 1026-1027 **(1996)**.

[20] S.Rabouan-Guyon, P.Courtois, D.Barthes; Determination of heptaminolacephyllinate in pharmaceutical preparations by high-performance liquid chromatography, Farmaco., **51(11)**, 739-746 **(1996)**.

[21] H.S.I.Tan, M.A.Manning, M.K.Hahn, H.G.H.Tan, U.R.Kotagal; Determination of benzyl alcohol and its metabolite in plasma by reversed-phase high-performance liquid chromatography, J.Chromatogr, Biomed-Appl., 106(1 (J. Chromatogr., 568)), 145-155 **(1991)**.

[22] G.N.Subba-Rao, J.W.Sutherland, G.N.Menon; High-performance liquid-chromatographic assay for thyrotrophin-releasing hormone and benzyl alcohol in injectable formulation, Pharm-Res., **4(1)**, 38-41 **(1987)**.

[23] D.Gormand, J.L.Brazier, F.Comet, D.Lecompte; High-performance liquid-chromatographic determination of benzyl alcohol and its degradation product benzaldehyde in a pharmaceutical cream, J.Chromatogr., **355(1)**, 345-349 **(1986)**.

[24] T.D.Wilson, M.D.Forde, A.V.R.Crain; Simultaneous liquid-chromatographic determination of glutaric acid, phenylephrine and benzyl alcohol in a prototype nasal spray with application to di- and tri-carboxylic acids, J.Pharm-Sci., **74(3)**, 312-315 **(1985)**.

[25] N.G.Goger, L.M.Zahtep, A.Berkkan, Z.Senturk; Simultaneous spectrophotometric determination of piroxicam and benzyl alcohol in gel forms, Chem-Anal (Warsaw,-Pol)., **50(6)**, 1059-1067 **(2005)**.

[26] J.Ghasemi, A.Niazi, S.Ghobadi; Simultaneous Spectrophotometric Determination of Benzyl Alcohol and Diclofenac in Pharmaceutical Formulations by Chemometrics Method, J.Chin.Chem.Soc., **52**, 1049-1054 **(2005)**.

[27] Y.C.de-Micalizzi, N.B.Pappano, N.B.Debattista; First and second order derivative spectrophotometric determination of benzyl alcohol and diclofenac in pharmaceutical forms, Talanta., **47(3)**, 525-530 **(1998)**.

[28] Authority of the United States Pharmacopeial Convention, The United States Pharmacopoeia (USP 34), National Formulary (NF 29), Maryland, USA, **(2011)**.

[29] The European Agency for the Evaluation of Medical Products, ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures, Methodology GPMP/ICH/281/95, **(1996)**.

[30] D.Woolfson; TheBritish pharmacopeia, London: the stationary office, UK, **(2013)**.